Ecological Functions and Ecosystem Services of Overwash Ponds on North Core Banks, NC

THE UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL
UNC Institute of Marine Science Field Site, Morehead City, NC

Fall 2021 Capstone Report

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Chapter 1: Introduction

North Core Banks is a stretch of protected barrier island shoreline, falling between Ocracoke Inlet and Portsmouth Island (Riggs, S. R. et al. 2007). The barrier islands of North Carolina provide the inland with a vital buffer between the open ocean, diminishing the effects of storms and reducing coastal erosion along the mainland (Dolan, R. 1973) (Dolan, R. et al. 1973). As a result, the barrier islands experience drastic changes in geography with landforms being added, removed, and displaced with every hurricane season. In 2019, one such season brought with it 110 mph winds, tornados, and up to 7 ft of wave swell in the form of Hurricane Dorian, which made landfall at Cape Hatteras in early September (U.S. Department of Commerce, N. 2019). The rising water levels in Core Soundinundated sections of Core Banks, shifting and creating new landscapes. This increased water level created more than 90 inlets across North Core Banks (Sherwood et al. 2020). Over time the beach accreted and sealed off inlets creating dozens of new ponds. Many of these Dorian-created ponds exist in areas that were formerly sand dunes; washed away by the sound during the storm. This contrasts with many of the older, pre-existing ponds on the island, which are mostly surrounded by vegetation or marsh (Kling, G. W. 1986). Three years later, the new ponds remain, their function largely understudied.

The National Parks Service, founded in 1916, works to manage and protect national parks and monuments along with many other natural and historical locations of value. Its mission statement reads as follows: “The National Park Service preserves unimpaired the natural and cultural resources and values of the National Park System for the enjoyment, education, and inspiration of this and future generations. The Park Service cooperates with partners to extend the benefits of natural and cultural resource conservation and outdoor recreation throughout this country and the world.” (U.S. National Park Service)

In 2021, the National Parks Service proposed a study on a set of pre- and post-Hurricane Dorian ponds that would investigate the ecosystem services and ecological functions of the ponds. To address this, we measured parameters such as hydrology, primary productivity, and biological diversity. The four main goals of this project were to:

1. Measure the effects of overwash and precipitation events on the ponds as well as the main drivers of sediment transport
2. Measure water quality and carbon sequestration
3. Compare diversity and productivity of macrophytes and microalgae
4. Compare species abundance, diversity, and biomass between the ponds.

Understanding the ecosystem services and ecological functions of these new ponds is essential information for the NPS as North Core banks is a popular recreational area. Results of this study can help guide future infrastructure implementation and public safety. Additionally, this information can be used to better protect the ponds’ diverse ecosystems and contribute to surrounding ecosystem stability.

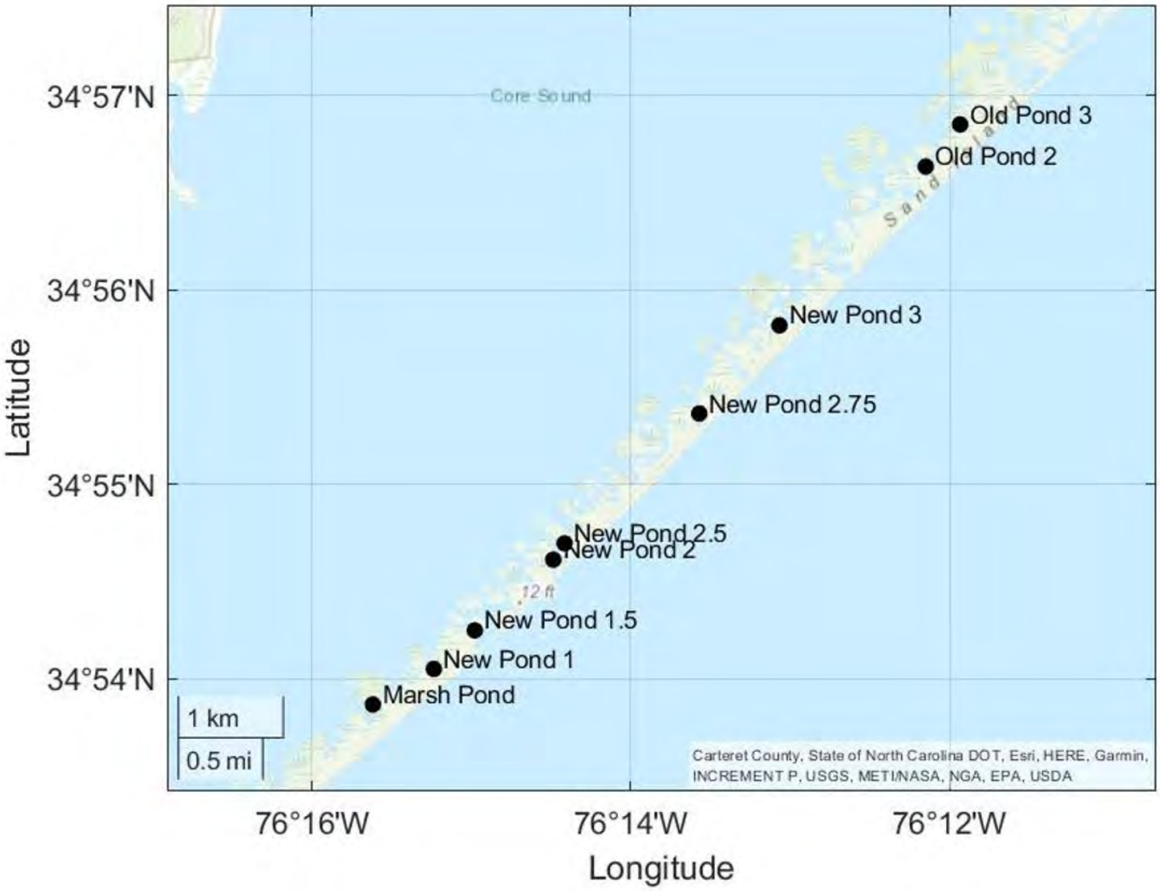

Figure 1.1. Map of the different old and new ponds used for this study.

Chapter 2: Geomorphology and Hydrology

Geomorphology and Hydrology of Ponds Found at Core Banks, NC

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Fall 2021

2.1 Introduction

Hurricane Dorian cut several inlets through North Core Banks and over time sediment has been transported and deposited on the beach sealing most of these inlets off, leaving behind several ponds. The mechanisms that are driving this sediment transport process and influencing pond morphology on North Core Banks are not well known. This study aims to identify key drivers of geomorphological change and attempt to understand the future geomorphological trajectories of the ponds and surrounding areas. Our findings can inform design decisions regarding placement of buildings and roads in a manner that minimizes negative interactions between anthropogenic features and natural features.

To investigate the key drivers of change, we had three main goals. Our first objective was to determine the overwash recurrence interval for these ponds and if there was a temporal pattern of overwash events among ponds. We hypothesize that the overwash recurrence interval for each pond would be variable depending on its proximity to the ocean and the topography between the pond and the ocean. Ponds with flat and low-elevation topography between the pond and the ocean are more likely to experience overwash than the ponds with dunes or a high-elevation berm between them and the beach. Second, we aimed to understand how the berm elevation between the ponds and the ocean would affect the depth of the ponds. We hypothesize that a more extreme difference in berm height and pond height (steeper slope of the wash platform) would result in higher infill due to a more drastic slope between the berm and the ponds which would facilitate water/sediment movement downhill. Ultimately, this will lead to infill which will directly affect average pond depth. The wash platform is the area between the edge of the pond and the berm. Third, we model how the slope of the wash platform changes over time. We hypothesize that the slope of the wash platform would become less extreme in magnitude over time due to sediment transport resulting from mechanisms such as wind, overwash, and precipitation.

Previous research in this area has found that overwash and inundation events on barrier islands can cause drastic changes in island geomorphology (Sherwood et al., 2014). Overwash is when wave surge elevation exceeds ground elevation. An overwash event on a barrier island can carry a significant amount of sediment towards the sound side of the island (Rodriguez et al., 2020). This is relevant to our research as Hurricane Dorrian initially transported sand from the sound side of the island towards the ocean side (which resulted in inlets). However, overtime, it appears that sediment has been moving from the ocean side of the island towards the sound side of the island as some of these inlets have closed and formed ponds.

Inundation of an island with water from either precipitation or overwash also plays a significant role in changing the geomorphology of the island by inducing sediment transport (Long et al., 2014). However, it is thought that there is not a linear relationship between the magnitude of the inundation event and the amount of sediment transported (Long et al., 2014) - instead Long et al. proposed that there exists an intermediate level of inundation that results in the greatest amount of sediment transport. This research is relevant to the questions we are asked, as similar trends could be impacting sediment in and around the ponds we are studying. Additional prior research establishes clear methodologies to consistently measure water elevation (VanDusen et al., 2016). Work has also been done to compile strategies to track beach profile evolution (Donnelly et al., 2006). In our research, we will draw upon these methods to test our hypotheses. We track sediment transport in and around the ponds using a variety of methods. We are particularly interested in how beach topography, overwash, and precipitation play a role in this transport process. We used a variety of survey methods to collect water elevation, pond bathymetry, ground elevation between September 15, 2021, and October 27, 2021.

2.2 Methods and Materials

We primarily focused our study on New Pond 1 (NP1), New Pond 2 (NP2), New Pond 3 (NP3). We also imaged Old Pond 2 (OP2), Old Pond 3 (OP3), New Pond 1.5 (NP1.5), New Pond 2.5 (NP2.5) and New Pond 2.75 (NP2.75). See appendix for map of ponds.

Hardware

Drones:

By selecting the area of interest in a phone application called "Maps made Easy", we can use drones to take a series of pictures which can then be used to build a 3D elevation model of the area. The drone automatically followed a preset flight track and took pictures in the desired location. The result is a series of photos of the desired location, which is then fed into a map creation software to generate a 3D high-resolution (1pixel per inch) raster map with x, y, and z coordinates after data processing. Drone maps can be made with ground control points (GPCs) for additional accuracy.

Fish Finder with Sonar System:

We attached a sonar system under the kayak to collect the bathymetry data for new pond 1. The sonar system supplied continuous and real-time elevation data as we kayak in the pond. This fish finder/sonar system was used to map roughly half of the bathymetry of new pond 1. The bathymetry data were collected in transects in the pond which were parallel to the beach spaced about 1 meter about.

Real-Time Kinematic (RTK):

RTK is a tool measuring the x, y, z coordinates of a specific location at a resolution <+/- 3 cm in real time. To use it, the person must make sure the receiver is stable and level. The receiver collects location readings from several satellites and a hand-held computer corrects those readings using measurements obtained at a nearby base station.

Because of the turbidity and reflectivity of the water in the pond, the drone imagery was not able to accurately measure elevation of the pond floors, therefore, we largely utilized the RTK for taking bathymetry measurements inside of the ponds. One person went into the pond and held the RTK to measure the points in the pond. The selected points were around 1.5-meter apart from each other and evenly distributed in the pond. Another person on the bank would record the data.

Water level loggers:

We used HOBO ware water level data loggers (3 U20L, 2 Titanium Water Level Data Logger), which measures pressure and temperature continuously. With a total of five loggers, we deployed two at New Ponds 1 and 2 and deployed one logger at New Pond 3. New Ponds 1 and 2 had a logger in a well that was submerged only when the water table was high and a logger that was submerged full-time in the pond itself. New pond 3 only had one sensor, which was deployed in the pond and submerged full-time. If a logger in a pond was observed in a location where high volume of sediment was recently transported, the logged was moved to a deeper location in the pond.

For logger deployment in well, we dug a hole to find the water surface and then put the water logger fixed within a permeable PVC pipe in the hole. For deployment in the pond, the sensor was affixed to a metal fence post. The detailed GPS information is recorded by RTK for each water logger. To calculate the precise elevation, we also measured the cap elevation (the top end of the PVC pipe) with the RTK and the distance between the sensor and the cap with a ruler.

Software

HOBO Ware:

HOBO Ware is the software used to process the water level logger data. We us this interface to compensate for the barometric pressure, from Davis Shore – KNCDAVIS3 weather station (38.6°N, 76.46°W), and convert the raw pressure data to depth of the sensor in meters. Calculations for actual water surface elevation occurred in excel using the elevation in NAVD88 of the top cap/ top of sensor from the RTK.

Pix4D:

Pix4D is a software which is used to create 3D orthomosaic maps from done imagery. We have used two methodologies to create maps. 1) We used ground control points during map generation to have greater location and elevation accuracy 2) We did not use ground control points when the map was only needed for a visual rather than a detailed topographic analysis of an area, to increase map-creation speed.

ArcGIS:

ArcGIS is a Geographic Information System software package. We used it to process initial bathymetry data and combine those data from RTK and sonar system.

Surfer:

Surfer is a scientific GIS software. It was used to visualize the map layers processed by Pix4D. It supplies multiple visualizations to show the elevation models. We used the software to compress the huge original files from Pix4D, zooming into the concerned study area and combining map layer from ArcGIS (bathymetry of pond) and drone collected data. We also exported transect data from elevation models.

Field Method History

Date (2021)	**Field Methods**
Sept. 15 & 16	• Water logger deployment (NP 1 well/pond, NP 2 well/pond, NP3 pond) • Drone imagery of NP 1, 2, 3 (w/ GCPs) • Bathymetry measurement with RTK in pond 1
Sept. 23	• Bathymetry measurement with RTK (NP 2, 3) • Collected water logger data (NP 1 well/pond, NP 2 well/pond, NP3 pond) • Relocated water logger (NP 1 pond, NP 2 pond, NP3 pond)
Sept. 29	• Collected water logger data (NP 1 well/pond, NP 2 well/pond, NP3 pond) • Drone imagery of NP 1, 2, 3 (w/ GCPs) • Drone imagery of NP 1.5, 2.5, 2.75 (no GPCs) • Fishfinder/Sonar bathymetry (NP 1)
Oct. 13	• Drone imagery of NP 1, 2, 3 (w/ GCPs) • Drone imagery of OP 2, 3 (no GPCs)
Oct. 27	• Drone imagery of NP 1, 2, 3 (w/ GCPs) • Collected water logger data (NP 1 well/pond, NP 2 well/pond, NP3 pond)

Data Analysis Methods

Map Creation:

Orthomosaic maps of new ponds 1, 2, 3 were created using drone imagery and Pix4D software. Drone images were uploaded into Pix4D and processed in tandem with data on ground control points to generate both an RBG orthomosaic for use as a base map as well as a digital elevation model.

Bathymetry Analysis:

To analyze bathymetry data collected by fish finder, we first examined imaging produced by soar system and excluded all the null or invalid data. Invalid data were collected in areas where depths were less than 1 meter and the sonar could not image the bottom. We discerned which data were null or invalid by visually examining the fish finder data in SonarTRX; when major jumps or discontinuities were spotted, those data were marked to be excluded. We then exported the filtered data into an excel spreadsheet.

We collected all the other bathymetry data by conducting manual transects in the ponds with the Trimble RTK GPS. All the X and Y coordinates are in UTM system (NAD83), and elevation data are in NAVD88. We exported this bathymetry data into an excel spreadsheet and then imported them into ArcGIS for geoprocessing. We created a continuous layer by combining data from sonar and RTK and using the kriging interpolation feature in Surfer to estimate elevations for areas lacking data points.

Water Elevation Calculations:

Data were obtained in the field via the HOBO Ware waterproof shuttle (see material section for model specification). Pressure data was offloaded from the waterproof shuttle and processed through the HOBO Ware Pro software, which used the barometric pressure compensation assistant to account for the local barometric pressure and converted the corrected pressure (in. Hg) to depth in meters. Barometric pressure data were collected from the Weather Underground KNCDAVIS3 station. For the well loggers, the depth in meters was converted to water elevation in NAVD88 by using the following equation:

$$E_w = (E_{TC} - (D_1 - D_2)) + D_w)$$

Where E_w = water elevation in NAVD88 (m), E_{TC} = elevation of the top cap in NAVD88 (m), D_1 = distance from the top of the cap to the bottom of the water logger, D_2 = distance from bottom of water logger to actual pressure sensor, and D_w = calculated water depth (m) from HOBO Ware. For the loggers deployed directly in the pond and submerged full-time, the water elevation in NAVD88 was calculated using the following equation:

$$E_w = (E_{TL} - (L_1 - D_2)) + D_w)$$

Where E_w = water elevation in NAVD88 (m), E_{TL} = elevation of the top of the logger in NAVD88 (m), L_1 = Length of the water logger, D_2 = distance from bottom of water logger to actual pressure sensor, and D_w = calculated water depth (m) from HOBO Ware.
Precipitation data were collected from the Weather Underground KNCDAVIS3 station and compiled using Microsoft Excel (Figure 2.11).

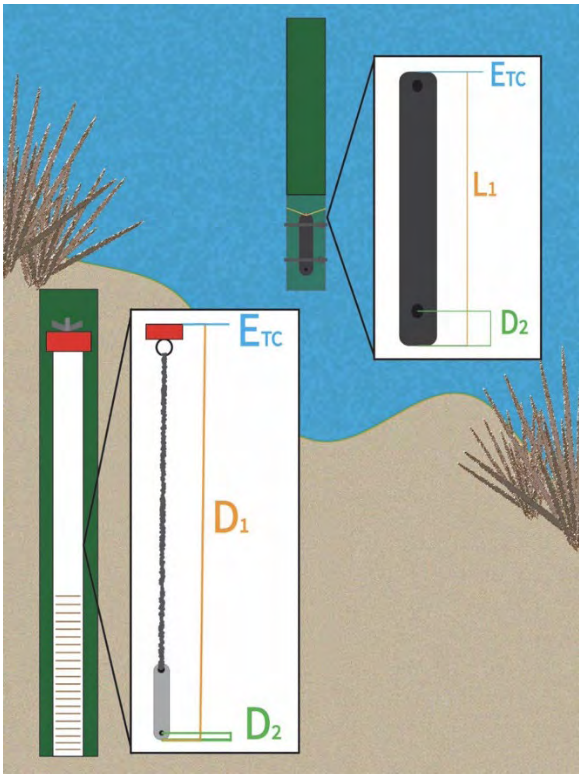

Figure 2.1. This schematic graphically illustrates, and labels key points of the installation of the water logger well (on land in front of the ponds), and water logger deployed in the pond it.

Elevation Transects:

Digital elevation models (DEM) of each pond and surrounding area, generated from Pix4D using the RGB drone images, were used for the purpose of generating beach elevation transects for each of the four dates that the ponds were imaged. These data were used to analyze the elevation of the wash platform over time.

For each DEM, a segment oriented perpendicular to the shoreline was selected for transect analysis in the Surfer software. We ensured there was no vegetation, dunes, or infrastructure on the line so that it could indicate areas where erosion and accumulation of sediment occurred based on the changing elevation. For each time step (four in total), we extracted elevations along the transects at the ponds. By comparing the slope of the elevation line across different timesteps, we can understand how beach topography changes and attempt to correlate this change with natural events such as overwash and precipitation.

Subtracting Elevation Models to Discern Temporal Elevation Change:

For new pond 1, 2, and 3, we selected the two elevations models generated across the longest time span (9/15/2021 and 10/27/2021) to understand how erosion and deposition of sediments operated for the duration of our study. In the Surfer software, we digitally subtracted the elevation model from 10/27/2021 to 9/15/2021, which shows the elevation differences of the same locations across the timesteps (Figures 2.8 - 2.10)

2.3 Results

We generated twelve RGB orthomosaics and associated DEMs (4 maps per New Ponds 1, 2, and 3). These maps, in conjunction with the bathymetry data and water elevation data, were used for further analysis of the geomorphology of the area.

We generated three bathymetry maps (for New Ponds 1, 2, and 3) which show the underwater topography of the ponds. See Figures 2.2, 2.3, 2.4 to see a graphical representation of the orthomosaic and bathymetry maps merged. We also calculated the average depth of each pond, using the bathymetry measurements (Table 2.2).

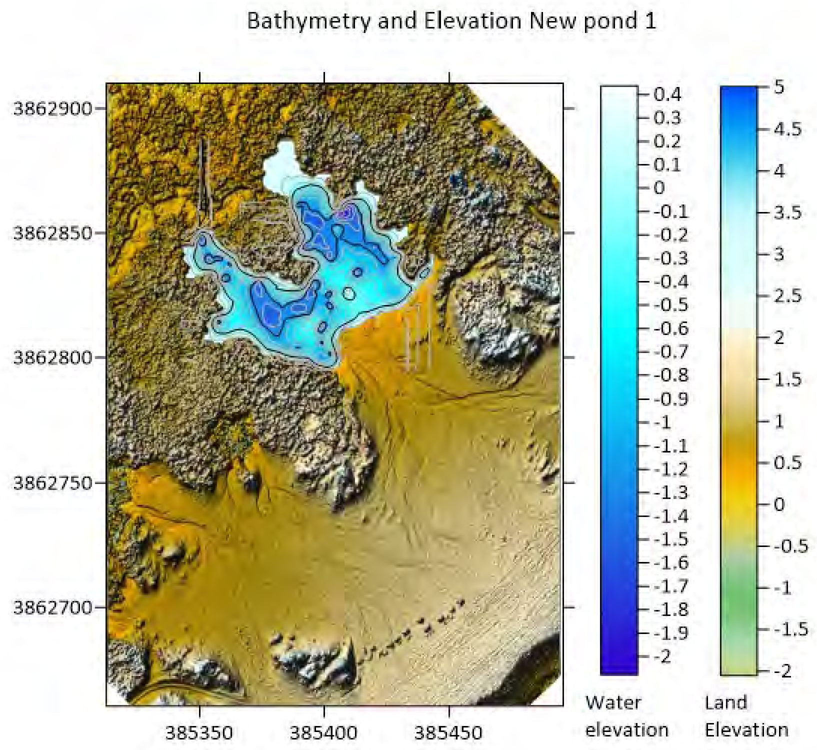

Figure 2.2. Map displaying the ground elevation and bathymetry of New Pond 1 in meters relative to NAVD88.

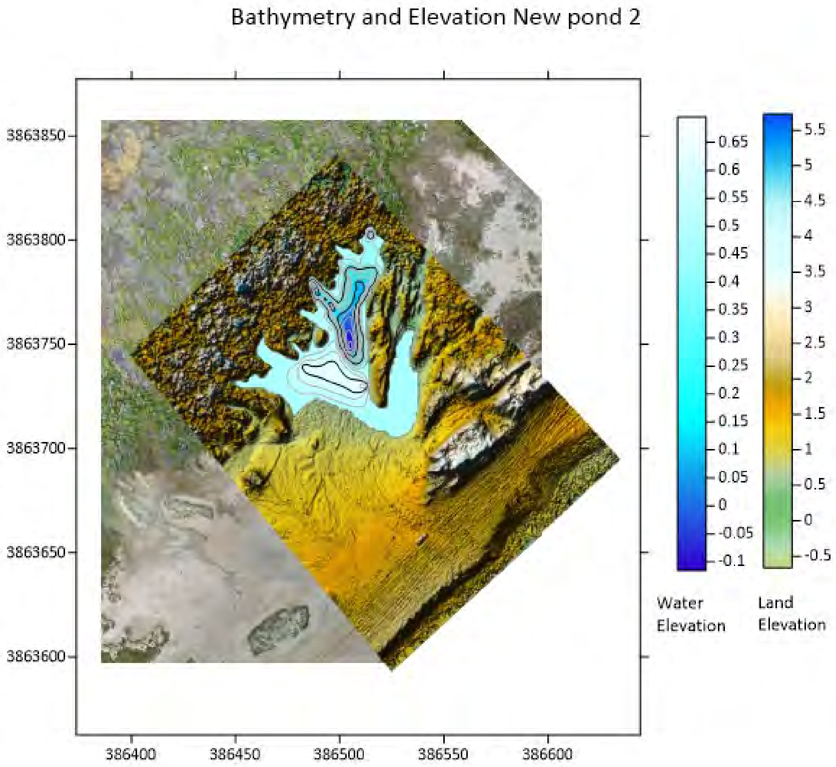

Figure 2.3. Map displaying the ground elevation and bathymetry of New Pond 2 in meters relative to NAVD88.

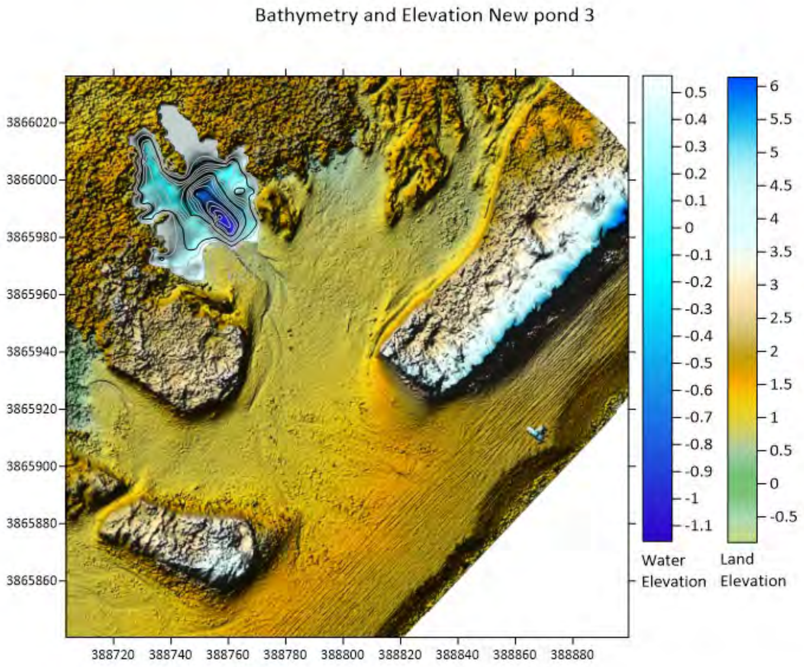

Figure 2.4. This map displays the ground elevation and bathymetry of New Pond 3 meters relative to NAVD88.

Average Depth Measurements	
New Pond 1	1.43 m
New Pond 2	0.37 m
New Pond 3	0.59 m

Table 2.2. Average depth measurements for each of the New Ponds. The pond depths for New Pond 2, 3 is the average depth from the bathymetric data collected on 9/23/2021. The pond depth for New Pond 1 is the average depth from the bathymetric data collected on 9/15/21 and 9/23/21; we had to split data collection of New Pond 1, due to its larger area.

We also learned that the wash platform, berm, and beach have a unique topography in front of each pond. From the pond to the berm, there is a rise in elevation, followed by a steep drop off from the berm to the ocean, based on our elevation transects in Figures 2.5, 6, and 7. From our elevation transects, we measured the elevation from the pond to the highest point of the berm across four time points (Figures 2.5 - 2.7).

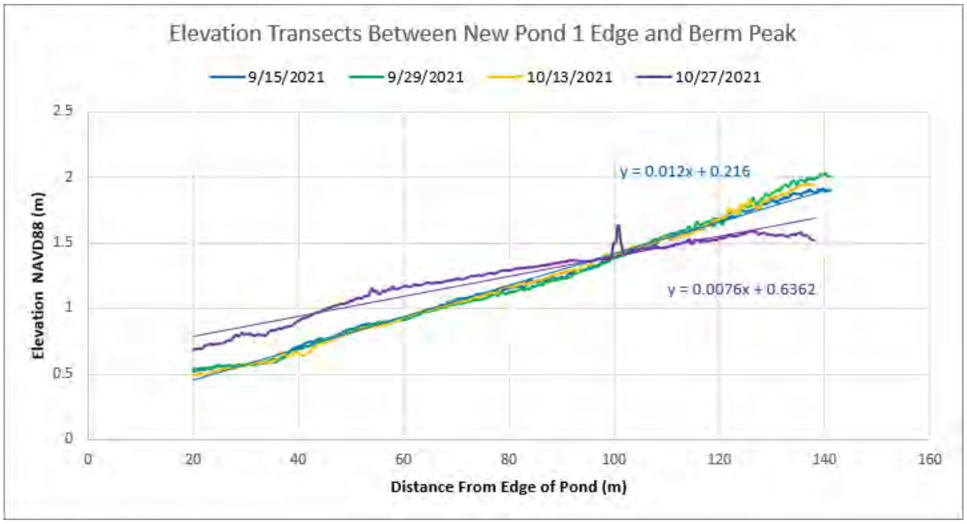

Figure 2.5. Wash Platform transects from edge of New Pond 1 to berm over period of the study. See Figure 2.8 for a visual rendering of the transect line.

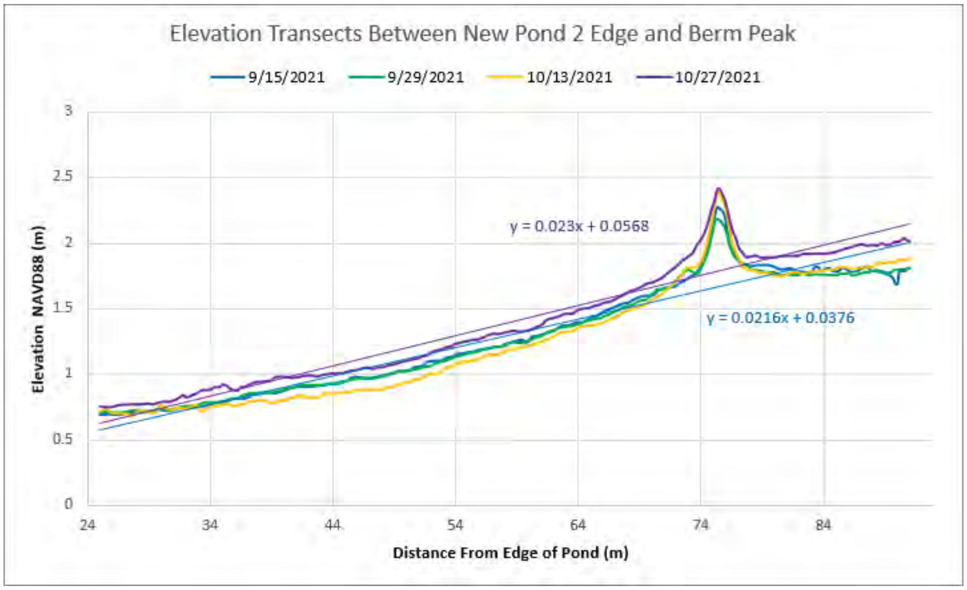

Figure 2.6. Wash Platform transects from edge of New Pond 2 to berm over period of the study. See Figure 2.9 for a visual rendering of the transect line.

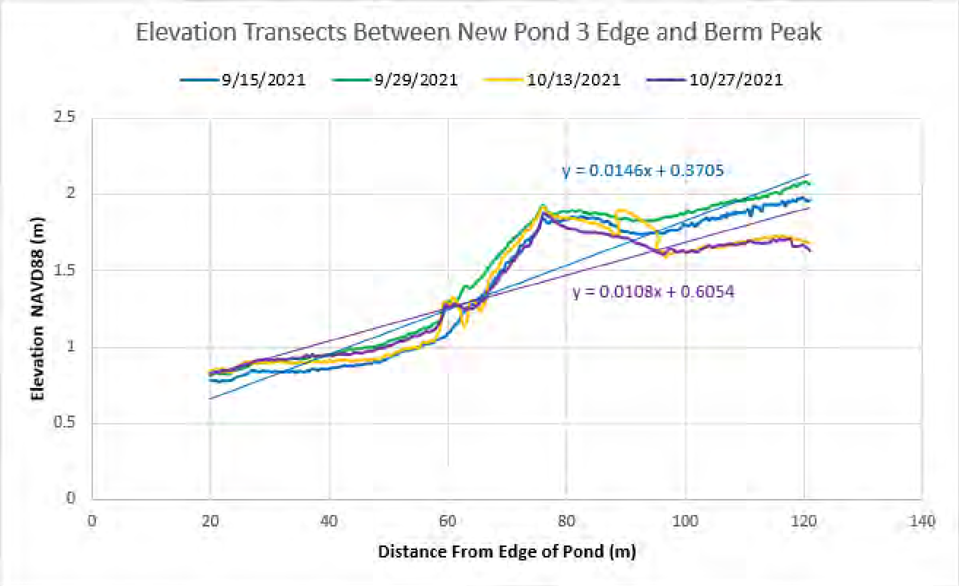

Figure 2.7. Wash Platform transects from edge of New Pond 3 to berm over period of the study. See Figure 2.10 for a visual rendering of the transect line.

The elevation transects in Figures 2.5 - 2.7 show that sediment is generally moving from high areas on the berm to lower areas near the ponds. To further quantify this sediment transport, we assessed the changing slope of the wash platform and elevation of the wash platform closest to the pond over time, see Tables 2.2 and 2.3 below. As sand moves, we expect the slope and elevation of these transects to change over time.

Slope of Wash Platform		
	9/15/2021	10/27/2021
New Pond 1	0.012	0.0076
New Pond 2	0.0216	0.023
New Pond 3	0.0146	0.0108

Table 2.3 This table documents the slopes of the wash platform on the first date that we took drone imagery (9/15/2021) and the last day we took drone imagery (10/27/2021)

Height of Wash Platform Closest to Pond (m)		
	9/15/2021	10/27/2021
New Pond 1	0.216	0.6362
New Pond 2	0.0376	0.0568
New Pond 3	0.3705	0.6054

Table 2.3. This table documents the height of the wash platform closest to the ponds on the first date that we took drone imagry (9/15/2021) and the last day we took drone imagry (10/27/2021)

We also directly observed areas of erosion and deposition based on our subtraction maps (Figures 2.8 - 2.10), which visually show changes in elevation over time between 9/15/2021 and 10/27/2021. Refer to methods section “Subtracting Elevation Models to Discern Temporal Elevation Change” for subtraction maps creation details. The red indicates where sediment was deposited, and the blue indicates where sand was eroded.

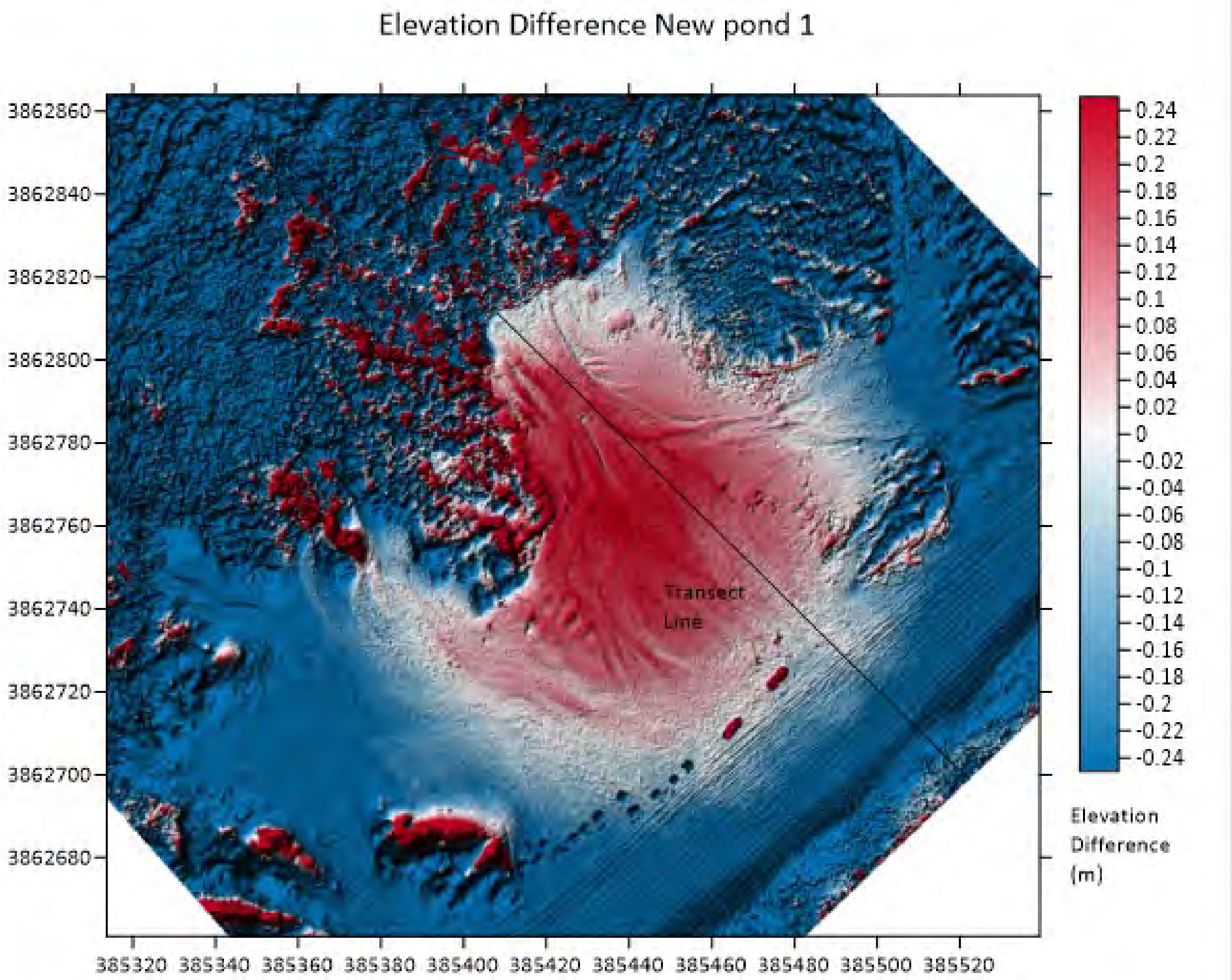

Figure 2.8. This is the result from subtracting the elevation map of new pond 1 between 9/15/2021 and 10/27/2021. Red indicates sediment deposits, while blue indicates erosion. The transect line is the marker along which we track elevation over time in Figure 2.5.

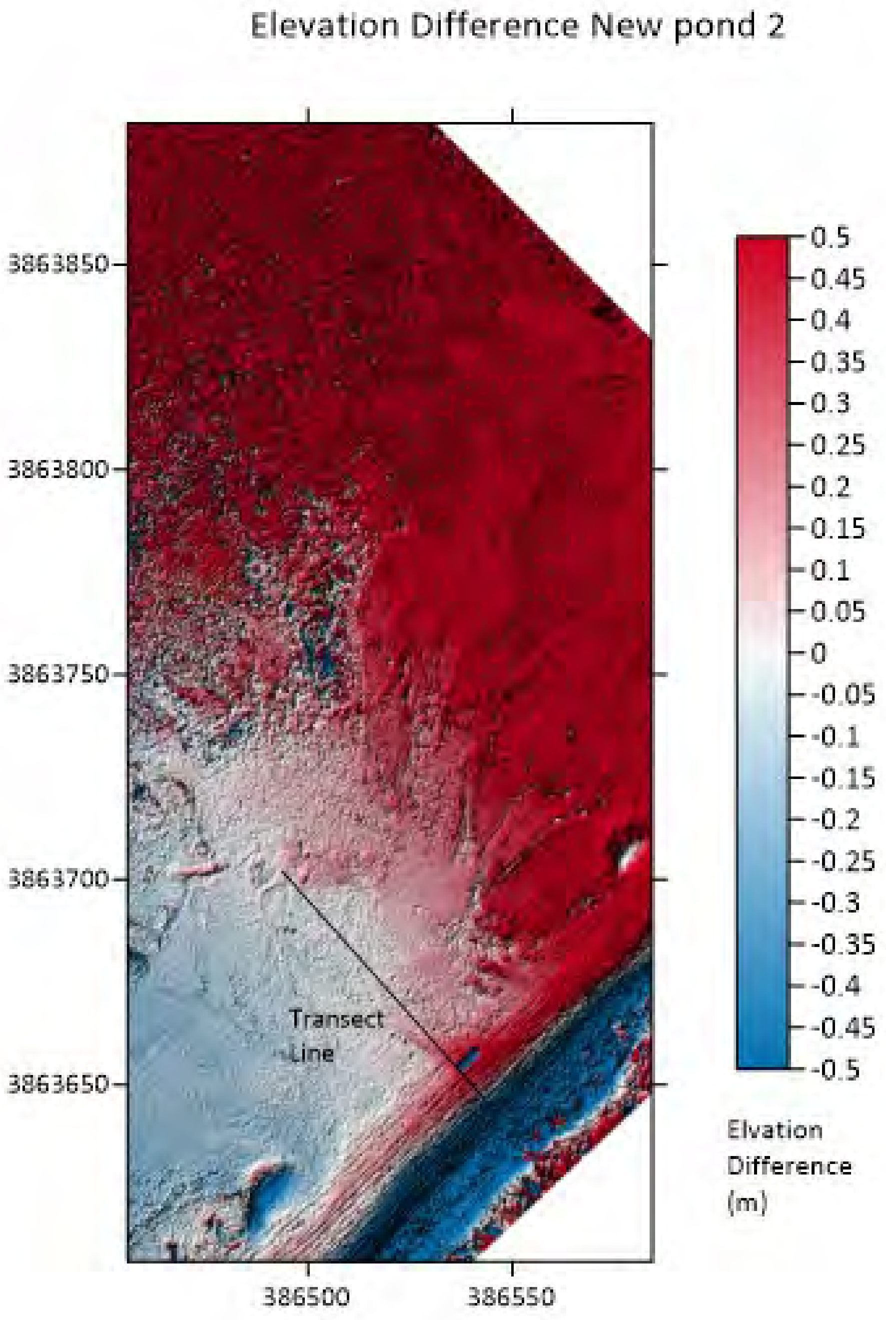

Figure 2.9. This is the result from subtracting the elevation map of New Pond 2 between 9/15/2021 and 10/27/2021. Red indicates sediment deposits, while blue indicates erosion. The transect line is the marker along which we track elevation over time in Figure 2.6.

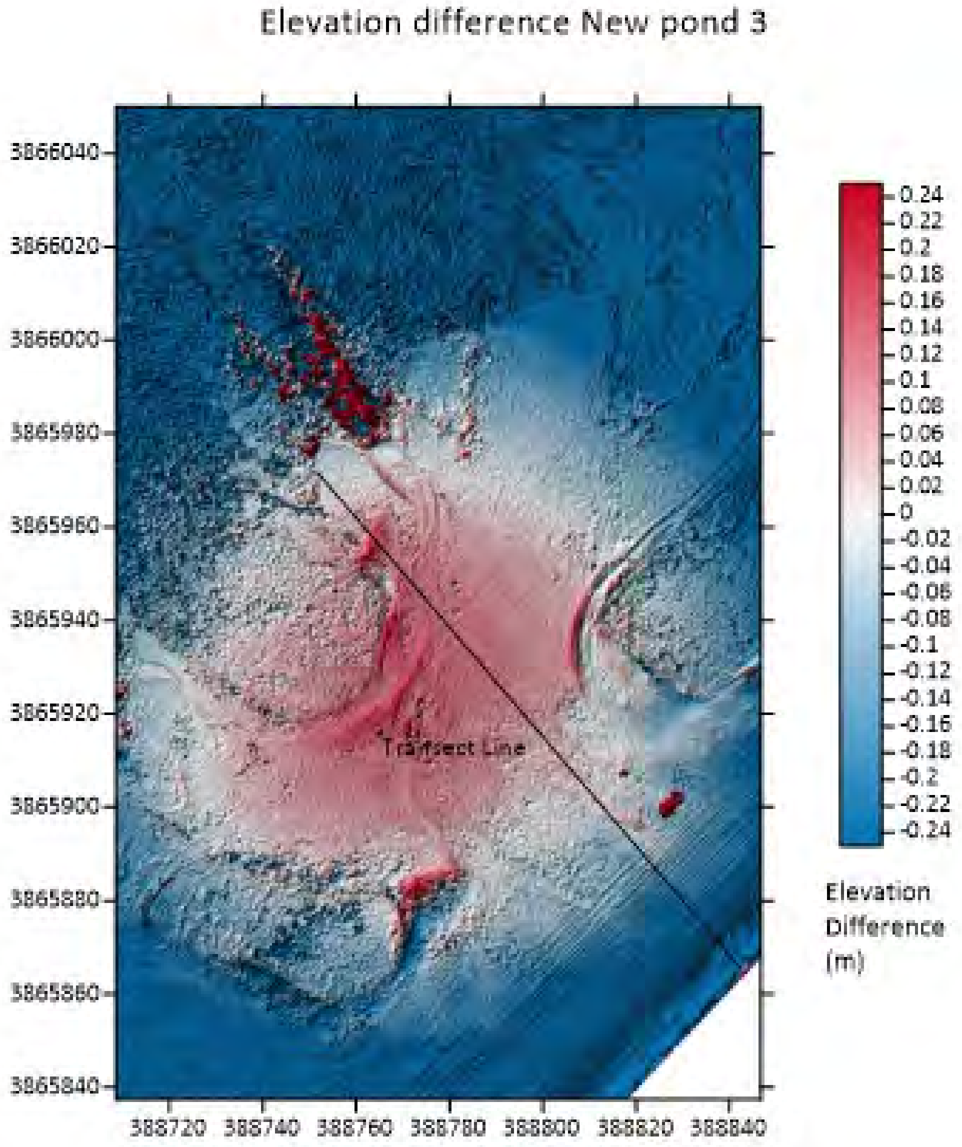

Figure 2.10. This is the result from subtracting the elevation map of new pond 3 between 9/15/2021 and 10/27/2021. Red indicates sediment deposits, while blue indicates erosion. The transect line is the marker along which we track elevation over time in Figure 2.7.

Based on Figure 2.11, water elevation appears to have a positive correlation with precipitation events. There is evidence of a time lag between precipitation event peaks and water elevation peaks. Some precipitation events were not reflected as strongly as others in the water elevation record. Baseline water elevation differed between each pond, which contributed to differences in the magnitude of certain water elevation events. Water elevation data exhibits slight oscillation, likely due to tidal influences. Furthermore, the water elevation baselines across the ponds differ. New Pond 2 has the highest water elevation, followed by New Pond 3, and New Pond 1.

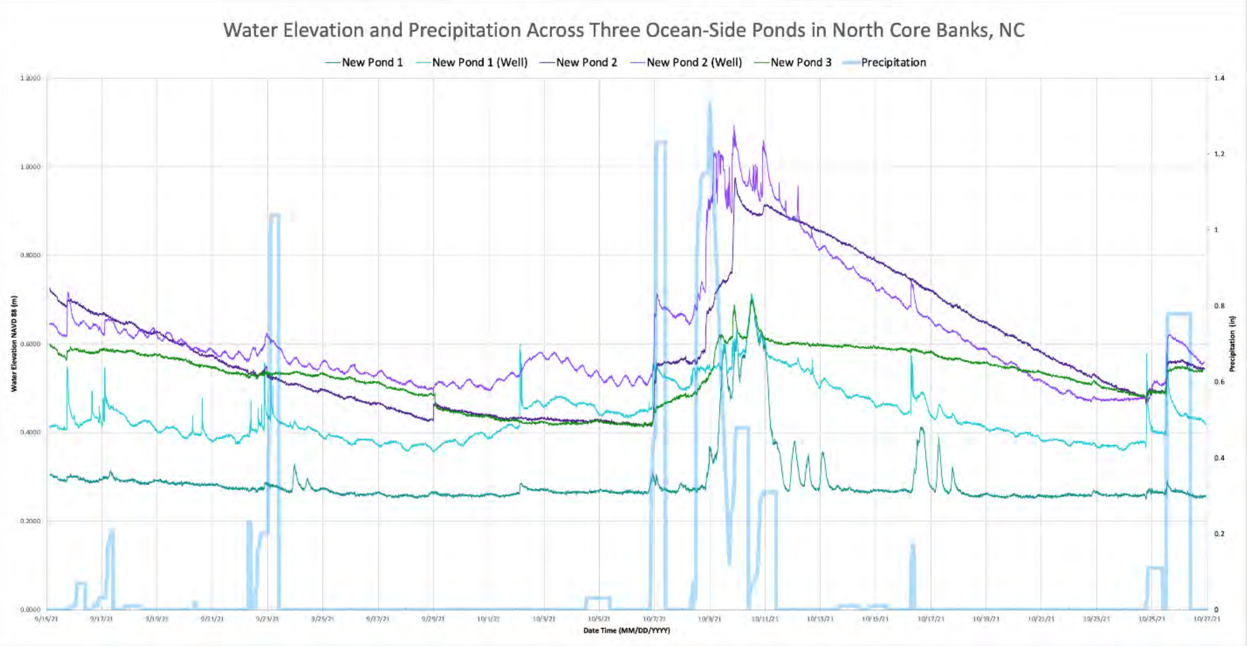

Fig 2.11. Pond water elevation data in NAVD88 in meters for New Ponds 1-3 (primary axis) mapped alongside precipitation data collected from a local weather station in Davis, NC (secondary axis). This figure was created using Microsoft Excel; it is a scatter plot with smooth lines.

2.4 Discussion

Based on our water elevation and precipitation data (Fig. 2.11), we concluded that precipitation was the primary factor influencing water level of the ponds. The water elevation recorded by the water loggers at each of the ponds never exceeded the elevation of the berm, which implies that this water was not entering the ponds from overwash. Our data shows overwash did not occur during the study period. Our first hypothesis, that the overwash recurrence interval would vary with the pond's proximity to the ocean, could not be assessed because the resistance to overwash was relatively high at each site and no major storms associated with high surge occurred over the study period. Based on the concurrence of precipitation events and increases in water level, we now believe that precipitation was the primary source of water feeding the ponds during our study period. Precipitation events were usually immediately followed by peaks in water elevation at the ponds (Fig. 2.11). We also believe that both wind and precipitation were the primary drivers of sediment transport in and around the ponds since overwash was not a factor and we visually observed sediment transport following large precipitation events.

Furthermore, based on our water elevation data, we were able to observe a different baseline water elevation for each of the ponds. New Pond 2 had the highest baseline water elevation, followed by New Pond 3, and then New Pond 1. Since the water table is a significant contributor to water elevation, it indicates that the water table may change in elevation across North Core Banks.

Our second hypothesis that depth of ponds negatively correlated with the slope of the wash platform was supported by the dataset. As you can see in table 4, New Pond 2 had the steepest wash platform slope and the highest elevation of the pond bed, New Pond 3 had the second steepest wash platform slope and the second highest pond bed, and New Pond 1 had the least steep wash platform slope and the lowest pond bed. This finding supports the theory that steeper wash platforms facilitate more sediment transport; the steeper the wash platform gradient, the easier it is for sediment transport to initiate as it moves down the slope and into the ponds. Ultimately, this would result in the largest volume of sand being transported downhill and into a pond at the location with the steepest wash platform.

	Elevation of Pond Bed (m)	Average Slope of Wash Platform from 9/15/2021 and 10/27/2021
New Pond 1	-0.07 (Averaged between 9/15 & 9/23)	0.0098
New Pond 2	0.42 (9/23/21)	0.0223
New Pond 3	0.32 (9/23/21)	0.0127

Table 2.4. Table displays the average elevation of the pond beds next to the averaged slope of the wash platform in front of each respective pond between the dates of 9/15/2021 and 10/27/2021. This average slope was computed by calculating the mean of the slopes on the dates 9/15/2021 and 10/27/2021.

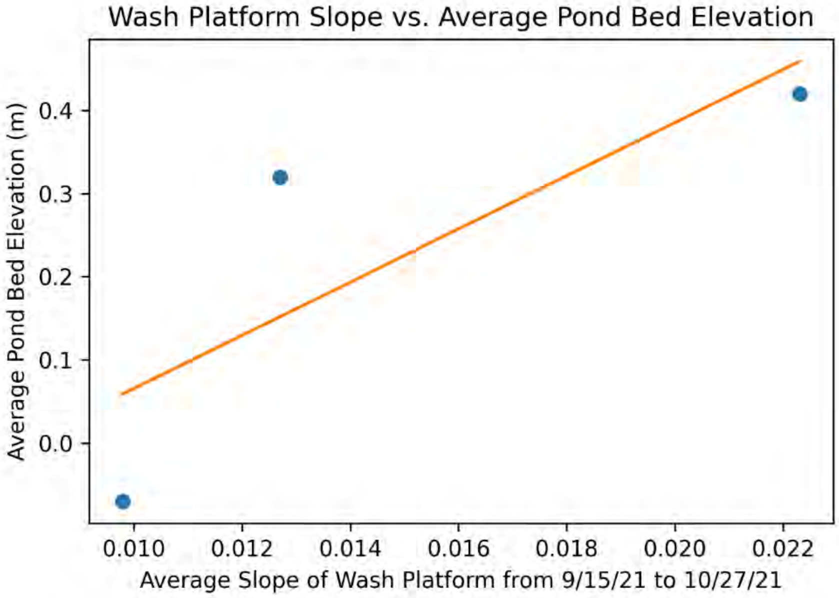

Fig 2.12. This graph shows the relationship between slope of wash platform versus the average elevation of the pond bed. There is a trend that as the slope of the wash platform increases, the average elevation of the pond bed also increases. (NAVD88)

The slope of the wash platform generally gets less steep over time, indicating that sand is being eroded from the higher parts of the wash platform and berm, and being pushed to the lower parts of the wash platform and into the ponds (Table 2.2). The height of the wash platform closest to each pond also gets higher over time (Table 2.3) which confirms this theory as well. This is further corroborated by our subtraction maps which all show a general trend of sediment deposit over the wash platform and erosion of the berm (Figs. 2.8 - 2.10). This evidence suggests that the sediment is moving towards and into the ponds over time. Therefore, it is likely that some of the ponds may be much shallower or completely filled with sediment in the future. Some ponds may persist if some drastic change occurs and moves a large volume of sediment (e.g., hurricane). For example, a hurricane may cut inlets through the island once again, as was the case with Hurricane Dorian.

Based on the highly dynamic nature of the wash platform and the water elevation’s frequent fluctuations, we do not recommend building any permanent infrastructure near the ponds. Permanent infrastructure, such as a road, will likely need constant repair as the ground underneath it will always be accreting sediment. Evidence suggests that the wash platform is generally becoming less steep over time, so it may be possible to build permanent infrastructure after sediment transportation has minimized/come to a stop which may happen when the wash platform has zero slope. However, the wash platform in front of each pond accretes sand differently, and the rates at which this accretion occurs and wash platforms level out differ. See appendix for calculations and estimates for when the wash platform in front of each pond will stabilize. If the wash platform was to ever stabilize it may lead to persisting new ponds transitioning into old ponds. Some new ponds may dry up before the wash platforms stabilize.

Appendix

Map of ponds studied:

Pond ID	Long.	Lat.	Date Visited
New Pond 1	34.5402 N	76.1514 W	9/15, 9/23, 9/29, 10/13, 10/27
New Pond 2	34.5523 N	76.1335 W	9/15, 9/23, 9/29, 10/13, 10/27
New Pond 3	34.5549 N	76.1304 W	9/15, 9/23, 9/29, 10/13, 10/27

*We have visited OP3, OP2, NP2.75, NP2.5, NP1.5 for other groups to collect drone imagery

<u>Calculation Time Until Wash Platform has Slope of Zero</u>

1. Calculating equation to define berm elevation over time, where b(t) = berm elevation, t = time
    a. NP1: b(t) = -0.82(t) + 186.5
    b. NP2: b(t) = -0.11(t) + 201.5
    c. NP3: b(t) = -0.74(t) + 185

Maximum Berm height (m)	**9/15**	**9/29**	**10/13**	**10/27**	**Avg (y-intercept)**
NP 1	1.92	2.03	1.95	1.56	186.5 cm
NP 2	2.06	2.11	1.88	2.01	201.5 cm
NP 3	1.98	2.02	1.73	1.67	185 cm

Rate of Berm Height Change per day (cm/day)	**9/15 - 9/29 (14 days) rate**	**9/29-10/13 (14 days) rate**	**10-13-10-27 (14 days) rate**	**Avg Rate (slope)**
NP 1	0.79 cm/day	-0.57 cm/day	-2.7 cm/day	-0.82cm/day
NP 2	0.36 cm/day	-1.6 cm/day	0.92 cm/day	-0.11 cm/day
NP 3	0.29 cm/day	-2.1 cm/day	-0.42 cm/day	-0.74 cm/day

2. Calculating equation to define edge of pond elevation over time, where p(t) = pond edge elevation, t = time
    a. NP1: p(t) = 1(t) + 42.56
    b. NP2: p(t) = 0.0457(t) + 4.72
    c. NP3: p(t) = 0.559(t) + 48.8

Height of Wash Platform Closest to Pond (m)					
	9/15/2021	10/27/2021	Avg Heigh of Wash Platform (cm)	Elevation change in 42 days (m)	Rate of Change
NP1	0.216	0.6362	42.56	0.4202	1 cm/day
NP2	0.0376	0.0568	4.72	0.0192	0.0457 cm/day
NP3	0.3705	0.6054	48.8	0.2349	0.559 cm/day

3. When will berm elevation equal edge of pond elevation? When the equations modeling berm elevation and pond edge elevation intersect.
    a. NP1 – 79 days (2.6 months)
    b. NP2 – 1229 days (41 months)
    c. NP3 – 105 days (3.5 months)
4. Assumptions Made: These calculations were made assuming that pond conditions remain similar to the conditions observed over the course of the study from 9/15/21 to 10/27/21. If an extreme event, such as overwash or a hurricane, were to occur, these calculations would no longer remain true.
5. Speculatively, once the wash platforms become level, it could be an indication that the island has “healed” itself from Hurricane Dorian’s effects. Therefore, the remaining new ponds would begin to transition into old ponds (discussed in later chapters). Some new ponds will inevitably dry up.

Chapter 3: Biogeochemistry and Bacteria

The Biogeochemistry and Bacterial Presence at Ponds and the Surrounding Environments found at Core Banks, NC

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Fall 2021

3.1. Introduction

The ponds' general health determines their ability to survive and supply services to the people and organisms who use them. The overwash ponds formed in the wake of Hurricane Dorian are relatively young, so knowledge about their current state and any anthropogenic impacts that could alter their well-being in the future is vital for management decisions. Microorganisms, such as bacteria, and biochemicals play a key role in the energy and nutrient cycling of the ponds. Aquatic bacteria assist in the decomposition of detritus, turning dead organisms into organic material that can be released back into the system to be utilized again. Many species are also nutrient fixers, taking elements like carbon, nitrogen, and phosphorus that are locked away and converting them to a form that can be used in the ecosystem. Successful decomposition is necessary for nutrient release and the facilitation of reproduction, growth, and primary production. Nutrient cycling serves as a bottom-up limitation to the entire ecosystem and the availability of these key elements regulates the production and activity of the entire trophic system (Howarth 1988). However, too much of these key nutrients can lead to out-of-control growth. Nitrogen and phosphorus loading in particular can lead to eutrophication and hypoxia, killing off plants and animals alike by depriving them of dissolved oxygen (Paerl 2009). Eutrophication can also drive the growth of harmful algal blooms, which may contain toxins harmful to humans.

Therefore, we aimed to examine the biogeochemical processes occurring in several ponds and their surrounding environments on North Core Banks and what effect the ponds’ persistence on the island might have on the barrier island ecosystem and interacting organisms, particularly humans. To accomplish this goal, we identified four potential factors that could lead to a positive or negative shift to the ecosystem services provided by the ponds and, by extension, Core Banks.

First, we examined the water quality of the ponds and compared their water quality to that of the sound and ocean. Because the new ponds were formed from a sound overwash event, we hypothesize the new ponds will have similar water quality to that of the sound rather than the ocean. Since the old ponds are isolated from the sound by dense marsh and the ocean by wide dunes, we hypothesize the water quality and general chemical conditions to be vastly different in the old ponds compared to the sound and the ocean. Moreover, because of this isolation, we expect the new ponds to be more saline than the old ones, as any additional overwash in the time since the hurricane is likely from the ocean.

Second, we examined the abundance of *Vibrio spp.* bacteria in both new and old ponds. Although bacteria are vital for decomposition and the recycling of organic matter and other compounds, some come with harmful attributes to humans. *Vibrio spp*. are known to cause life threatening infections such as necrotizing fasciitis (CDC 2019). *Vibrio spp*. can enter the bloodstream via open wounds and ingestion, so they pose a major health concern to anyone who might be using the ponds. Previous research shows the damage that *Vibrio spp.* presence has on ecosystems, not only due to its harmful effects on fish stock in aquaculture but also its adverse effects on humans (Moriarty 1998). It is known that *Vibrio spp*. grow best in high salinities (30-35 ppt), warm temperatures (20-40 ℃), and at a pH between 6.5-9. (Percival and Williams 2014; Randa et al. 2004). Therefore, we hypothesize that the newer ponds will have higher *Vibrio spp.*

concentrations than the older ones, as any additional tidal washover is more likely to fill the new ponds.

Next, we determined the limiting nutrient for phytoplankton primary production within the ponds. Since most marine environments are nitrogen limited (Segal et al. 2009), we hypothesize that we will see similar trends across all ponds. However, we expect old ponds to be more eutrophic since they are more connected to the marsh compared to the new ponds. The vegetated areas may lead to increased nitrogen fixation and nutrient runoff into the old ponds (Cloern et al. 2001). However, there is a growing interest on the impact of phosphorus in freshwater systems, and how the nutrients present in the system and the productivity of the water body can impact its ability to support life, and environmental stressors like eutrophication and anoxia (Elser et al. 2007). These stressors can be measured and tracked through rates of dissolved oxygen and bioavailable nutrients present in the water column.

Finally, we examined the percent organic biomass as a proxy for carbon sequestration in several areas to make predictions as to the future influences the ponds might have on carbon in the ecosystem. Carbon sequestration is a vital ecosystem service carried out by marine systems. The ocean, wetlands, and marsh systems all serve as carbon sinks, sequestering carbon into their biomass and removing it from the atmosphere and the surrounding ecosystems (Gilbert et al. 2021). We hypothesize that if the ponds are deep enough to sequester carbon, then they will act as a sink and will assimilate more carbon than a previously existing dune system.

3.2. Materials and Methods

Sampling Site Locations

Several sites along North Core Banks, NC were chosen in this study (Table 1). Pond sites were selected based on whether they were formed during or prior to Hurricane Dorian in 2019. Ponds termed “new” were formed during Dorian and “old” ponds were formed pre-Dorian. The old ponds were formed at least 30 years prior to Hurricane Dorian according to historical Google Earth images and GIS imagery. For comparison, the sound, the ocean, and a marsh pond were all tested (Table 1; Fig 1-3). The ocean samples were taken adjacent to both New Pond 1 (NP1) and New Pond 2 (NP2). Dune and take soil transects for carbon sequestration analysis. Samples were taken on six different occasions from September 10th, 2021 through October 27th, 2021.

Nearby the New Pond 1, there are several abandoned cabins that were destroyed during Hurricane Dorian. All the cabins were on septic tanks, which are still present but inactive. However, there is one functioning septic system at the bath house adjacent to the cabins.

Table 3.1. Longitude and latitude of all the sites analyzed for this project.

Site Geographical Locations		
Site Name	**Latitude**	**Longitude**
Marsh Pond	34°53’52’’N	76°15’37’’W
Sound	34°53’59’’N	76°15’30’’W

New Pond 1 (NP1)	34°54’03’’N	76°15’14’’W
Ocean - New Pond 1	34°54’00’’N	76°15’09’’W
New Pond 2 (NP2)	34°55’23’’N	76°13’35’’W
Ocean - New Pond 2	34°55’20’’N	76°13’29’’W
New Pond 3 (NP3)	34°55’49’’N	76°13’04’’W
Old Pond 2 (OP2)	34°56’38’’N	76°12’09’’W
Old Pond 3 (OP3)	34°56’51’’N	76°11’56’’W
Dune	34°56’36’’N	76°12’05’’W
Marsh	34°55’23’’N	76°13’35’’W

Figure 3.1. Satellite imagery from the Carteret County GIS database from 2020 of the Marsh, Sound, New Pond 1, and New Pond 1 - Ocean sites.

Figure 3.2. Satellite imagery from Carteret County GIS from 2020 of the New Pond 2, New Pond 2 - Ocean, and New Pond 3 sites.

Figure 3.3. Satellite imagery from the Carteret County GIS database from 2020 of the Old Pond 2 and Old Pond 3 sites.

Water Quality Sampling

A YSI 6600 meter was used to measure the conditions of the water such as temperature, pH, salinity, specific conductance, turbidity, and dissolved oxygen (in percentage and mg/L). The YSI was submerged in the shallow area of each pond to take water quality measurements, but a deep-water sample (~1m) was also collected at NP1 and NP2 once. The exception to this methodology was Old Ponds 2 and 3, where a water sample was taken instead of submerging the YSI due to the extremely low water levels (~1-2 cm).

Vibrio spp. Sampling and Testing

In the field, 1 L samples from each site were collected in acid-washed polypropylene bottles. We sampled for *Vibrio spp.* in the ponds twice to assess temporal variability of *Vibrio spp*. Abundances. For the first sampling on 9/16/2021, samples were collected from a new pond (NP1), an old pond (OP2), the marsh, and the sound. For the second sampling on 9/23/2021, samples were collected at two new ponds (NP1 and NP2), two old ponds (OP2 and OP3), and a marsh pond. After the samples were collected in the field, they were placed in a cooler and maintained at in-situ temperature. Samples were then brought back to the lab for processing within 8 hours in accordance with US EPA guidelines.

In the laboratory, all samples were diluted at three different concentrations (A, B, and C) using (1x) Phosphate Buffer Solution (PBS) (Table 2). Once the samples were properly diluted, they were filtered using vacuum filtration. We placed a 47 mm MCE Milipore filter at the base of each funnel. We then poured 10 mL of diluted sample into the funnel and filtered before transferring the filter paper to a 50x9 petri dish containing Chrom-Agar Vibrio (CAV). We ensured the filters were positioned on top of the agar and avoided producing any air bubbles. We then repeated this filtering process for each sample in order to get duplicates for each dilution at each site tested. Once all the filters were placed on the agar, the plates were inverted and incubated at 37℃ for 24 hours. After 24 hours, the plates were removed from the incubator and the individual colonies of *Vibrio parahaemolyticus*, *Vibrio vulnificus*, and *Vibrio alginolyticus* were counted. The *Vibrio parahaemolyticus* colonies are mauve in color on the plate whereas *Vibrio vulnificus* are cyan and *Vibrio alginolyticus* are creamy white. The colony counts were determined based on the number of colonies in each color present on the plate.

To quantify the concentration of *Vibrio parahaemolyticus*, *Vibrio vulnificus*, and *Vibrio alginolyticus* at each pond, we calculated the CFU per mL and the CFU per 100 mL. We calculated those concentrations by taking the average colony count between the duplicate plates for each *Vibrio spp*. However, we disregarded average colony counts that were less than 5 based on prior research in Dr. Rachel Noble's lab (UNC-IMS). Additionally, if the colony count was listed as “Too Numerous to Count” we replaced those values with 200 because we are assuming there is a minimum of 200 colonies on the plate. Once the averages were calculated, we used the following formula to determine the CFU per 100mL (Equation 3.1).

Equation 3.1. CFU/ 100 mL = 100 * ((Average Colony Count) / (amount of sample on filter (mL)))

Table 3.2: Dilution Concentrations for Vibrio Analysis				
Analysis Date	**Dilution**	**Amount of (1x) PBS added (mL)**	**Amount of Sample Added (mL)**	**Amount of Sample on Each Plate (mL)**
9/15/2021	A	0.36	29.64	0.12
9/15/2021	B	3	27	1
9/15/2021	C	10	0	10
9/23/2021	A	0.16	29.84	0.06
9/23/2021	B	0.3	29.7	0.1
9/23/2021	C	3	27	1

The amount of PBS and Sample added to each dilution (A, B, C) from both testing days. The last column shows how much of each sample is expected to be on the petri plate after filtration.

Nutrient Sampling and Testing

The growth-limiting macronutrient (N or P) was assessed three separate times using nutrient addition bioassay experiments from water collected from Old Pond 3, Old Pond 2, New Pond 2, and New Pond 1. A 2 L sample bottle was rinsed with pond water and filled at each site. The bottle was then stored in a covered bin and brought back to the lab. On two occasions 100 mL, and one occasion 50 mL, of pond water from each site was vacuumed filtered through 25 mm Whatman GF/F glass, fiber filters. The filtered water was placed in a Falcon tube and frozen at -20°C prior to analysis of nitrate, ammonium, phosphate, and total dissolved nitrogen via standard colorimetric methods on a Lachat Quickchem 8000 nutrient autoanalyzer (Peierls et al. 2012). The filtered was saved and used to measure initial phytoplankton biomass as chlorophyll *a*. The remaining water was divided into 100 mL triplicate sub-samples in glass bottles for the control with no nutrients added and three nutrient addition treatments: addition of nitrogen(+N), addition of phosphorus(+P), and a combined nitrogen and phosphorus addition(+NP). 11.67µL of 0.025M potassium phosphate and 16.67µL of 0.29M sodium nitrate were added to the corresponding treatments. Thus, in the 100 mL bottles, the concentration of added nitrate was 48.3 µM and the concentration of phosphate was 2.92 µM in the respective treatment bottles. In the 50 mL bottles, the concentration of nitrate added was 96.6 µM and the concentration of phosphate was 5.84 µM in the respective treatment bottles. Sample bottles were then corked and incubated in the UNC IMS artificial ponds under environmental temperatures and natural lighting for 42, 90, and 42 hours for experiments 1, 2, and 3, respectively.

For analysis of the final phytoplankton biomass as chlorophyll *a*, the sample bottles were filtered using vacuum filtration onto 25 mm GF/F filters. The filters were folded and placed in a labeled piece of aluminum foil and stored frozen at -20°C. Later, samples were removed from the freezer and foil and placed in test tubes wrapped in aluminum foil and tape. Tubes were filled with 10-13 mL of 90% acetone and placed in the freezer at –20°C for 24 hours. After 24 hours, the tubes were removed from the freezer, and ~3 mL of each solution was placed in a small vial and analyzed for chlorophyll using the EPA fluorometric method 445.0 (after Welschmeyer et al. 1994) on a Turner Trilogy fluorometer. The chlorophyll *a* (µg/L) of each sample was determined by multiplying the volume of acetone used for extraction (L) by the fluorometer value (µg/L) and dividing that value by the volume filtered (L).

We calculated the total Chlorophyll-*a* concentration for each sample using Equation 3.2 which follows analysis procedures found in Welschmeyer et al. (1994). We determined which nutrient was limiting based off the chlorophyll-*a* concentration deviation of each treatment from the initial and the control. The pond was determined to be potentially nitrogen-limiting if the +N treatment was much higher than the control and the +P treatment was similar to the control. The pond was determined to be potentially phosphorus-limiting if the +P treatment was much higher than the control and the +N treatment was similar to the control. The pond was determined to be both phosphorus and nitrogen co-limiting if both the +N and +P treatments were similar to the control while the +NP treatment was much higher. Lastly, the pond was determined to have potentially no nutrient limitation if all three treatments had similar chlorophyll-*a* concentrations to the control. In general, the determination of the limiting nutrient is highly subjective though as there are many different methodologies and ways to interpret the data. Furthermore, the initial calculations measured by using the Lachat nutrient autoanalyzer were exported and converted from the original units of µg/L to µmol/L or µM. First, the original sample data was combined by summing together the compounds with a shared element (e.g. Nitrate and ammonium). If one compound contained a reading below the Lechat standard detection limit, the detection limit was substituted in its place for future calculations, and if more than one reading was below the replicate was excluded from analysis. The summarized N and P values µg/L were divided by the standard molar mass and then used to construct a ratio.

*Equation 3.2. Fluorometer value (µg/L) * Volume Extracted (L) / Volume Filtered (L)* Dilution Factor*

Carbon Sequestration Sampling and Testing

Three sediment samples on a transect were taken from a new pond, an old pond, the dunes, and the marsh using a Russian peat core. In the ponds, the transect was taken from the shallow, middle, and deepest areas. At the dune, a transect was taken from the ocean-side of the dune to the dune line on the opposite side. Lastly, the marsh transect was taken from the edge of the marsh to the middle. Approximately 5 g of the top portion of the core was collected for lab sampling. Only the top portion was sampled because we wanted to quantify how these sites were sequestering carbon currently, not historically. Once in the lab, all samples were placed in separate circular tins. Following the loss on ignition (LOI) method used in Heiri et al. (2001), the samples were put in an oven at 105°C for 24 hours to evaporate all of the water. After the 24-hour period, samples were weighed using an analytical balance. Samples were then placed back in the oven at 550°C for 4 hours. After this second heating period, samples were reweighed using the same analytical balance. To determine the amount of organic mass present in the samples, the weight measured after the second heating was subtracted from the initial weight. The amount of organic mass provides an estimate of the amount of carbon being sequestered.

Statistical Analyses

All analyses were performed using JMP Pro version 16.0 and the statistical significance was set at $P < 0.05$. For nutrient analysis, two points (NP2 control from 29 September 2021 and OP2 +NP from 13 October 2021) were imputed by randomly generating values from a normal distribution with the mean of existing duplicates and a standard deviation equal to the average standard deviation of all treatment replicates with the experiments. Data were assumed to be normal for the completion of One-way ANOVA tests. If the One-way ANOVA revealed significant treatment effects on final Chlorophyll-*a* concentrations, Dunnett's post-hoc tests were completed for that sample site on the specific day to determine which treatments were significantly different from the control.

For the percentage organic biomass data, an arcsine transformation was completed before doing ANOVA and a Tukey-Kramer HSD post-hoc test to determine if any of sample sites' values were significantly different.

3.3. Results

Water Quality

Over the course of 5 sampling days, the YSI instrument was deployed 27 different times at 8 different sites to measure basic water quality conditions. Out of the 8 sites, 4 were sampled 5 times, 1 was sampled twice, and 3 were sampled once. Of the sites that were sampled two or more times, the water quality data was averaged to distinguish trends across sites (Table 3). On average, OP2, OP3, and NP3 had the highest temperature with values of 27.78℃, 27.42℃, and 27.08℃ respectively. The lowest average temperature was observed at NP2 (25.76℃) and NP1 (25.14℃). Regarding salinity, the highest average salinity was observed at NP3 (30.62 ppt) while the lowest was at the old ponds with OP3 having an average salinity of 4.39 ppt and OP2 having an average salinity of 5.89 ppt. The highest average dissolved oxygen percentage and mg/L was observed at NP2 with values of 141.08% and 9.61 mg/L. The lowest average dissolved oxygen percentage and concentration was at OP3 with values of 88.02% and 6.83 mg/L. The highest average pH was seen at the new ponds (NP3: 8.27; NP2: 8.19; NP1: 8.14) whereas the lowest average pH was seen at the old ponds (OP3: 7.73; OP2: 7.85). When looking at the age of the ponds (new versus old), similarly aged ponds had similar characteristics. The old ponds consistently had a higher temperature and lower salinity, pH, and dissolved oxygen compared to the new ponds. Additionally, amongst the new ponds, NP3 had a higher average temperature, salinity, and pH compared to the other new ponds. All three new ponds had similar dissolved oxygen percentages and concentrations.

On October 13th, 2021, two new ponds, two old ponds, the ocean, and the sound were sampled in order to see how the water quality from the ponds compared to the ocean and the sound (Table 4). The highest temperature recorded was at OP2 at 26.24℃, whereas the lowest was 23℃ at both OP3 and the sound. The highest salinity was observed at both ocean sites (NP1-Ocean and NP2-Ocean) ranging from 30-31 ppt. The lowest salinity observed was recorded at the old ponds ranging from 6-10 ppt. The highest dissolved oxygen percent and concentration was recorded at NP2 with values of 129.2% and 9.27 mg/L. The lowest dissolved oxygen percent and concentration was seen at the old ponds with values ranging from 64-65% and 5 mg/L. Lastly, the pH was the highest at the new ponds with values around 8 and was the lowest at the old ponds with values around 7. In general, the new ponds had temperatures closer to the values recorded in the ocean and the salinity of the new ponds differed from the ocean by ~7-8 ppt. The new ponds DO% was near the value recorded at the ocean whereas the sound had a value ~10% lower than the new ponds. The DO (mg/L) concentration for the new ponds, the sound, and the ocean were all very similar ranging from 98 – 110 mg/L, but NP2 had a much higher value at 129.5 %. Lastly, the pH of the new ponds was the same as the ocean ranging from 7.94-8.07. Regarding temperature, both the sound and OP3 had a temperature of 23℃., but OP2 had a much higher temperature at 26.24℃. The low temperature recorded at OP3 on 10/13/2021 differed extensively from the average temperature of 27.42℃ (Table 2). Despite the similarity in temperature between the old ponds and the sound, the old ponds had significantly different values for salinity, DO%, DO (mg/L), and pH. The salinity for both old ponds was >20 ppt lower than both the sound and the ocean. The DO% was ~50% lower, the DO mg/L was ~2 mg/L, and the pH was ~0.5-1 lower at the old ponds as well. The dissimilarities between the old ponds and the sound and the ocean leads us to conclude that the old ponds are hydrologically disconnected from both the ocean and sound.

Table 3.3. The average water quality parameters throughout the entire project from the ponds.

Site	Number of Times Sampled	Temperature (°C)	Salinity (ppt)	DO %	DO (mg/L)	pH
OP3	5	27.42	4.39	88.02	6.83	7.73
OP2	5	27.78	5.89	98.56	7.41	7.85
NP3	2	27.08	30.62	107.90	7.64	8.27
NP2	5	25.76	28.32	141.08	9.61	8.19
NP1	5	25.14	25.99	108.58	7.74	8.14

Table 3.4. Water quality data collected on 10/13/2021 from all sites

Site	Temperature (°C)	Salinity (ppt)	DO %	DO (mg/L)	pH
OP3	23.17	10.17	65.5	5.28	6.94
OP2	26.24	6.4	64.2	5	7.29
NP2	25.84	22.75	129.5	9.27	8.03
NP2 - Ocean	24.7	30.76	109.5	7.64	8.07
NP1	25.19	21.75	108.7	7.91	7.94
NP1 - Ocean	24.19	30.85	109.9	7.73	8.03
Sound	23.12	25.52	98.6	7.28	7.86

Vibrio Abundances

The three *Vibrio spp*. exhibited different patterns of abundance by site type and sampling date that may relate to the ecological niche of each species. The marsh had the highest average concentration of *V. parahaemolyticus* for both sampling days. It was significantly greater on the first day at 30,208 CFU per 100 mL (Figure 3.4). As for *V. vulnificus*, OP2 had the greatest relative concentrations for both days, although the second sampling day's results were more than five times greater than the first (Figure 3.5). On the first of two sampling days, the sound had the highest CFU per 100 mL of *V. alginolyticus* followed by the marsh and OP2. For the second day, OP2 had the highest average concentration of *V. alginolyticus*, more than twice as much as any other sample site (Figure 3.6). Overall, *V. alginolyticus* was the most abundant *Vibrio spp.* found on the first day, while *V. vulnificus* had the greatest sum of CFU per 100 mL for the second sampling day.

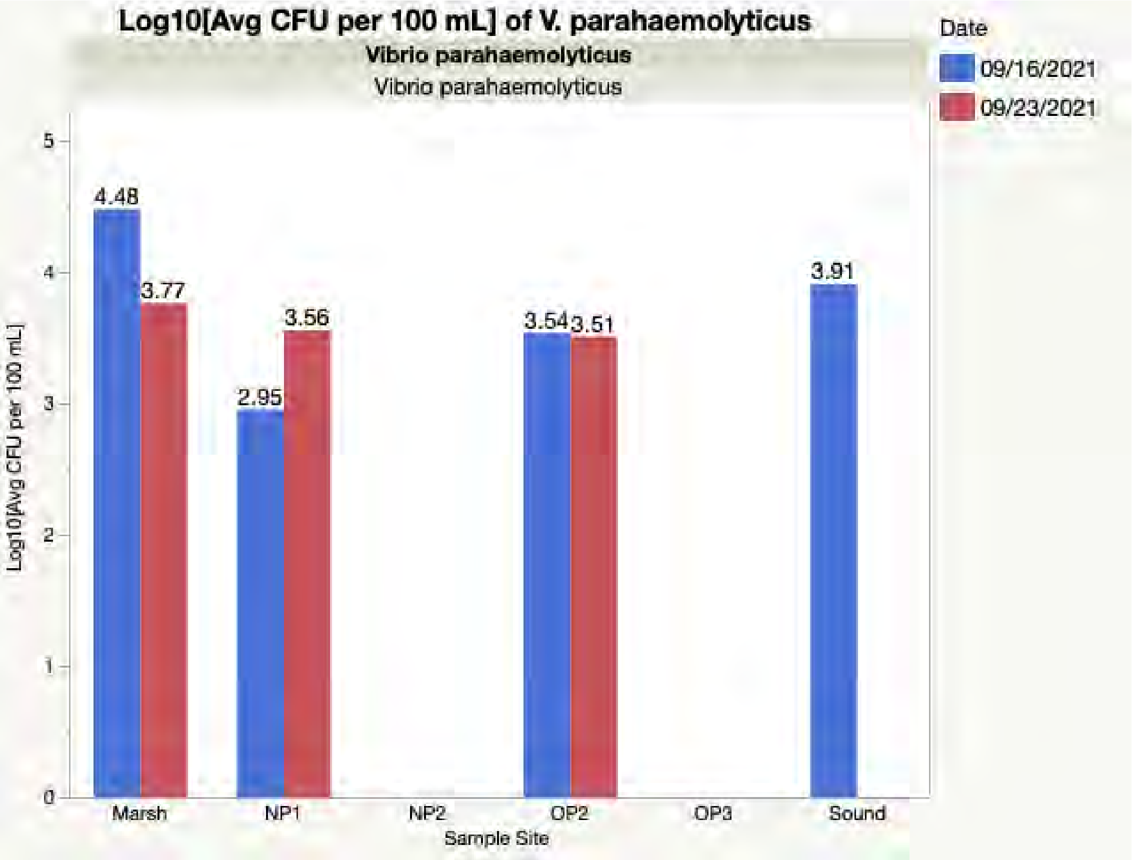

Figure 3.4. The log transformed CFU per 100 mL mean concentration of V. parahaemolyticus at several sample sites on two separate sampling days. The sound was only sampled on the first day. NP2 and OP3 were only sampled on the second day. For the first day, their values were unquantifiable due to low colony counts.

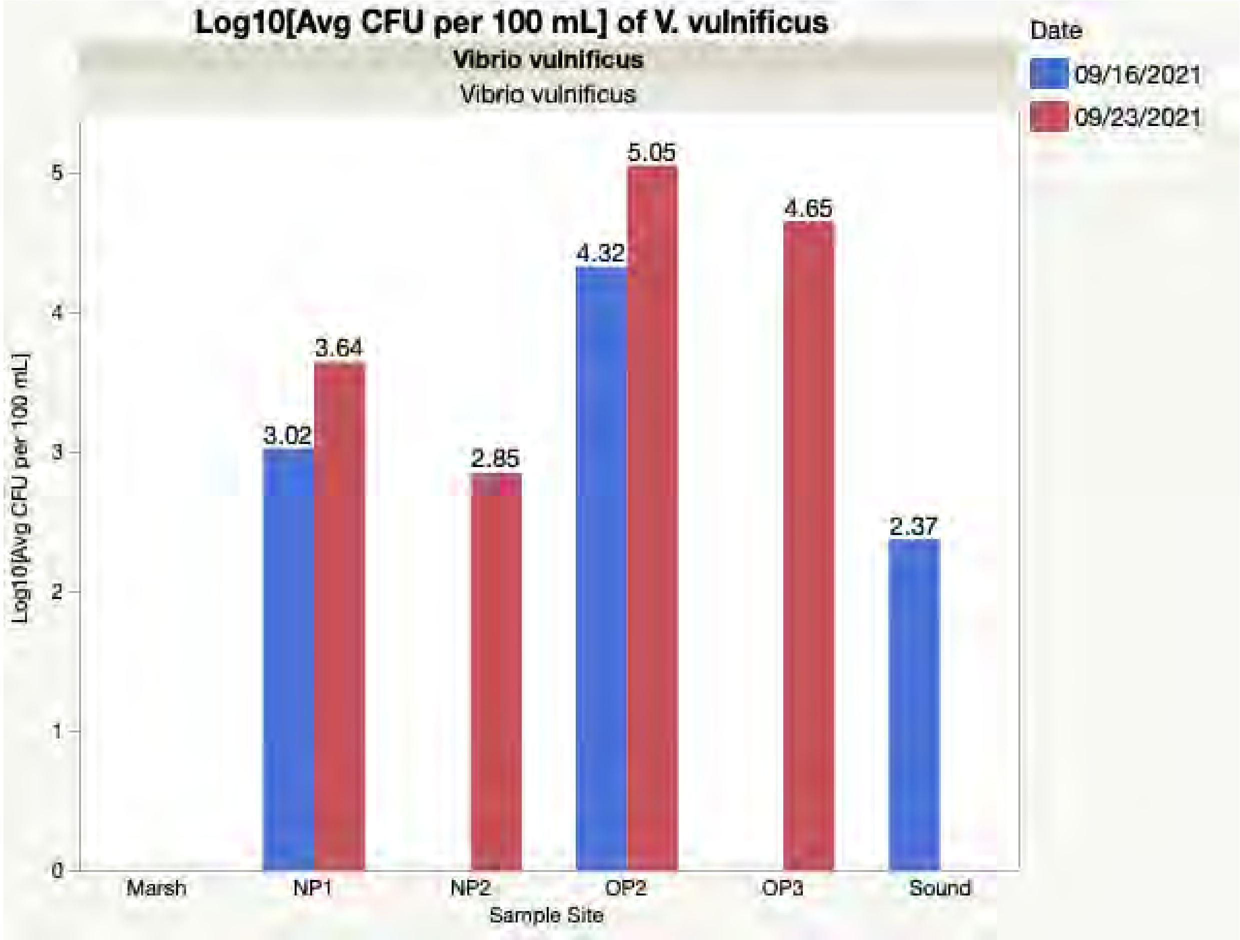

Figure 3.5. The log transformed CFU per 100 mL mean concentration of V. vulnificus at several sample sites on two separate sampling days. Marsh was unquantifiable due to low colony counts. The sound was only sampled on the first day. NP2 and OP3 were only sampled on the second day.

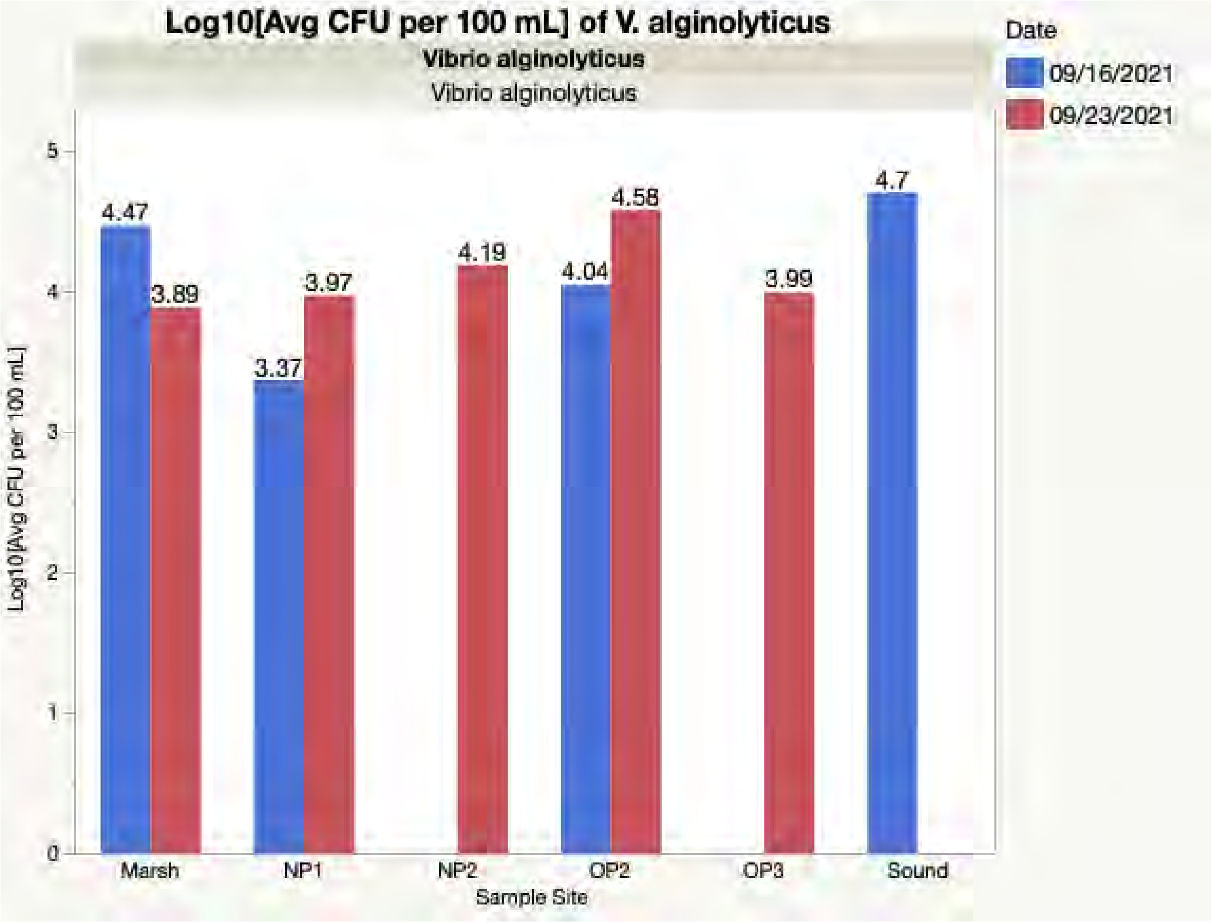

Figure 3.6. The log transformed CFU per 100 mL mean concentration of V. alginolyticus at several sample sites on two separate sampling days. The sound was only sampled on the first day. NP2 and OP3 were only sampled on the second day.

Limiting Nutrients

On the first sampling day, 29 September 2021, Old Pond 2 had the greatest initial Chlorophyll-*a* (µg/L) concentration amongst the ponds at 53.23 µg/L, while New Pond 1 and Old Pond 3 had the lowest initial concentrations (Figure 3.7). After 42 hours of incubation, for New Pond 1, the greatest Chlorophyll-*a* occurred in samples spiked with nitrate (+N) and combined nitrate and phosphate (+NP). Dunnett's post hoc test found these two treatments to be significantly different compared to the control (P = 0.0017 for +N; P = .0001 for +NP). For New Pond 2, the initial value was significantly lower than the control (P = .0214). Additionally, the +NP treatment was significantly higher from the control (P = .0451 for +NP). Old Pond 2's +P treatment had the greatest Chlorophyll-*a* readings, while the initial and +NP groups had the lowest values, but these values were not significantly different from the control. Lastly, during this first experiment, Old Pond 3's greatest final Chlorophyll-*a* readings came from the +NP treatment; however, the +N and +P groups had similar final concentrations (Figure 3.7).

For the second experiment started on 13 October 2021, initial Chlorophyll-*a* readings were similar between all ponds (Figure 3.8). New Pond 1's highest final experimental Chlorophyll-*a* concentrations came from +N, and +NP treatment groups. The +NP treatment group had the highest Chlorophyll-*a* concentrations for New Pond 2, with +P being greater than +N on average. Old Pond 2's +NP treatment group also had the highest Chlorophyll-*a* concentration; however, the +N treatment group had higher values than the +P group. For Old Pond 3, all treatment groups including the control grew by 372% from the initial Chlorophyll-*a* value. Final Chlorophyll-*a* of the +N was slightly higher than other treatments (Figure 3.8).

On the third sampling day, 27 October 2021, new ponds (i.e., New Pond 1 and New Pond 2) had lower initial Chlorophyll-*a* concentrations than old ponds (i.e., Old Pond 2 and Old Pond 3). Nitrate and phosphate treatments all had similar final values and were greater than control and initial values for New Pond 1. All treatments had very similar Chlorophyll-*a* value for New Pond 2. Old Pond 2's +NP treatment had the greatest Chlorophyll-*a* concentration followed by +N and control. The phosphate addition treatment was slightly lower. The initial value was significantly lower than the control and subsequent treatments in Old Pond 3 and followed a similar pattern to Old Pond 2. The slightly higher final values Chlorophyll-*a* in the +NP treatment but similar values between the +N and +P treatments indicate co-limitation by N and P (Figure 3.9).

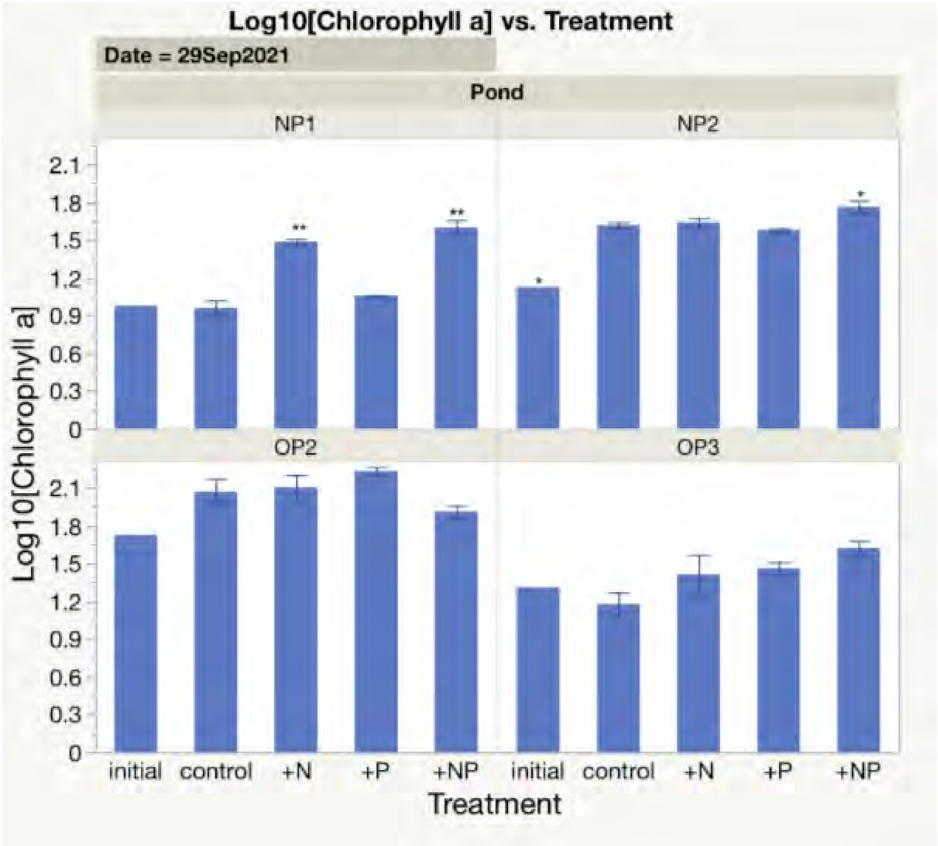

Figure 3.7. The Chlorophyll-a concentrations amongst the three treatments and initial compared to the control at four testing sites for the first sampling day: 29 September 2021. Standard error is represented by error bars.

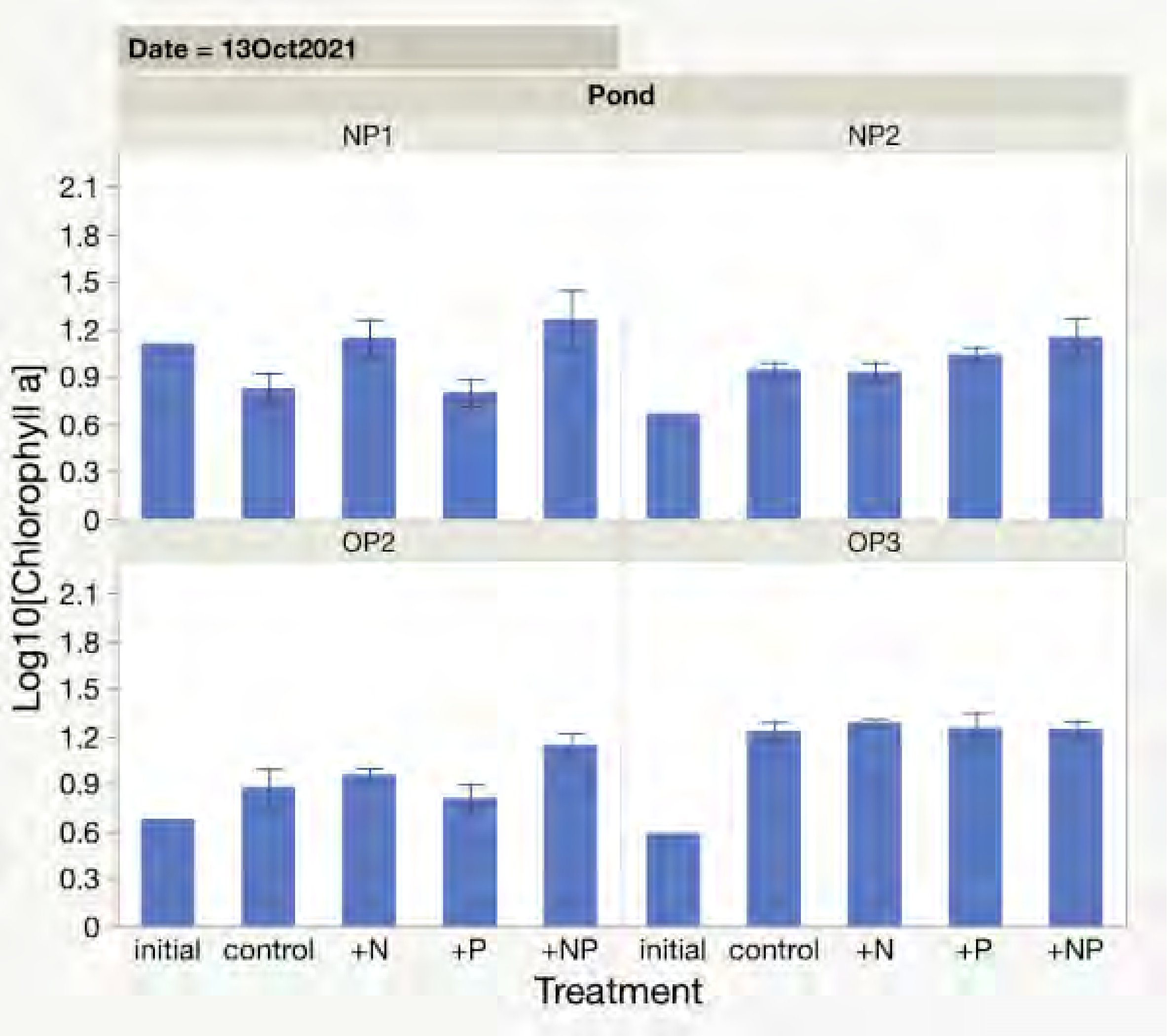

Figure 3.8. The Chlorophyll-a concentrations amongst the three treatments and initial compared to the control at four testing sites for the second sampling day: 13 October 2021. Standard error is represented by error bars.

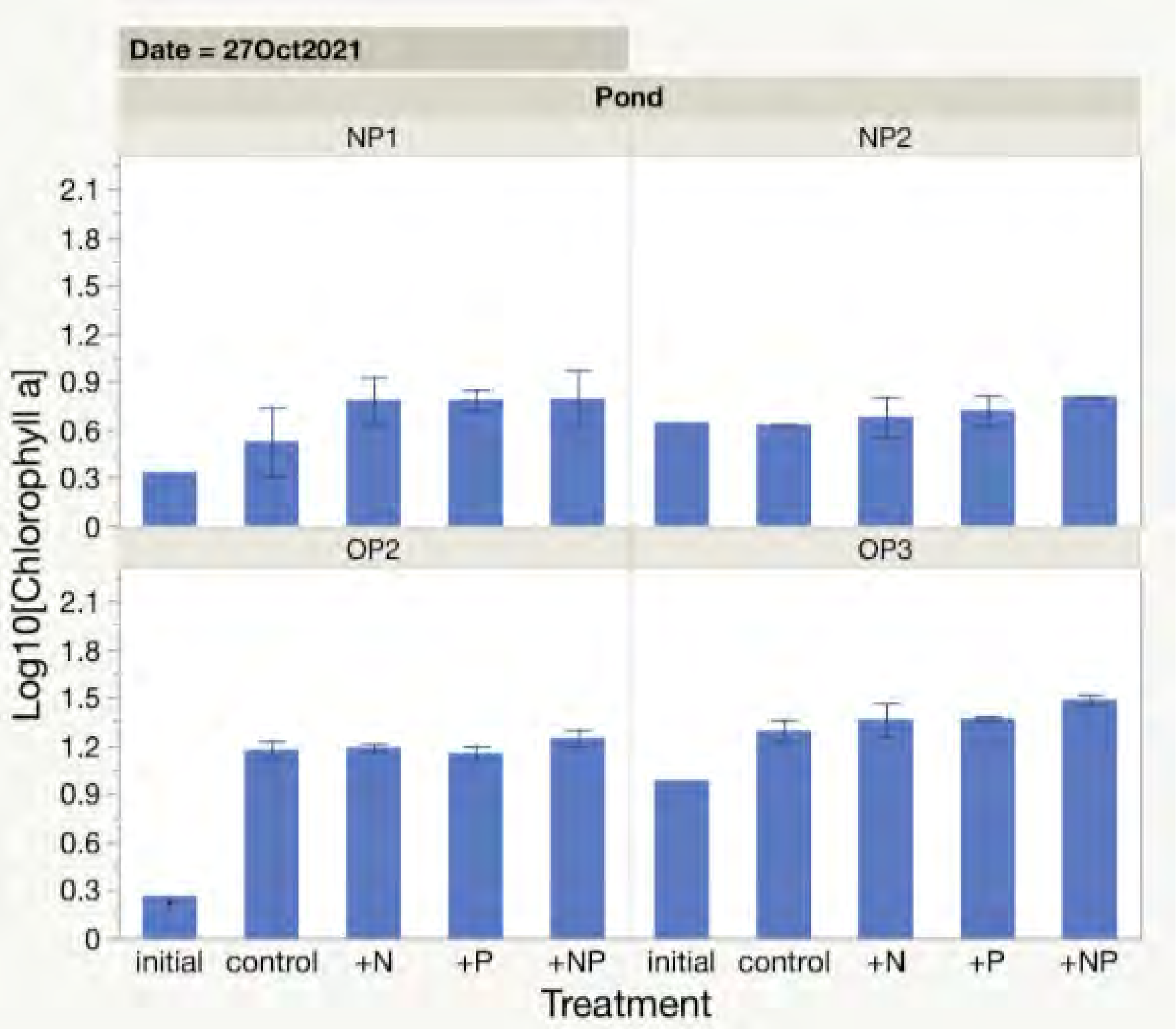

Figure 3.9. The Chlorophyll-a concentrations amongst the three treatments and initial compared to the control at four testing sites for the third sampling day: 27 October 2021. Standard error is represented by error bars.

Additionally, initial water samples were collected from the ponds before treatment and frozen for further analysis using the aforementioned standard colorimetric method, and then the data converted to micromolar values. The concentrations of the various forms of nitrogen and phosphorus were consolidated and compared against the Redfield ratio, a constant concentration of elements found in marine phytoplankton biomass (Redfield 1958). Deviation from this ratio, which is 16:1 N: P is assumed to be indicative of limitation with values lower and higher than 16 indicating N and P limitation, respectively. In Table 3.4, values in the rightmost column, the estimated ratio values, which are less than 16 indicate nitrogen limitation and values greater than 16 indicate phosphorus limitation. The ratios indicate that most ponds on both sampling dates were nitrogen-limiting, however two ponds, New Pond 1 and Old Pond 3 on the second sampling date, 10/27/2021, were phosphorus-limited.

Table 3.4. Nutrient concentration levels in initial water samples from ponds

Date	Sample	Concentration uM/L N	Concentration uM/L P	Ratio N/P
10/13/21	NP1	3.14	1.40	2.23
10/13/21	NP2	1.29	--	--
10/13/21	OP2	7.58	1.32	5.75
10/13/21	OP3	4.92	1.19	< 4.13
10/27/21	NP1	7.39	0.09	> 80.05
10/27/21	NP2	2.10	0.09	> 22.74
10/27/21	OP2	2.52	0.26	9.54
10/27/21	OP3	8.72	0.26	33.76

Note: Values colored red included a data point registering below the detection limit and were corrected during calculations by substituting the appropriate lower detection limit value. Values were excluded from the second row due to multiple compound readings being below the detection limit.

Carbon Sequestration

Each pond was sampled at three sections: shallow/edge, middle, and deep. No significant differences were found between sections within a sample site. Surface sediments of OP3 had the greatest percent organic matter for each section overall (22.46%). The second greatest percent organic matter for each section was observed at OP2 (3.76%), but the value was significantly lower than that of OP3. The percent organic matter found in samples from NP2, the marsh, and the dunes was minimal with all values less than 1%. A post-hoc Tukey Kramer test found a significant difference between OP3 and all other sample sites. The P-values were all less than 0.01 (Table 5). All other sample site comparisons were statistically indistinguishable (Figure 3.10).

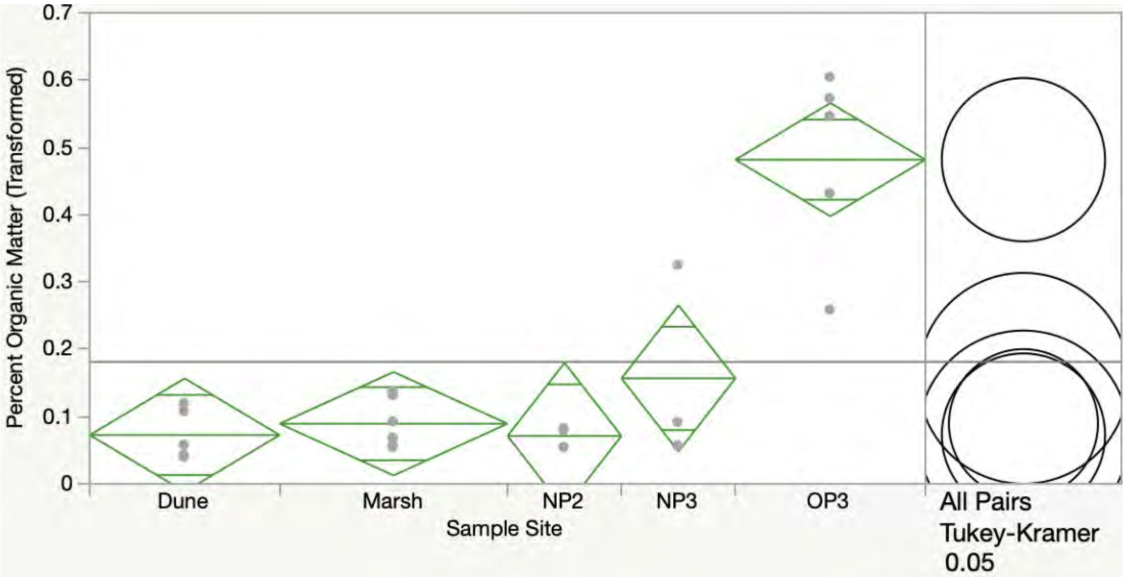

Figure 3.10. One Way analysis of transformed percent organic matter vs. sample site, including post-hoc Tukey-Kramer test on the right. Note: The grand sample mean is represented by the grey horizontal line. The mean for each

individual group is shown by a green horizontal line in the middle of the diamond. The diamonds represent the confidence intervals for each group.

Table 3.5. Ordered differences report from post-hoc Tukey Kramer test.

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
OP3	NP2	0.4106298	0.0651542	0.212400	0.6088599	<.0001*
OP3	Dune	0.4092866	0.0564252	0.237614	0.5809589	<.0001*
OP3	Marsh	0.3924218	0.0540230	0.228058	0.5567855	<.0001*
OP3	NP3	0.3250077	0.0651542	0.126778	0.5232378	0.0009*
NP3	NP2	0.0856221	0.0728446	-0.136006	0.3072501	0.7648
NP3	Dune	0.0842789	0.0651542	-0.113951	0.2825090	0.6985
NP3	Marsh	0.0674141	0.0630852	-0.124521	0.2593495	0.8197
Marsh	NP2	0.0182081	0.0630852	-0.173727	0.2101435	0.9983
Marsh	Dune	0.0168649	0.0540230	-0.147499	0.1812286	0.9977
Dune	NP2	0.0013432	0.0651542	-0.196887	0.1995733	1.0000

Note: Orange p-Value indicates statistical significance.

3.4. Discussion

Water Quality

We found that the new ponds were most similar to the ocean and most different to the old ponds, as initially hypothesized. As for specific water quality attributes, the temperature varied seasonally with each sample site recording the highest temperature on the first sampling day and the lowest on the last day. In September, the older ponds tended to be warmer; however, in late October, the older ponds had the coldest water. This is likely due to the shallow depth of water in the old ponds making the ponds more influenced by changing air temperature compared to the deeper new ponds.

It is difficult to compare findings from the new and old ponds with the sound and ocean because we only sampled at the latter sites once. Therefore, we are unable to account for seasonal variances in the sound and ocean. From the data we do have, it appears that the salinity of the new ponds was more similar to the sound than the ocean. This is likely due to precipitation events decreasing the salinity of the new ponds, which were originally created by overwash from the sound. As for dissolved oxygen and pH, the new ponds are actually more similar to the ocean.

Regarding the old ponds, their dissolved oxygen and pH measurements were also most similar to the sound. The old ponds are organic matter rich, which could be driving oxygen consumption, particularly with the sediment disturbance that likely occurred while measuring the old pond water quality. In general, understanding the water quality variation amongst the ponds, sound, and ocean is vital because the water quality parameters could be related to other aspects of the ponds such as terrestrial wildlife, marine organisms, and plant diversity. These connections can help determine the ecosystems services of the ponds found at Core Banks which is the goal of this entire project.

Vibrio

There are distinct species of vibrio present in both old and new ponds. Due to the higher salinity of the new ponds, we expected there to be higher abundances of *Vibrio spp.* at those sites, but we found a similar presence of *Vibrio spp.* at the old ponds that had much lower salinity. The species most prevalent at the new ponds were *V. parahaemolyticus* and *V. alginolyticus,* whereas the old ponds had a large presence of *V. vulfnicus*. The large presence of *V. vulfnicus* at the old ponds was quite surprising considering the seemingly unfavorable water quality conditions there. Many studies have shown that *V. vulnificus* prefers to grow in temperatures ranging from 22 – 30℃ and moderate salinities ranging from 15-20ppt (Chart 2012). While the average temperature at the old ponds (~27 ℃) was favorable, the salinity was extremely low ranging from 4-6 ppt. *V. vulfnicus* is a halophilic species, so it is very unlikely to find them in such minimally saline conditions.

However, some studies, such as Randa et al., have found *V. vulnificus* thriving in low salinity environments (5-10 ppt) and temperatures ranging from 15-30℃. The variance in ideal growth conditions comes from the lack of understanding of the relationship between *V. vulnificus* abundance with salinity and temperature. Randa et al. (2004) and Lin et al. (2021) have found *V. vulnificus* population dynamics to be strongly correlated with temperature, yet large *V. vulnificus* populations have been found in both warm and cold water. Additionally, some speculate there is an inverse relationship between abundance and salinity while others have suggested the range of salinities play a larger role (Randa et al. 2004). Since the ideal growth temperature and salinity for *V. vulnificus* is so widely contested, it is difficult to say whether or not the presence of *V. vulnificus* in the old ponds is an anomaly or not. The abundance of *V. parahaemolyticus* and *V. alginolyticus* was much lower at the old ponds compared to *V. vulnificus* likely because they are less resistant to low salinities (Lin et al. 2021).

The concentrations of *V. parahaemolyticus* and *V. alginolyticus* were significantly higher in concentration in the sound and marsh compared to both new ponds studied. *V. parahaemolyticus* and *V. alginolyticus* tend to grow most successfully at 20-42℃, high salinities (>30 ppt), and a pH range from 7.4 to 9.6 (Percival and Williams 2014). Both new ponds had an average temperature of 26℃, a salinity of 27 ppt, and a pH of 8.2. All of these water quality parameters were met at both new ponds, so it is not surprising there was a large presence of both *V. parahaemolyticus* and *V. alginolyticus*. Moreover, the sound had a similar salinity and temperature to the new ponds compared to the old ponds. This trend shows that *V. parahaemolyticus* and *V. alginolyticus* prefer high salinities, moderate temperatures, and neutral pH, but can still thrive in slightly basic conditions.

V. vulnificus and *V. parahaemolyticus* are two of the most dangerous *Vibrio spp*. bacteria as they are associated with severe infections and health conditions (Chart et al. 2012). *Vibrio spp.* can enter the bloodstream through open wounds and from eating raw uncooked seafood. *V.*

vulnificus is the most dangerous because it can cause life-threatening infections such as necrotizing fasciitis (CDC 2019). Any concentration of *Vibrio spp*. present can pose a health concern to humans. Since all of the ponds, and even the sound, had detection of one or two of the most harmful *Vibrio spp*., it is imperative to study the microbial community at these ponds, so tourists can be adequately warned of the potential health risks.

Limiting Nutrients

The nutrient addition experiments showed a trend which supports the hypothesis of nitrogen limitations in the ponds. However, strong limitation by N or P was rarely observed in the ponds, particularly as the weather cooled. Significant differences from the control were shown across multiple ponds for the combined nitrogen-phosphorus treatment and the occasional nitrogen treatments, but consistently the control triplicates produced similar quantities of Chlorophyll *a*. Some ponds did show at times potential phosphorus limitation in their Chlorophyll *a* production (Old Pond 2 and New Pond 2 on individual occasions) and their nutrient concentrations, but this is likely due to seasonal variations in sediment phosphorus production. Other studies over a longer time frame have noted similar phosphorus limitation occurring in the fall in low salinity systems and attributed to reaeration of bottom sediments (Fisher 1982, 1999). The seasonal trend may not have a noticeable effect on the overall chlorophyll production because total nutrient limitation is not a strong enough constraint in the ponds (Cloern 2001).

There are some potentially confounding factors in the study to be addressed. First, the second and third experimental incubations were conducted after notable precipitation increased water levels, particularly in the old ponds. This may have led to a dilution effect in the ponds leading to a potential decrease in overall Chlorophyll-*a* production from the first to second week. Additionally, the amount of water sampled and filtered, and the length of incubation were not held consistent throughout the study, but previous studies on this topic indicate that these methodological differences should have little effect on the experimental outcomes (Elser et al. 1990, Spivak et al. 2010). The salinity measured during the water quality section also showed varying salinity levels, which could affect the phosphorus limitation seen during the study and precipitation could increase atmospheric nitrogen deposits into the ponds.

Similar studies into nutrient limitation continue to find persistent nitrogen limitation among marine and coastal environments (Paerl 2018), and further management intervention is necessary to control nutrient loading as a result (Paerl 2009). Human impacts, such as increased pollution or runoff, on ecosystems may increasingly throw natural nutrient cycles out of balance. Additional trials and replicates would be helpful to clarify our results but would likely only reinforce the trends revealed in our study. Knowledge of the nutrient levels and nutrient limitation status of the phytoplankton in both the old and new ponds provides information regarding not only primary production, but also the ability of the ponds to sustain additional trophic levels. Tracking when key limiting nutrients become more available also allows for management to determine the effects of runoff and the potential for eutrophication, which could lead to harmful algal blooms and hypoxia. In the future, we recommend that the National Park Service continues to monitor nutrient limitation in their ponds before installing new septic tank systems. Also, when installed, these systems should be placed downslope of the ponds to reduce nutrient loading and potential algal blooms.

Carbon Sequestration

Our results do indicate that carbon sequestration is occurring, especially in the deepest or deeper areas of the ponds. Activity is most pronounced, however, in the old pond and not in the marsh as we expected. This is likely due to human error during data collection. Our methodology involved collecting one sample from the edge, middle, and deep/center of each zone. However, all three marsh samples were collected in a relatively small storm-influenced section compared to the entirety of the marsh because deeper areas of the marsh were not able to be sampled. Each of the marsh samples are essentially an ‘edge’ sample and not truly indicative of the sequestration rates likely to be found in a true ‘deep’ marsh sample.

A healthy marsh can contain over 15 kg C/m^2 and serves as one of the most efficient carbon storage systems in a coastal ecosystem (Drake et al. 2015). That being said, if the new ponds maintain steady water levels and continue to be pervasive, it is likely that they will eventually take on characteristics like the old ponds and become a greater carbon sink. Previous research into recently converted marshland shows that wetland and marsh habitats quickly become net sinks as the vegetation fixes atmospheric carbon into their biomass or are broken down and sequestered into the soil (Shiau et al. 2019). Overall, the conversion of areas from dune to pond did increase their ability to sequester carbon, but not to the level of ponds that had been converted from marsh areas. If left undisturbed, this trend will continue in the new ponds, however further research and more refined methods are needed to know the true extent of transition and sink potential.

Chapter 4: Primary Productivity

Evaluation of macrophyte and microalgae composition and phytoplankton productivity in and around the Ponds

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Fall 2021

4.1. Introduction

In evaluating the ecosystem services and ecological functions of these ponds, it is crucial to consider the role of primary producers. These organisms serve as the foundation of all food webs, producing food and energy via photosynthesis. As such, primary producers are bottom-up drivers in determining community composition, structure, and function in higher trophic levels (Scherber et al., 2010). In coastal ecosystems, such as the ponds we are investigating, primary producers are composed of macrophytes, phytoplankton and benthic microalgae.

Macrophytes are aquatic plants, which may grow in or near water. It is important to evaluate the role of macrophytes because they play a significant role in supporting local ecosystems. Macrophytes often provide cover, oxygen, and food for fish as well as other species of wildlife. Macrophytes are often good indicator species, are easily sampled, and can easily be used to calculate species abundances, diversity, etc. The density, evenness, diversity, and species richness of macrophytes in an area can also indicate the health of a local waterbody (EPA, 2021).

Phytoplankton are microalgae that are suspended in the euphotic zone (NOAA, 2021). Phytoplankton convert inorganic materials into organic carbon through photosynthesis, during which they contribute about 50-80% oxygen production and half of the net primary productivity in the world. Additionally, they also play a significant role in aquatic secondary production because phytoplankton are the most important source of biomolecules that are essential for the growth of zooplankton (Peltomaa et al., 2017).

Benthic microalgae, also known as microphytobenthos, are frequently overlooked in evaluations of primary productivity as sediment layers often do not receive sufficient irradiance to support photosynthesis. However, in shallow coastal environments, abundant light penetration coupled with nutrient rich sediment can result in microphytobenthos dominating biomass and production (Jacobs et al., 2021; Light & Beardall, 1998). Benthic diatoms and bacteria specifically play a significant role in trophic relationships and secondary production as they are an important food source for many deposit-feeding and suspension-feeding macrofauna (Miller et al., 1996). Given that the ponds we surveyed are all less than two meters deep, it is prudent to consider the contribution of microphytobenthos in each pond.

The diversity of primary producers has been shown to correlate positively with higher productivity. Carbon and nitrogen uptakes are higher within communities of greater diversity (Behl et al., 2011; Bracken & Stachowicz, 2006). Although ecosystem processes are more sensitive to functional diversity rather than species or alpha diversity, higher species diversity increases the likelihood of a stable supply of ecosystem services such as nutrient cycling and production of organic matter (Hooper et al., 2005). Additionally, high species diversity can also lead to an increase in positive interspecies interactions, such as facilitation, which further enhances productivity (Cardinale et al., 2002). With that in mind, we hypothesize that a more diverse pond will also be more productive. To evaluate this hypothesis, we examined microalgae abundance, composition and diversity via microscopy and taxa-specific pigments, and compared diversity with estimates of microalgae-driven primary productivity within the ponds measured using ^{14}C radiolabeling.

As these ponds are located on a barrier island, they are frequently being exposed to disturbances ranging such as erosion, overwash and hurricanes (Conery et al., 2018). The amount of disturbance that these habitats are exposed to is an important factor in explaining the species composition and community structure of the ponds. The intermediate disturbance hypothesis (IDH) asserts that the greatest diversity of a community occurs when there is a moderate amount of disturbance within the ecosystem. In other words, when disturbances are intermediate in both frequency and intensity, diversity is thought to be higher. As the period between disturbances increases it is proposed that diversity will increase, as the longer interval allows time for the settlement of more species. However, if the period between disturbances is too long then the more dominant species will out compete other species, leading to lower diversity. These disturbances also need to be strong enough to severely damage or kill any preexisting species, allowing for the colonization of new species, but not too intense to the point that important habitat is destroyed (Connell, 1978). Here we will attempt to use the IDH to explain species diversity and richness of the primary producers found both in and surrounding these ponds.

In order to predict how the ponds will evolve over time, we are comparing two new ponds to two old ponds. Following the intermediate disturbance hypothesis (Connell, 1978), we hypothesize that species diversity is higher at the new ponds as various species colonized the area after the disturbances caused by Hurricane Dorian. At the old ponds, we hypothesize that an equilibrium is likely established with more competitive species dominating the environment, resulting in lower diversity. Consequently, we expect the new ponds to have higher productivity as well.

4.2. Methods

We surveyed two new ponds (New Ponds 1 and 2) and two old ponds (Old Ponds 2 and 3) as shown in Figure 4.1. All ponds were located landward of the dune line on North Core Banks. The new ponds had sandy bottoms and vegetation was mainly located on either sides and the "back" of the pond (i.e sound-facing side). The old ponds had marsh-like characteristics with muddy bottoms and thick vegetation surrounding the entire pond.

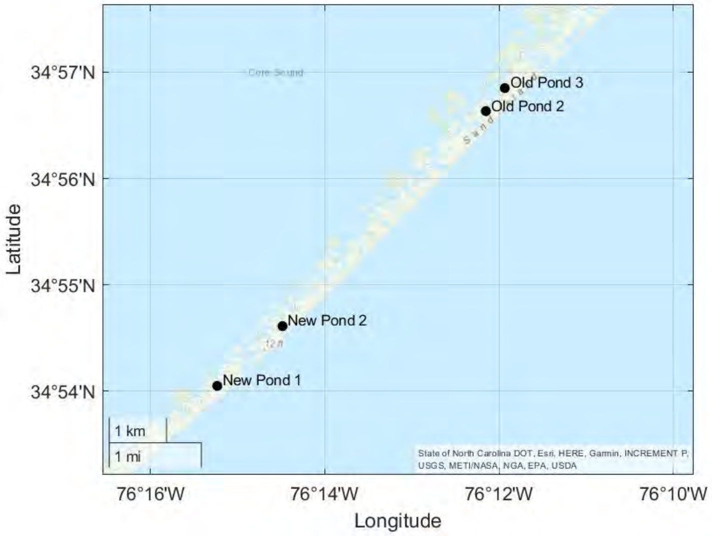

Figure 4.1. Map showing the four ponds sampled in this chapter

Macrophyte Survey

Macrophyte surveys were conducted on September 23rd, September 29th and October 13th. A 0.5m by 0.5m quadrat was used to survey the macrophytes surrounding the ponds. At each pond, four sites were selected by tossing the quadrat in random directions. Macrophytes within the quadrat were then identified and the number of stalks of each species were counted. Unknown plants were photographed and identified later with reference to Palus (2020).

Plant density was calculated by dividing the total number of each species by the area surveyed at each pond ($0.25\text{m}^2 \times 4 = 1\text{m}^2$). Simpson's Reciprocal Index was then used to quantify biodiversity of the macrophytes, which considers both species richness and evenness. The first equation used was Simpson's Index (D):

$$\mathrm{D} = \frac{\sum \mathrm{n}(\mathrm{n}-1)}{\mathrm{N}(\mathrm{N}-1)}$$

where n is the total number of organisms of a particular species and N is the total number of organisms of all species.

Simpson's Reciprocal Index quantifies biodiversity by considering both species richness and evenness of abundance among the species present. Specifically, it measures the probability that two individuals randomly selected from an area will belong to the same species. Within this index, 0 represents infinite diversity and 1, no diversity. However, when Simpson's Reciprocal Index (1/D) is used, 1 is the lowest possible value, and higher values indicate greater diversity. The maximum value for 1/D is the total number of species in the sample and occurs when all species are equally abundant.

Collection of water and sediment samples

Water and sediment samples were collected from each pond to examine the microalgae composition and productivity levels. Three sets of samples were collected for 1) microscopic identification and enumeration of microalgae, 2) photopigment measurements by high pressure liquid chromatography (HPLC), and 3) measurements of primary productivity by ^{14}C uptake.

Microscopy samples were collected on September 16th, September 23rd and September 29th. From each pond, a 20mL scintillation vial was filled with surface pond water. A 10mL syringe was modified to form a mini core by cutting off the tip and filing the surface flat. The syringe was used to collect a 1mL sediment core from each pond. The core was placed into a separate clean vial and topped off with pond water. These samples were immediately fixed with 1% Lugol's solution and stored at room temperature until microscopy analysis.

Samples for HPLC analysis of photopigments were collected on September 29th, October 13th, and October 27th. Water samples were collected in triplicate at random locations throughout the pond using 125mL dark bottles. Sediment samples were also collected in triplicate using the modified syringe corer as described above. The sediment cores were placed directly into 15mL falcon tubes. All samples were immediately placed on ice until returned to the lab for processing.

Water and sediment samples were collected for measuring phytoplankton and benthic microalgal primary productivity on 13th October and 27th October. At each pond, 500mL dark bottles were filled with pond water. The modified syringe was used to place five 1mL cores in a separate bottle before filling it up with pond water. The samples containing sediment and pond waters will be referred to as benthic samples from here on out. Water samples were used to measure phytoplankton productivity whereas benthic samples were used to measure the combined productivity of phytoplankton and microphytobenthos. Subsequently, phytoplankton productivity was subtracted from benthic values to obtain microphytobenthos productivity. Water and benthic samples were stored at ambient temperature until 14C primary productivity incubations which were performed the next day.

Enumeration of microalgal communities

Each sample was inverted several times to ensure homogeneity, then 1mL of each sample was pipetted into a settling chamber. The samples were left to settle for at least one hour before enumeration. The samples were then observed under a Leica DM IRB inverted light microscope (Leica Microsystems Inc., Illinios USA) and counted at 200x or 400x magnification depending on the size of cells. At least 400 cells or 100 fields of view (FOVs) were counted for each sample. Subsequently, the counts were converted into units of cells per milliliter by taking into account the number and surface area of the FOVs, surface area of the chamber, and volume settled (Eaton et al., 1998). The calculation was done using the following formula:

$$C_{cell} = A_{settling\ chamber} \div (A_{FOV} \times N_{FOV}) \times N_{cell} \div V$$

where $A_{settling\ chamber}$ was the area of settling chamber (250mm^2), A_{FOV} is the area of field of view (0.25mm^2 for 200x magnification, and 0.0625mm^2 for 400x magnification), N_{FOV} was the number of fields of view, N_{cell} was the cell count, and V was the volume of sample settled.

The average species abundance between triplicate samples from each site was then used to calculate the Simpson's Reciprocal Index as mentioned above to quantify microalgae diversity. In addition, the species abundance data were grouped by taxonomic classes to calculate the class-level relative abundance, which was compared with microalgae composition calculated from accessory photopigment concentrations using CHEMTAX (Mackey et al., 1996) to corroborate microscopic enumeration. A repeated measures multivariate ANOVA test was performed to determine whether the composition and abundance of samples from different ponds had significant variability.

Pigment extraction and HPLC analysis

The sediments cores were immediately placed into the freezer at -20˚C when returned to lab. The water samples were filtered through 25mm GFF filters in reduced light conditions. One hundred milliliters of each water sample was filtered in most cases, however smaller volumes were filtered if high amounts of suspended sediment prevented 100mL from passing through the filter. The volumes filtered were recorded for subsequent calculations. The filters were then folded in half with content side inward, blotted dry, placed in 15ml centrifuge tubes and immediately frozen at -20ºC until extraction.

Pigments were extracted from the sediment cores and filters using 100% HPLC-grade acetone. The samples were kept on ice during the extraction and all work was performed in the dark to prevent degradation of pigments by light. One and a half milliliters of acetone was added to each sample and sonicated using the Sonics Ultrasonic Disruptor microtip for 15 to 30 seconds. The samples were then returned to the freezer to complete the extraction process for at least 24h. Filtered extracts were injected into a Shimadzu HPLC system equipped with a Varian Dynamax Microsorb guard column (0.46 x 1.5 cm, 3 µm) followed by a single monomeric reverse-phase C18 column (Kinetex, 0.46 x 10 cm, 5 µm) and two polymeric reverse-phase C18 columns (Vydac 201TP5, 0.46 x 25 cm, 5 µm). A nonlinear, variable flow, binary gradient adapted from van Heukelem et al. (1994) was used for pigment separation. Solvent A consisted of 80% methanol: 20% ammonium acetate (0.5 M adjusted to pH 7.2) and solvent B consisted of 80% methanol: 20% acetone. Absorption spectra and chromatograms (at 440nm) were acquired using a Shimadzu SPD-M20A UV/Vis photodiode array spectrophotometric detector. A multi-point calibration curve is generated by injection volumes of known quantities of pure pigment standards (obtained from DHI, Denmark) and calculating the peak areas of those pigments. The peak areas are used to calculate the slope (response factor) for that pigment. Pigments extracted from the samples are then quantified (usually in ug L^{-1}) by multiplying the peak areas of a chromatogram by the response factors.

Total biomass was determined using the sum of chlorophyll-α and chlorophyllide-α concentrations. To standardize units, volumetric concentrations for phytoplankton samples were multiplied by the average depth of the respective ponds to obtain units of mg m^{-2}.

Microphytobenthos samples were converted from µg cm^{-2} to mg m^{-2} by multiplying the initial values by ten. Given the shallow depths of the pond, we felt it reasonable to assume that all ponds were well-mixed, and that phytoplankton biomass was evenly distributed throughout the water column. Subsequently, a repeated measures ANOVA was performed to determine significant differences in biomass between the four ponds.

Taxa-specific photopigment concentrations measured by HPLC were also used to determine class-level benthic microalgal community composition using CHEMTAX (Mackey et al., 1996). Using an initial input pigment matrix, CHEMTAX estimates the contributions of different phytoplankton classes by comparing the amount of each taxa-specific pigment to overall chlorophyll-α. Because the ponds had estuarine characteristics, we used input pigment ratios developed by Lewitus et al., (2005) for use in southeastern U.S. estuaries.

Primary Productivity Incubations

Primary productivity of phytoplankton and microphytobenthos of each pond was measured using ^{14}C radiolabeling (Lewis & Smith, 1983). Because the benthic samples contained both microphytobenthos and phytoplankton, it was necessary to subtract the productivity of the water samples from the productivity of the benthic samples to assess the activity of the benthic microalgae. Two methods of incubation were utilized. First, we incubated our samples using photosynthetrons as described by Lewis and Smith (1983). We performed this incubation on samples from New Pond 1 and Old Pond 3 collected on 13th October, with 10 light levels ranging from 0 to 650 µmol photons $m^{-2}s^{-1}$ for each water and benthic sample. However, as light intensity varied between the chambers, it was not possible to directly subtract phytoplankton productivity from the benthic samples. Given that, we subsequently incubated phytoplankton and benthic microalgal samples collected on 27th October in the UNC Institute of Marine Science's experimental pond. Using neutral-density, window screen, we obtained five irradiance levels (100%, 50%, 25%, 12.5% and 6.25%). Since both water and benthic samples were incubated at the same light level, microphytobenthos productivity could be determined by subtracting the productivity of the water samples from that of the benthic samples. The method described below is that of the pond incubation, however the photosynthetron incubation followed a similar protocol, with the only difference being the number of light levels and location of incubation.

Each sample was thoroughly mixed and 50mL was measured out into a beaker. 100µL of radioactive bicarbonate (^{14}C) label was added to the beaker and the solution was stirred to ensure homogeneity. Five milliliters of the resultant solution were pipetted into 8 separate 20mL scintillation vials. Five vials were placed in containers and covered with screens to obtain the five light levels. The remaining three vials were placed in a dark container to account for non-photosynthetic 14C uptake. A light meter was placed in the pond over the course of the incubation and the average light intensity was used to determine 100% irradiance. The light levels to which samples were exposed were calculated accordingly based on the number of screen layers.

The 14C labelled samples were incubated in the ponds or photosynthetron incubator for 6.5 hours. After incubation, the samples were acidified with 0.5mL of 6N HCl, shaken well and left uncapped in a fume hood overnight. This step ensured that any residue inorganic 14C is released as carbon dioxide. The next day, the samples were neutralized with 0.5mL 6N NaOH and 10mL of Ecolume scintillation cocktail was added. The activity of the ^{14}C label was determined by adding 0.5µL of phenylethylamine to 0.5µL of the ^{14}C bicarbonate label, then topping the solution off with 5mL of Ecolume. The radioactivity of each sample and the ^{14}C label was then measured using a Beckman LS 6500 Multi-Purpose Scintillation Counter. Dissolved inorganic carbon (DIC) from each sample used to measure primary productivity was determined using a Shimadzu 5000A Total Organic Carbon (TOC) analyzer.

Carbon uptake rate (mg C l^{-1} hr^{-1}) was calculated using the following equation:

$$C_{uptake} = \text{Naturally occurring DIC} \times 1.05 \times \frac{\text{DPM}_{\text{sample}} - \text{DPM}_{\text{dark}}}{\text{DPM}_{\text{added}}}$$

The volumetric C uptake rate was then multiplied by depth of pond to obtain a rate based on area (mg C m^{-2} h^{-1}). This C uptake rate was plotted against irradiance and a non-linear regression was performed using the photosynthesis and light equation described by Jassby and Platt (1976):

$$\text{P} = \text{P}_{\text{max}}\tanh\,(\alpha\text{I}/\text{P}_{\text{max}})$$

where P is C uptake, I is irradiance and α is the slope of the curve at low light levels (linear range). Subsequently, P_{max} was divided by chl-α concentration to obtain biomass-normalized productivity. The resultant value was plotted against Simpson's Reciprocal Diversity Index to determine the relationship between diversity and productivity.

Light measurements were taken at varying depths in New Ponds 1 and 2. Light attenuation coefficient (*k*) and mean water column irradiance (I_x) were calculated according to Mallin and Paerl (1992).

$$k = (ln\text{I}_0 - ln\text{I}_\text{z})/\text{z}$$
$$\text{I}_\text{x} = \text{I}_0[1 - e^{-k\text{z}}]/k\text{z}$$

where z is water column depth (m), I_0 is surface irradiance, and I_z is irradiance at depth z. The I_x values were then compared to the productivity vs irradiance plots to determine the potential for light limitation of microalgal growth in the ponds.

4.3. Results

Macrophyte Survey

Simpson's Reciprocal Index diversity values (Figure 4.2) were highest at New Pond 1 and New Pond 2 at 2.88 and 2.04, with the highest total possible values (i.e., observed species richness) being 13 and 10, respectively. Lower values were calculated for Old Pond 2 and Old Pond 3 at 1.83 and 1.33 out of maximum values of 10 and 8, respectively. On average, the new ponds were 55% more productive than the old ponds.

Spartina patens (salt meadow cordgrass) was the dominant macrophyte at all ponds (Figure 4.3) with *Juncus roemarianus* (black needle rush) and *Hydrocotyle bonariensis* (large leaf pennywort) being second and third most abundant. Overall, Old Pond 3 had the highest macrophyte abundance and New Pond 1 had the lowest.

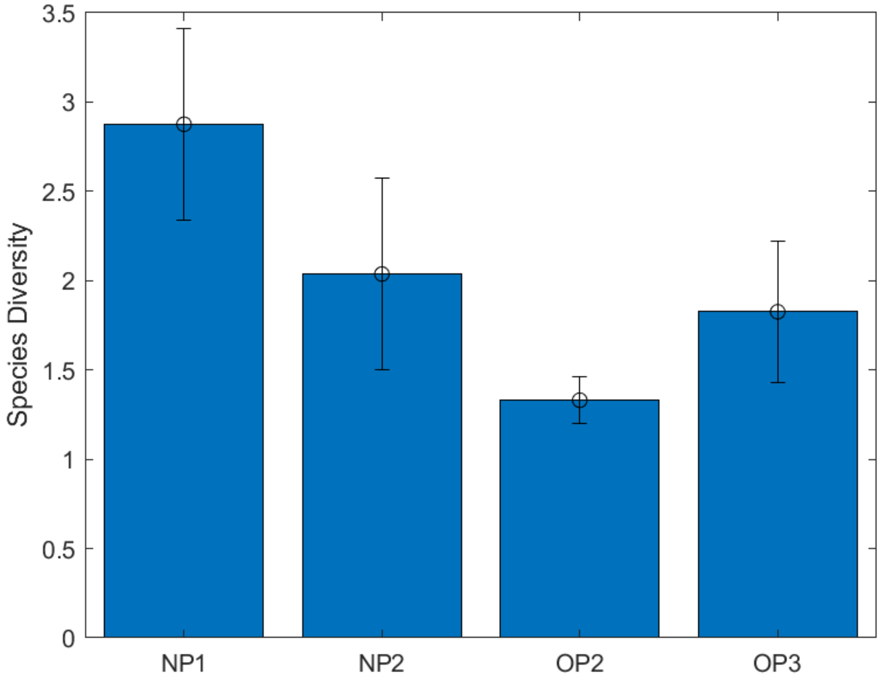

Figure 4.2. Simpson's Reciprocal Diversity Index Values based on plant density averages at each pond.

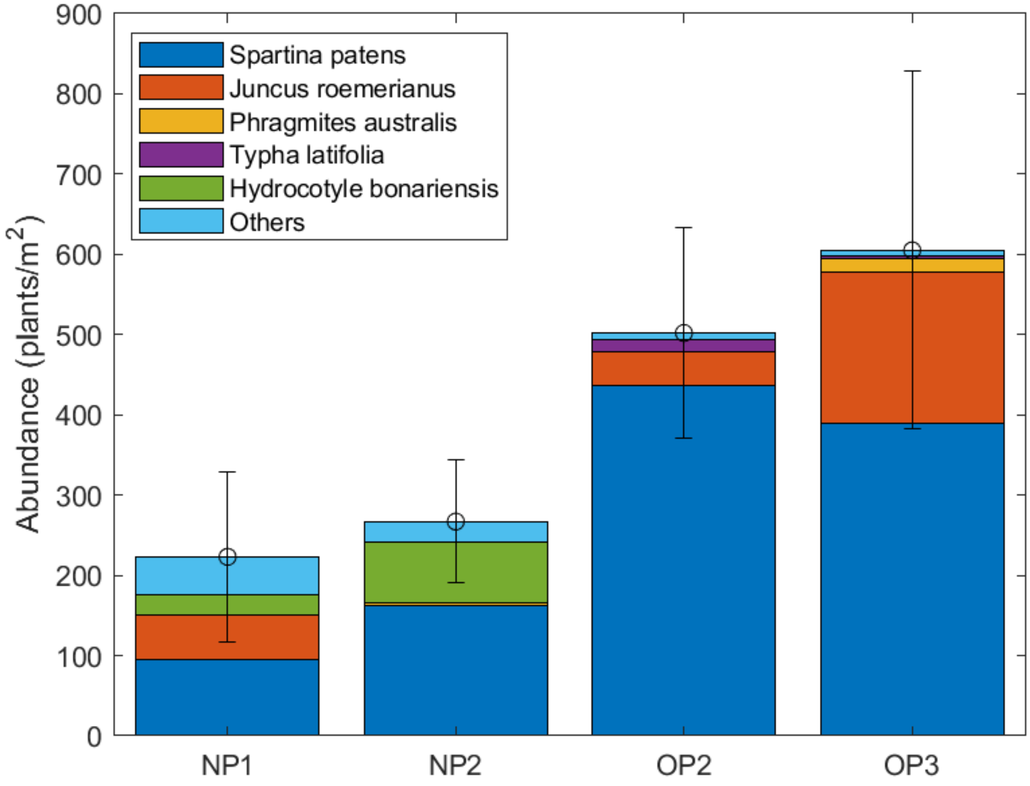

Figure 4.1. The average plant density at each pond overlaid with the species composition.

Microalgae composition and species diversity

Microscopic Enumeration

Simpson's Reciprocal Index diversity values for phytoplankton were higher than that of benthic microalgae in all ponds except Old Pond 3. Phytoplankton diversity indices were 3.06 for New Pond 1, 3.19 for New Pond 2, 3.60 for Old Pond 2 and 3.78 for Old Pond 3. Microphytobenthos diversity indices were 2.96 for New Pond 1, 2.99 for New Pond 2, 3.13 for Old Pond 2 and 4.03 for Old Pond 3. On average, the microalgal communities of old ponds were 19% more diverse than new ponds.

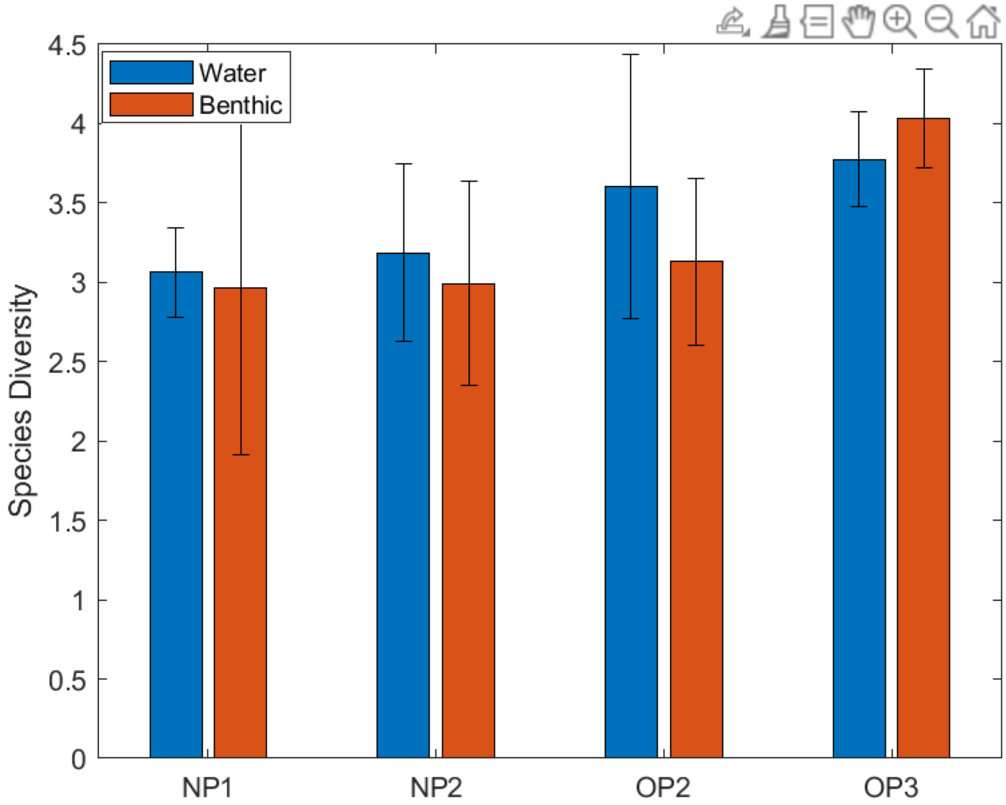

Figure 4.2. Simpson's Reciprocal Diversity Index of microalgae at each pond.

Microalgae were dominated by pennate diatoms for both water and sediment samples. Chlorophytes were also common within Old Pond 3’s phytoplankton community. Microphytobenthos biomass was significantly higher than phytoplankton biomass, contributing to 71% of the total cells counted. Old Pond 3 had the most phytoplankton whereas New Pond 2 had the most benthic microalgae. Repeated measures MANOVA (Table 4.1) indicated no significant differences between the microalgae composition and abundance of the four ponds. However, most variability was observed between New Pond 1 and New Pond 2 (p-value = 0.57). This variability can mostly be attributed to the microphytobenthos population (Figure 4.6) as both new ponds had comparable phytoplankton assemblages (Figure 4.5)

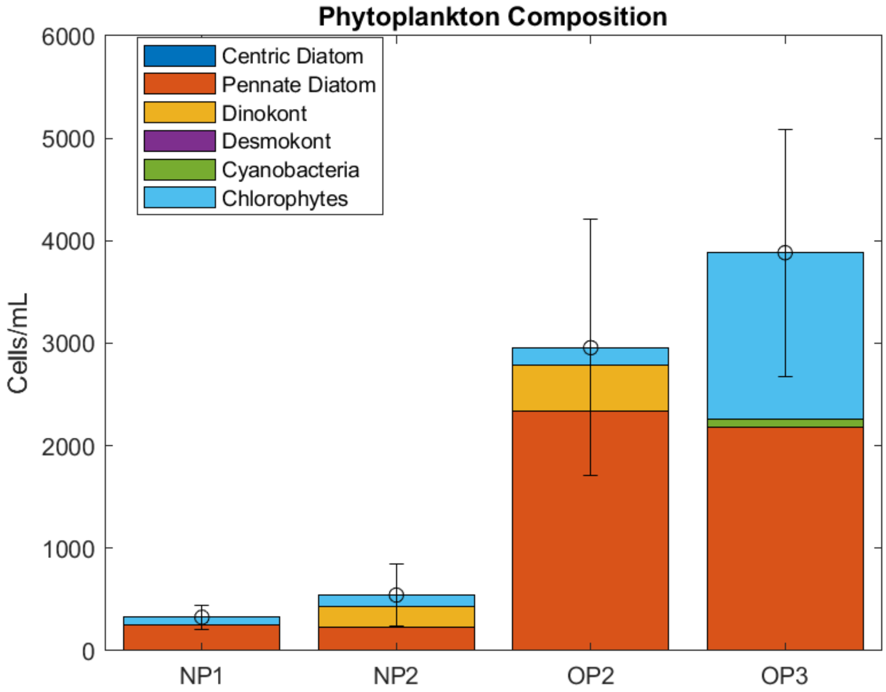

Figure 4.3. Phytoplankton abundance and composition across all ponds. Error bars shown are standard deviation.

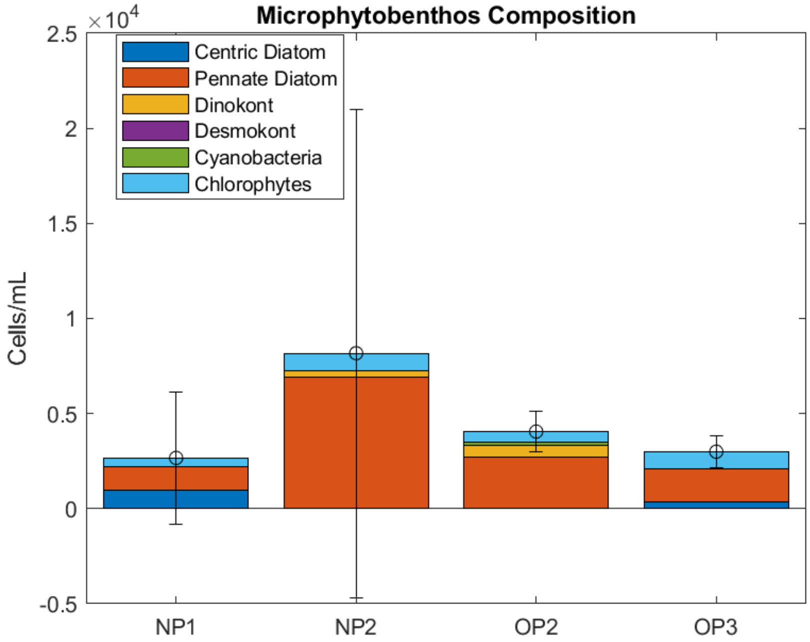

Figure 4.4. Microphytobenthos abundance and composition across all ponds. Error bars shown are standard deviation.

Table 4.1. Repeated Measures MANOVA results of microalgae composition and biomass based on microscopic enumeration.

Pond 1	Pond 2	p-value
NP1	NP2	0.57
NP1	OP2	0.80
NP1	OP3	0.82
NP2	OP2	0.98
NP2	OP3	0.98
OP2	OP3	1.00

Phytoplankton Community Composition

Chlorophytes and cyanobacteria were the dominant phytoplankton taxa throughout the four ponds based on CHEMTAX (Figure 4.7). New Pond 1 had a higher proportion of cyanobacteria and chlorophytes and a lower proportion of dinoflagellates and diatoms compared to the remaining three ponds.

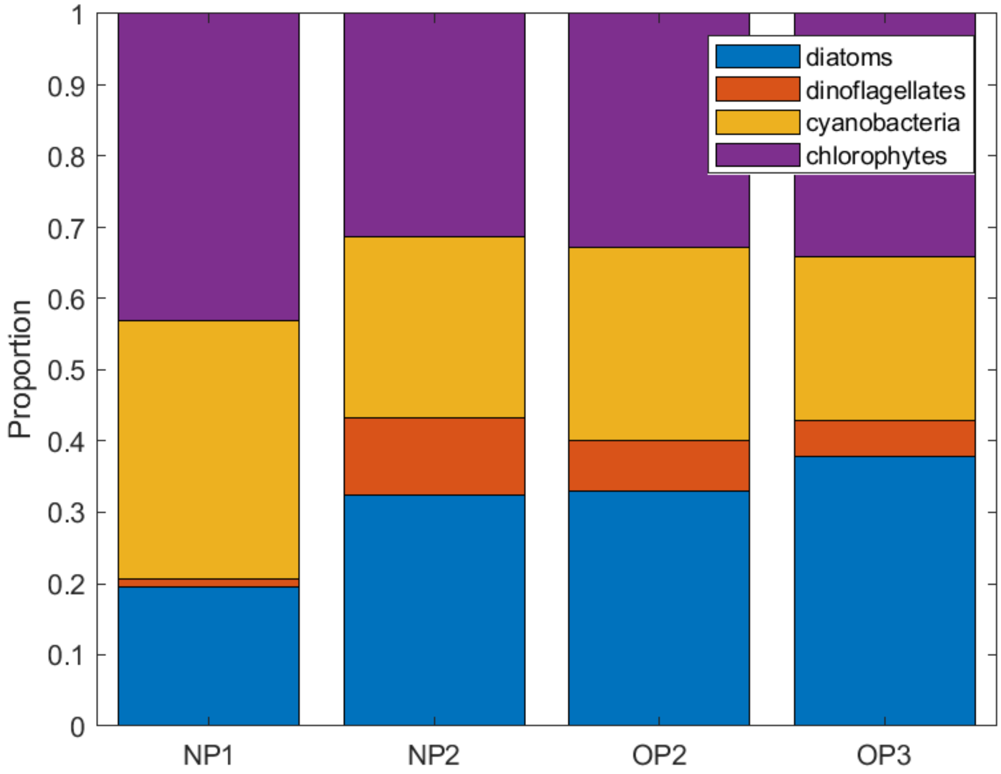

Figure 4.7. Phytoplankton community composition determined via ratios of accessory photopigments to total chlorophyll-a. Results showing phytoplankton composition at each pond.

Diatoms were the dominant microphytobenthos taxa across the four ponds (Figure 4.8). However, New Pond 2 had a lower proportion of diatoms and more chlorophytes compared to the other three ponds. New Pond 1 had the highest proportion of dinoflagellates of the four ponds although dinoflagellates were still the least prevalent taxa overall.

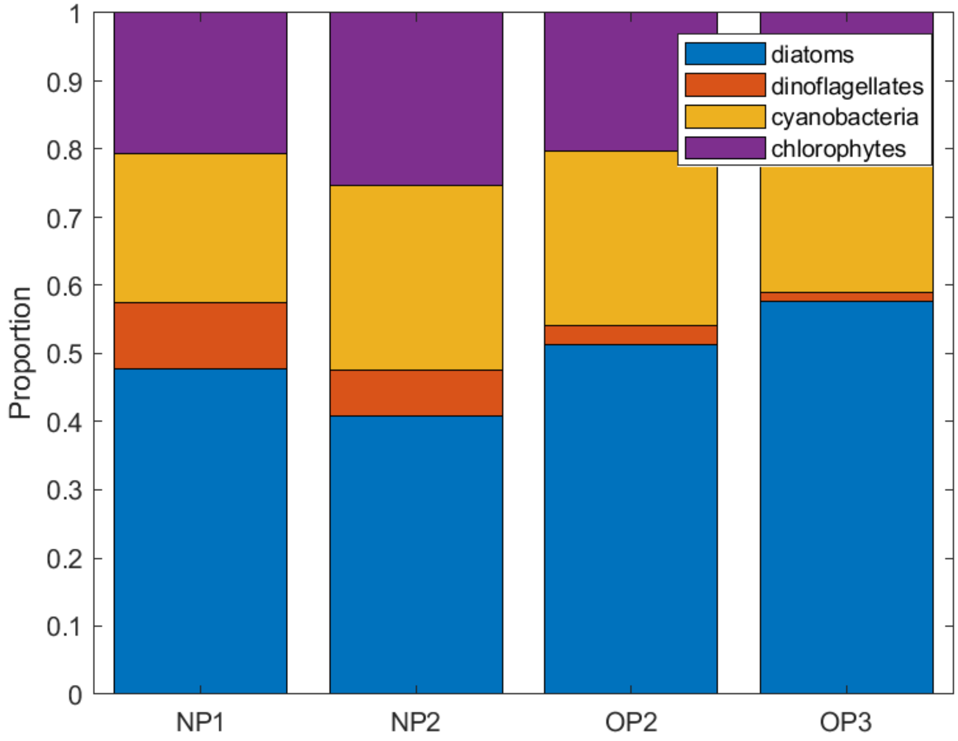

Figure 4.85. Results showing benthic microalgae community composition at each pond.

Microalgae biomass

Microalgae biomass was dominated by microphytobenthos at all ponds, with sediment biomass accounting for >70% of total biomass at the four ponds. New Pond 1 had the highest biomass for both water and sediment samples. In terms of total microalgae biomass, New Pond 1 had 46% greater biomass than New Pond 2, 53% greater than Old Pond 2 and 62% greater than Old Pond 3. ANOVA test returned significant differences between New Pond 1 and the three other ponds, with p-values listed in Table 4.1.

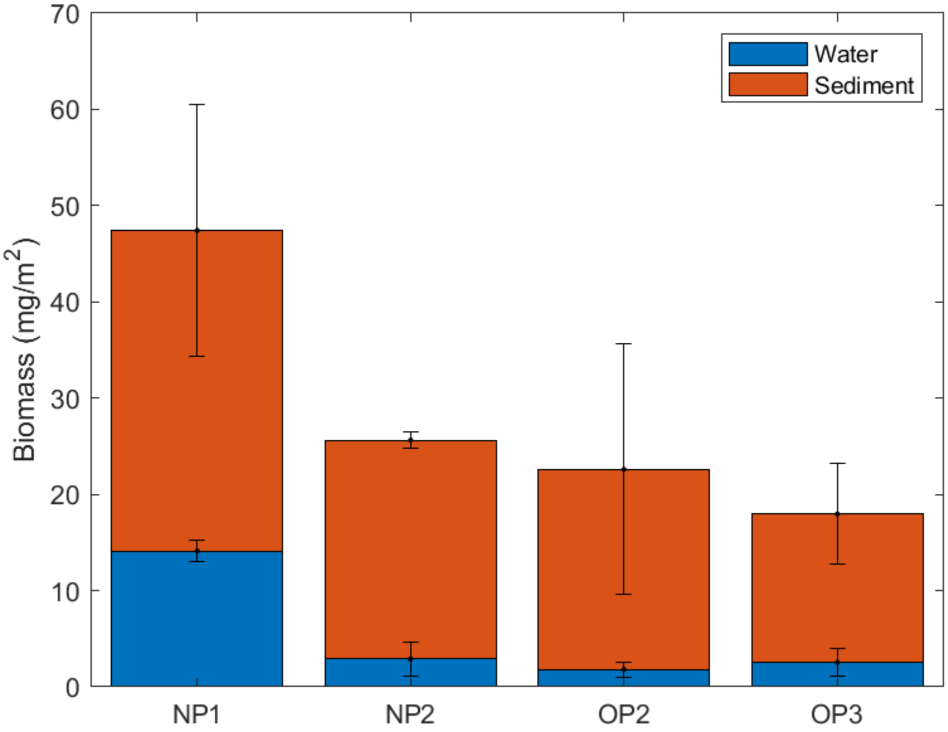

Figure 4.96. Averaged biomass (chlorophyll-α) across the four ponds. Error bars are standard deviation of water and sediment biomass over the three dates measured.

Table 4.2. Repeated measures ANOVA results of microalgae biomass at each pond based on HPLC results. Asterisked values show significant difference (p-value ≤ 0.05).

Pond 1	**Pond 2**	**p-value**
NP1	NP2	0.05*
NP1	OP2	0.04*
NP1	OP3	0.02*
NP2	OP2	0.9
NP2	OP3	0.5
OP2	OP3	0.8

Microalgae primary productivity

Results from photosynthetron incubations performed on 13th October are shown in Figures 4.10 and 4.11. Maximum water productivity observed was 422.5mg C m^{-2} h^{-1} in New Pond 1 and 23.8 mg C m^{-2} h^{-1} in Old Pond 3. In order to estimate the productivity of the microphytobenthos, maximum water productivity was subtracted from maximum benthic productivity, yielding −39.4mg C m^{-2} h^{-1} New Pond 1 and 85.1mg C m^{-2} h^{-1} for Old Pond 3. Maximum productivity occurred when light intensity exceeded ~100-200μmol photons m^{-2}s^{-1}.

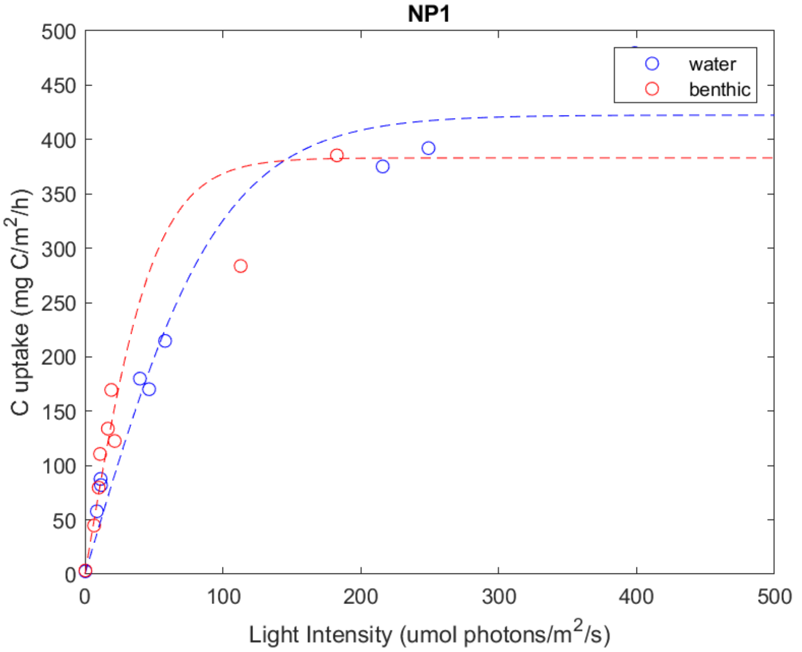

Figure 4.107. Carbon uptake rate for water and benthic samples in New Pond 1 on 13th October.

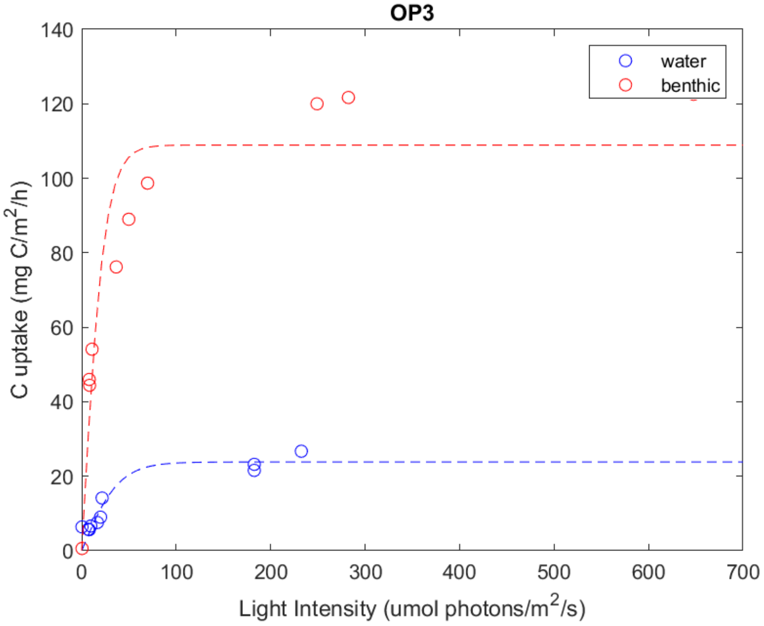

Figure 4.118. Carbon uptake rate for water and sediment samples in Old Pond 3 on 13th October.

Results from pond incubations performed on 27th October are shown in Figures 4.12 to 4.15. Because microphytobenthos productivity was calculated by subtracting productivity of water samples from that of benthic samples, some values were negative. Strangely, microphytobenthos productivity did not fit the expected productivity vs irradiance curve for any of the ponds. As such, we only evaluated phytoplankton productivity. Maximum water productivity was 69.1 mg C m^{-2} h^{-1} for New Pond 1, 8.2mg C m^{-2} h^{-1} for New Pond 2, 12.2mg C m^{-2} h^{-1} for Old Pond 2 and 27.4mg C m^{-2} h^{-1} for Old Pond 3. On average, water column productivity is 49% lower at Old Ponds compared to New Ponds.

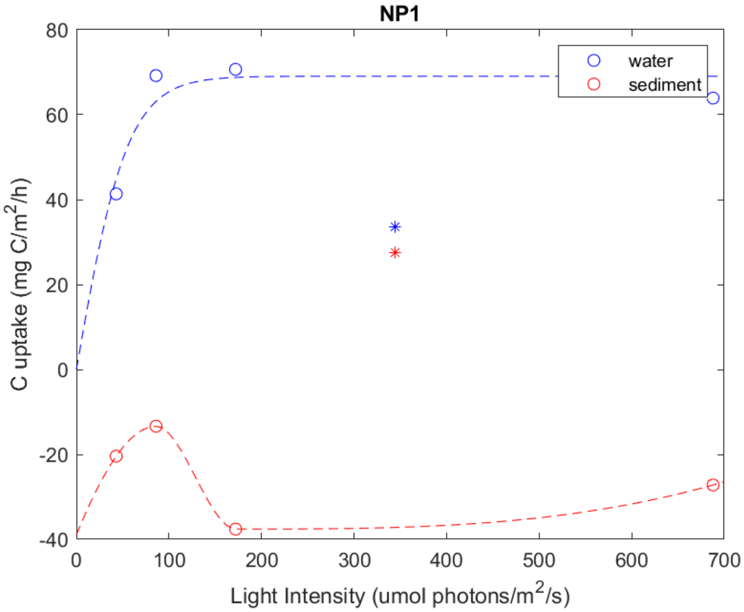

Figure 4.129. Carbon uptake rate for water and sediment samples in New Pond 1 on 27th October. The asterisked points were not included in the fit as they deviated significantly.

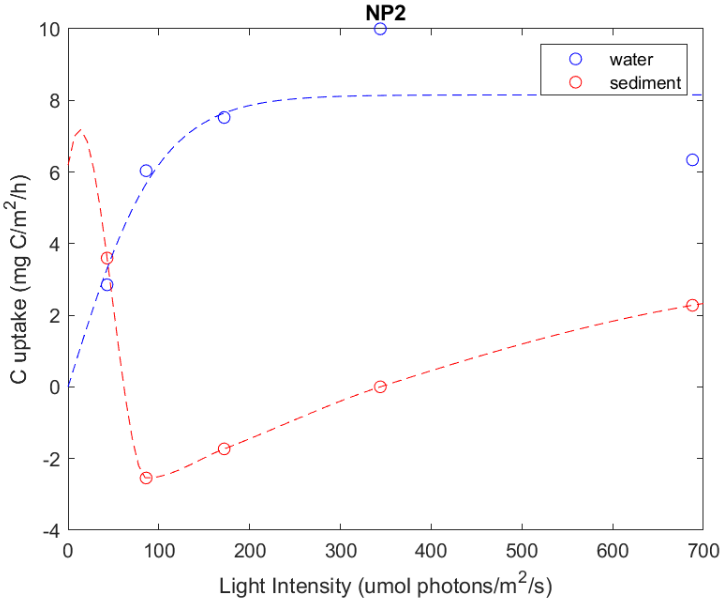

Figure 4.1310. Carbon uptake rate for water and sediment samples in New Pond 2 on 27th October.

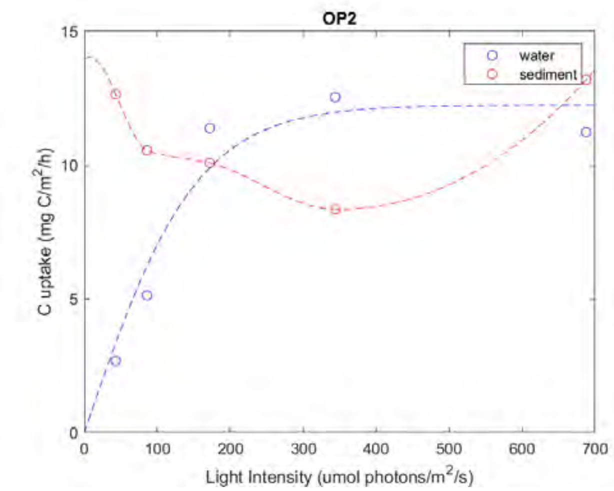

Figure 4.1411. Carbon uptake rate for water and sediment samples in Old Pond 2 on 27th October.

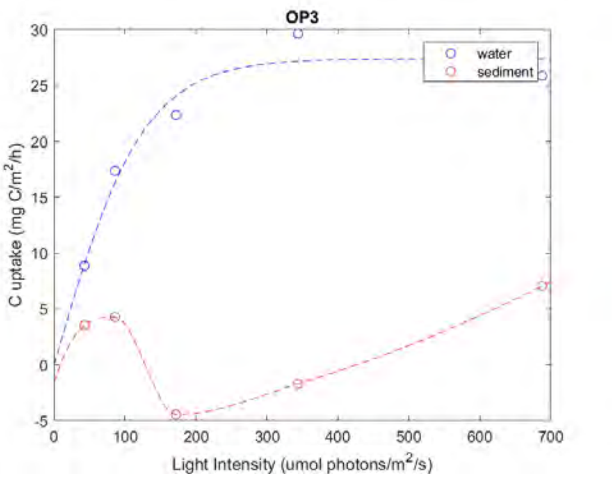

Figure 4.1512. Carbon uptake rate for water and sediment samples in Old Pond 2 on 27th October

A linear regression between diversity and biomass-normalized P_{max} (mg C/mg chl-α/h) was performed and is shown in Figure 4.16. A positive relationship is observed with an R^2 value of 0.76.

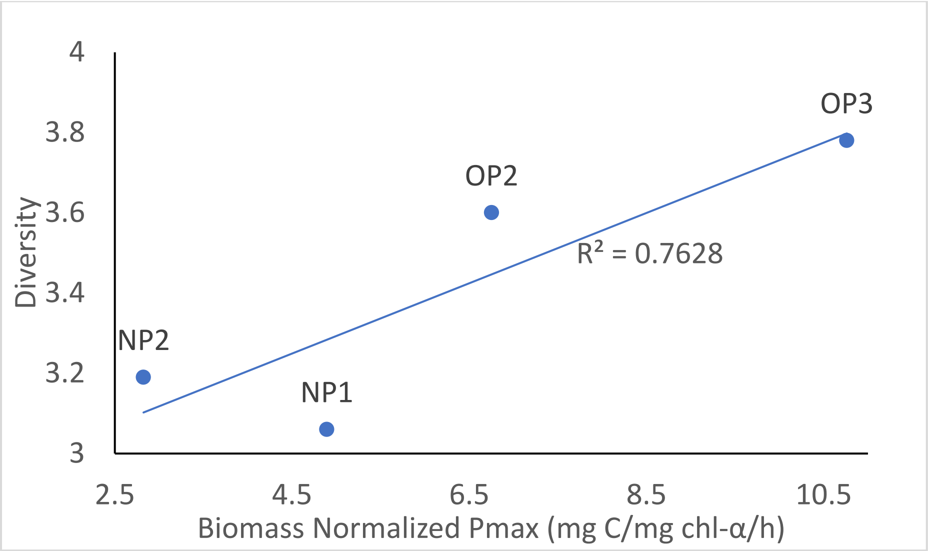

Figure 4.16. Linear regression of Simpson's Reciprocal Diversity Index and Biomass-normalized P_{max} of phytoplankton samples collected on 27th October.

Light attenuation coefficient (*k*) value of both New Ponds 1 and 2 were 2.17. Mean water column irradiance (I_x) was 233µmol photons $m^{-2}s^{-1}$ at New Pond 1 and 546µmol photons $m^{-2}s^{-1}$ at New Pond 2. Hence, neither pond is light limited. Light intensity was not measured at the old ponds as they were very shallow (<0.5m) and thus assumed to be light saturated.

4.4 Discussion

Intermediate Disturbance Hypothesis

Our main goal in sampling the macrophyte species surrounding the ponds on the North Core Banks was to determine how species richness and composition would vary between old and new ponds. We hypothesized that macrophytes at the new ponds would have greater species diversity because the new ponds are exposed to more intermediate disturbances, in both frequency and intensity. Barrier islands, such as North Core Banks, are exposed to severe winter storms as well as hurricanes and tropical storm events. Topography and vegetation are also strongly influenced by overwash events, which provides increased opportunity for species colonization (Stallins et al., 2003). We predicted that the old ponds would be less impacted by these events as they are surrounded by more vegetation and appear behind established dunes, making them better protected. This was supported by Odum, Smith, and Dolan (1987) who observed that the construction of artificial foredunes and increased transverse vegetation zonation led to lower levels of disturbance behind the dune line on the Outer Banks of North Carolina. Thus, we expected the old ponds to experience less frequent and less intense disturbances. Since barrier islands are often wave-dominated, the dunes are commonly short and discontinuous, allowing for barrier flats to develop (Stallins et al., 2003). Barrier flats are flat, low-lying areas that are exposed to the seaward edge of a barrier island and were observed in front of the new ponds on North Core Banks, suggesting that these ponds are not protected by the dunes. This exposure, without the protection of dune formations and their respective vegetation, allows for higher levels of disturbance than at the old ponds (Stallins et al., 2003). Following the Intermediate Disturbance Hypothesis proposed by Connell (1978), we would expect to observe lower diversity at the old ponds if they are in fact experiencing a low amount of disturbance.

The IDH is supported by our collected macrophyte data. As seen in Figure 4.3, the old ponds have greater average plant densities while the new ponds have a more diverse composition of species. A more diverse composition could indicate greater opportunities within this habitat for colonization by a variety of species, in other words, frequent exposure to intermediate disturbances. Species richness and evenness used to calculate Simpson's Reciprocal Index diversity values (1/D), as seen in Figure 4.2, indicated greater diversity at the new ponds as compared to the old ponds as well. Conversely, the diversity index values calculated for the microalgae community did not support the IDH as on average, the old ponds were 19% more diverse than new ponds. This was surprising as Flöder and Sommer (1999) previously tested the applicability of IDH on phytoplankton communities in field site experiments with artificial disturbance and observed that maximum diversity occurred at intermediate interval length of disturbance, supporting the IDH. One potential explanation is the rate of succession of phytoplankton in significantly faster than that of terrestrial plants. Padisak (1992) noted that several months of phytoplankton growth corresponds to decades in grasslands and centuries in forests. Given that, it is possible that the phytoplankton community within the new ponds have re-established their equilibrium after Hurricane Dorian in 2019 while the macrophytes have not, accounting for the similar levels of microalgae diversity at both new and old ponds.

Diversity vs Productivity

We examined microalgae composition using both microscopy and taxa-specific pigments using CHEMTAX. Pigment analysis detected higher proportions of cyanobacteria and chlorophytes than microscopy. On average, cyanobacteria and chlorophytes accounted for 21% of the cells enumerated through microscopy and 54% of the biomass analyzed using CHEMTAX. This type of deviation between microscopy and pigment analysis is well documented (Gong et al., 2020). Diatoms are frequently overrepresented in microscopy-based cell abundance, likely because their large size makes them easily detectable compared to smaller cyanobacteria and chlorophytes. Additionally, familiarity with diatom taxonomy may have contributed further to variations in microalgae composition. Diatoms were the dominant taxa within the microphytobenthos which was expected because pennate diatoms tend to be benthic is nature (Nakov et al., 2015). On the other hand, cyanobacteria and chlorophytes were dominant within the phytoplankton community at all four ponds.

The mean water column irradiance for both New Ponds 1 and 2 fell within the light saturation range (>200 µmol photons m^{-2} s^{-1}). The old ponds were extremely shallow (<0.5m), thus were assumed to be light saturated. As such, we can estimate the productivity of microalgae within the ponds using the maximum productivity rate determined by the productivity-light non-linear regression (Jassby & Platt, 1976). As maximum primary productivity was not obtained for benthic samples during the October 27th incubations, we will only be focusing on the phytoplankton community in our discussion of diversity and productivity. The variation in microphytobenthos productivity was likely the result of some methods error which will be discussed in more detail in section 4.3.

Microalgae diversity was determined using microscopic analysis and is described in Figure 4.4. Phytoplankton diversity was 19% higher in the old ponds than the new ponds. With that in mind, we expected phytoplankton productivity to be higher in the old ponds as well. Because the overall phytoplankton productivity is dependent on the total amount of phytoplankton present, it is important to account for the phytoplankton biomass. As such, P_{max} of phytoplankton samples collected on 27th October were biomass-normalized and plotted against diversity. Figure 4.16 demonstrates that there is a positive relationship between diversity and biomass-normalized P_{max}, supporting our hypothesis that increased diversity can result in higher productivity.

Improvements and Recommendations

The pond incubations allowed sediment and water productivity to be separated and compared. However, sediment samples did not follow the expected photosynthesis-light curve (Jassby & Platt, 1976) making it difficult to estimate the maximum productivity within these samples. The erratic variations may be due to chemoautotrophic activity within the dark and low-light samples (Boschker et al., 2014). However, previous studies have described microphytobenthos productivity increasing with light availability, following the same trends as the expected photosynthesis-light curve (Jacobs et al., 2021). Additionally, the lack of such variations within the photosynthetron incubations indicate that the results are more likely due to experimental errors instead of chemoautotrophic activity. In performing their ^{14}C incubations Jacobs et al. (2021) mixed 2mL of sediment with 75mL of What-man© GF/F-filtered seawater from the same location. This allowed them to directly measure the productivity of the microphytobenthos as they removed any phytoplankton present in the water samples. Should the primary productivity incubations be repeated, it would be prudent to filter water from the respective ponds to create a sediment slurry as described by Jacobs et al. (2021) as this would remove confounding factors caused by combining phytoplankton and microphytobenthos assemblages.

It is also important to note that while New Pond 1 had the highest productivity of the four ponds, New Pond 2 had the lowest productivity. As such, as the distinction of a pond as “new” or “old” may not be a strong predictor of phytoplankton productivity. This is also observed in the microalgae composition (Table 4.1) and biomass (Table 4.2) where New Pond 2 was more similar to both old ponds than New Pond 1. Thus, we must be wary of assuming that all new ponds behave similarly when evaluating the ecological function of the ponds on North Core Banks. Our preliminary results indicate differences between the ponds we surveyed in terms of biomass, diversity and productivity. However, to fully elucidate how these ponds will evolve over time, more monitoring should be implemented. We recommend that surveys be performed seasonally (at least 4 times a year) to understand how primary productivity varies according to seasonal changes. This will also help establish a baseline of the communities and allow us to evaluate how the ponds respond during and after disturbances such as hurricanes. Overall, it is evident that the primary producers in these habitats supply energy throughout multiple trophic levels. Therefore, given the significance of these ecosystem services, it is important to continue monitoring these ponds.

Chapter 5: Benthic Invertebrates

Benthic Invertebrate Assemblages Found in Newly Formed Ponds at Core Banks, NC

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University of North Carolina at Chapel Hill

Fall 2021

5.1. Introduction

Invertebrates such as polychaetes, oligochaetes, molluscs, and crustaceans, are common in marsh and saline environments (Radler, 1984). These organisms form the base of benthic food webs and as such, are an important part of the community structure of these environments. For example, the common marsh fish *Fundulus heteroclitus* eats small crustaceans and polychaetes (Kneib and Stiven 1978). Polychaetes, in particular, can be critical for the distribution of shore birds including threatened species such as the Piping Plover and Red Knot (SC Department of Natural Resources, 2015).

Benthic invertebrate assemblages are influenced by physical factors of the habitat as well as stochastic or cyclic disturbances (Kneib, 1984). Salinity, sediment structure, depth, and temperature also influence the distribution and abundance of benthic invertebrates such as polychaetes (Hutchings, 1998). The new ponds at Core Banks formed after hurricane Dorian, thus represents a habitat that may have unpredictable factors and disturbances. The new ponds may change over time under different conditions, and changing water levels have already been observed in the ponds during our study in the Fall of 2021 (Ch 2).

The diversity and abundance of benthic invertebrates serves as an important measure of habitat quality for other members of the community. Highly disturbed systems tend to have low diversity of benthic organisms consisting mostly of small generalist species that have fast population growth. More established ecosystems tend to have higher diversity and more species that have more complex life cycles and feeding habits. Understanding what types of benthic invertebrates exist in the new ponds of North Core Banks is important for understanding the overall community structure and food chain, and provides insight into how the island ecosystem may change as the new ponds change or disappear.

This study aims to provide quantitative evidence of the biodiversity and distribution of benthic invertebrates in different newly formed ponds across Core Banks. The diversity and abundance of benthic invertebrates was measured at six new pond sites. Size, depth, salinity, sediment grain size, and degree of connectivity to marsh was also measured for each pond to relate to the abundance and diversity of benthic invertebrates. The goal of this study is to determine the community structure of benthic invertebrates in the new ponds of North Core Banks and the abiotic factors contributing to their abundance and diversity. We hypothesized that salinity would be the major factor affecting diversity of benthic invertebrates, while depth will have the largest impact on the abundance of invertebrates. We also hypothesized that ponds with greater connectivity to salt marsh would have a higher abundance of benthic invertebrates as nutrients from the salt marsh would enhance productivity and boost food availability for invertebrate communities. Results of this study may be used to better understand the structure of the benthic invertebrate community present on North Core Banks and how this community supports the new ponds’ ecological role on the island.

5.2. Methods

We selected 6 new ponds located across North Core Banks with varying depths, sizes, and marsh connectivity (Figure 5.1.).

Figure 5.1. Aerial photo of pond sites sampled on Core Banks.

Ponds were sampled either 2 or 3 times due to time constraints. Multiple sampling allowed us to account for differences in environmental parameters and to increase sample sizes. To compare the physical factors of each new pond, we measured the perimeter and area, the amount of perimeter connected to marsh, the maximum depth, and the salinity of each pond. Depth of New Ponds 1, 2, and 3 were averaged across time within each pond. All depths were taken throughout each pond from a fish finder attached to a kayak by the Geomorphology group (Ch 2). New Ponds 1.5, 2.5, and 2.75 depth measurements were not measured due to lack of time. For each sampling day, we took salinity measurements at a shallow site and a deep site of each pond using a ProSolo digital water quality meter YSI. These salinities were averaged per pond, and the maximum and minimum salinities for each pond were taken as well.

Perimeter and area dimensions of the new ponds were collected via drone imagery from the Geomorphology group (Chapter 2). This imagery was used to calculate the perimeter of each pond and the perimeter that is connected to marsh. They were mapped in Google Earth Pro with .tiff images using the path tool (Figure 5.2).

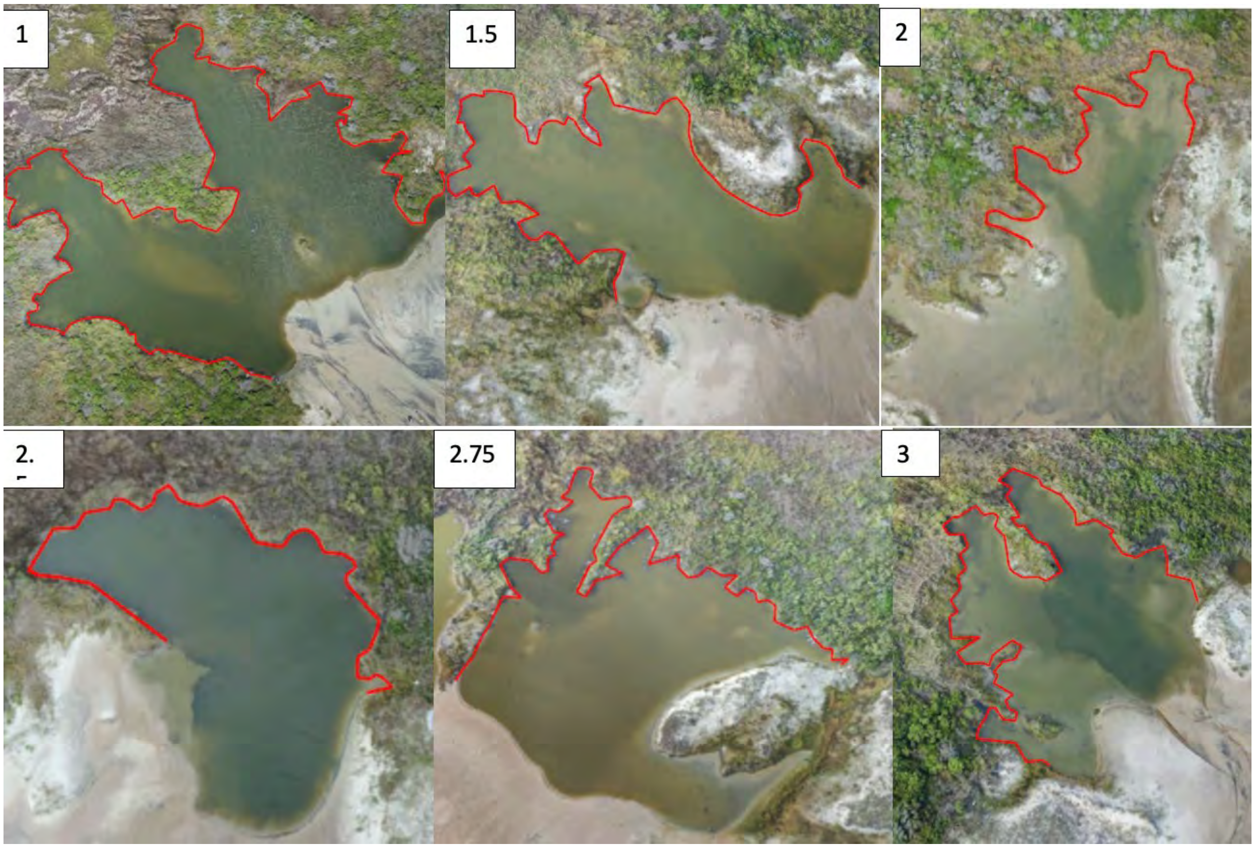


Figure 5.2. Pond Perimeters: Marsh Connectivity mapped via path tool on Google Earth Pro.

For our research we investigated infaunal and macroinvertebrate assemblage community composition, abundance, and diversity. Infaunal benthic invertebrates can be categorized as macrofauna, which are organisms retained on a 500 μm mesh sieve, and microfauna, which will pass through a 500 μm sieve but are retained on smaller mesh sizes (Kneib, 1984). We sampled the macrofauna by sieving through 500 μm mesh, and hand sorting the remaining invertebrates (Kneib, 1984, Radler, 1984). For our research we followed this method of coring using a 15.5 cm diameter core and sieving through a 500 μm sieve, and hand sorting the remaining invertebrates. Core samples were taken from 3 transects at each pond, with 2 cores per transect, one in a shallow area and one in a deep area. Depth measurements were taken at every coring site. Collected invertebrates were measured, recorded, and stored in a plastic bag on ice for later identification. We identified our collected samples to the lowest possible taxonomic level, typically family, with the help of online searches and benthic invertebrate identification books (Brinkhurst, 1971; Friese, 1973).

Sediment samples were also taken from a shallow and deep site at each pond. Depth measurements of each sample site were taken. Samples were collected by inserting an open-faced 100mL syringe into the sediment, similar to coring methods. Samples were stored in plastic bags on ice for later in-lab evaluation. Sediment grain size was measured in a CILAS 1180 Liquid laser diffraction particle size analyzer.

Minnow traps were used to sample larger macroinvertebrates in partnership with fish sampling (Chapter 6). Four minnow traps baited with 6 pieces of dog food were placed in each pond for a minimum of 30 minutes. Two traps were in a shallow area and two were in a deep area, each with measured depth. Any invertebrates captured were identified, measured, recorded, and released. In collaboration with the fish sampling, the ponds were each seined and any invertebrates caught were identified, measured, recorded, and released.

Data Analysis

We calculated the Shannon-Wiener Diversity Index ($H = -\sum[(p_i) * \ln(p_i)]$) for each date and pond sampled. For these analyses we used family identifications instead of species. For visualization and analysis, we averaged the diversity index values for each pond across all dates. In addition, we calculated and averaged family richness, the number of individuals, and family evenness (Table 5.2.).

Percentages were calculated for marsh connectivity using excel and dividing the meters of connectivity by the total perimeter. This was calculated for each pond so we could compare. An important note is that New Pond 2 grew and shrank in size multiple times during sampling visits. Perimeters were taken each time, but we averaged the numbers for our data analysis purposes.

5.3. Results

Depth

In the deep and shallow samples from the transects and traps, the results are nearly equal (Figure 5.3). In the deep cores, 69 individuals were found, and in the shallow cores, 68 individuals were found.

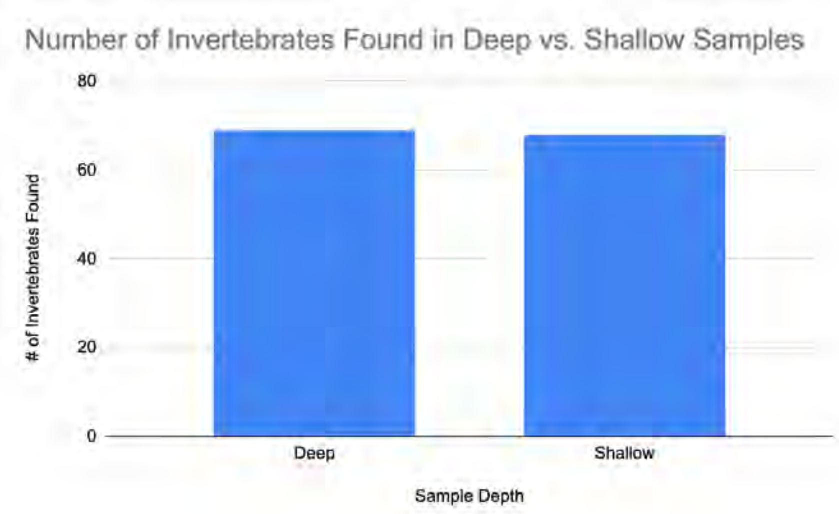


Figure 5.3. Depth Abundances Based on Coring Location

As depth increases, average marine worm abundance does not appear to be related, with an R^^{2} value of 0.021(Figure 5.4).

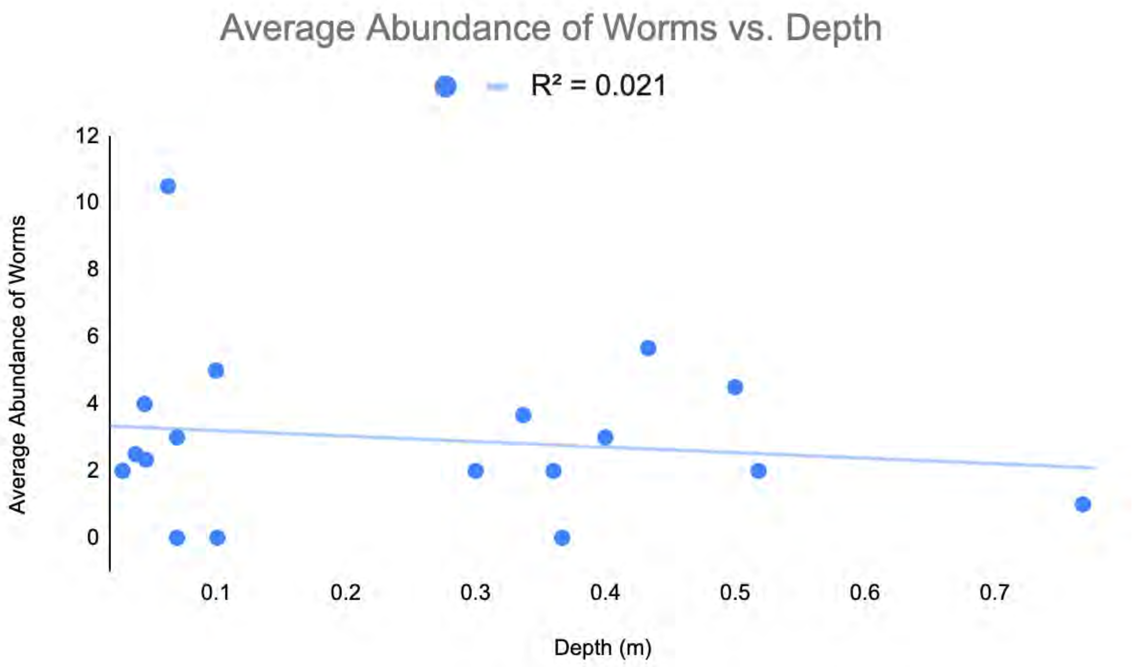


Figure 5.4. Average Abundance of Worms as a function of Depth

Abundance

In the new ponds, 4 families of invertebrates were found: *Naididae, Nereididae, Penaeidae*, and *Portunidae.* A category added in our data analysis is "Unidentified" for marine worms that we could not classify. Two families of marine worms, the *Naididae* (oligochaetes) and *Nereididae* (polychaetes), were the most abundant organisms sampled. However, across ponds this differed. At New Pond 2, no marine worms were found, but both *Penaeidae* (shrimp) and *Portunidae* (crab) were found. *Portunidae* was found at every pond sampled while *Penaeidae* was found only at New Ponds 2 and 2.5 (Figure 5.6). New Pond 2 and 2.5 also had the lowest overall invertebrate abundance (Figure 5.5). The highest average abundance was found in New Pond 2.75 with an average of 31.5 individuals (Table 5.1).

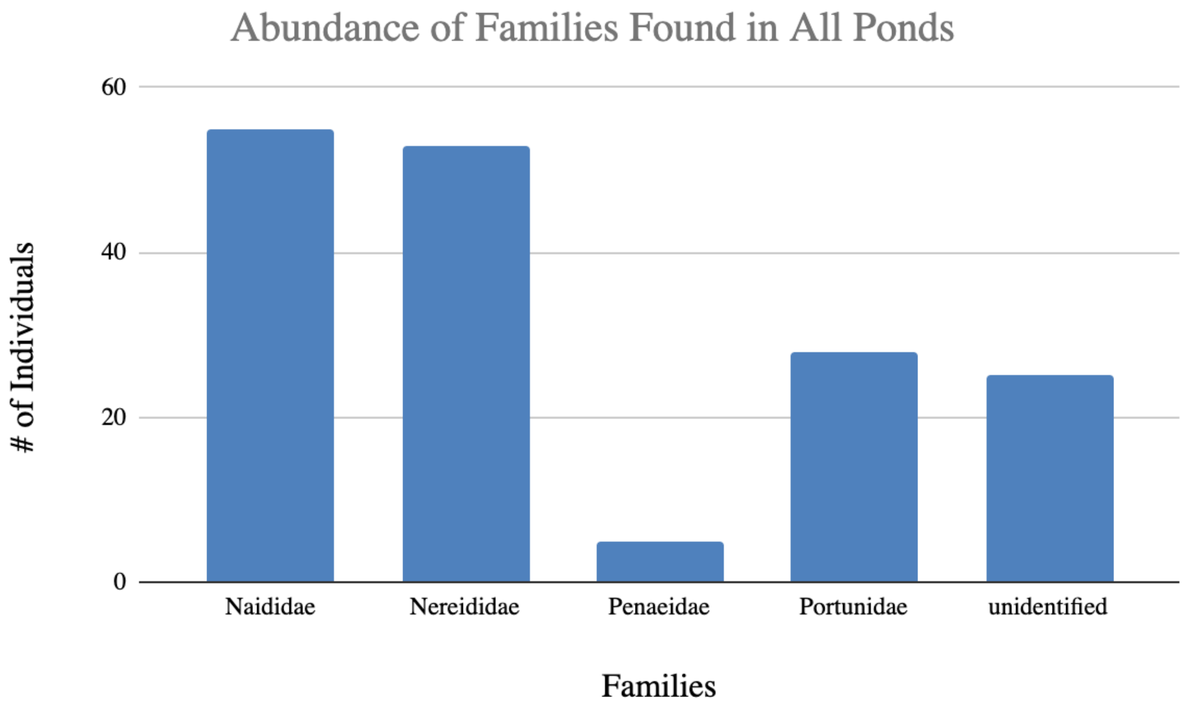


Figure 5.5. Abundance (number of individuals) by Family Found in All New Ponds

Table 5.**1.** Average Number of Total Individual Benthic Invertebrates per New Pond

Data type	Pond 1	Pond 1.5	Pond 2	Pond 2.5	Pond 2.75	Pond 3
Average # of Individuals	13	9.5	4	9.5	31.5	9.3
Standard Deviation	4.24	0.71	1	2.12	17.68	11.02

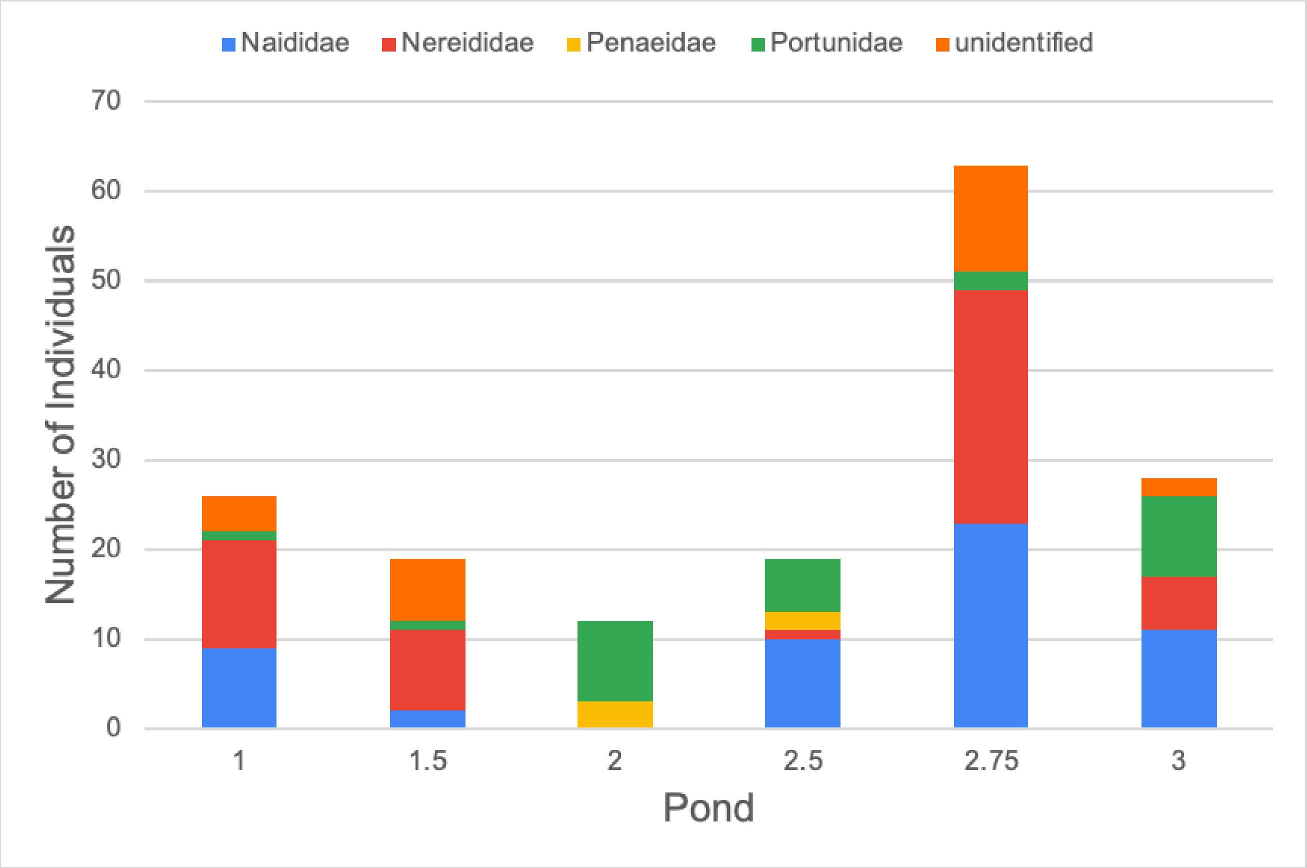


Figure 5.6. Abundance of Benthic Invertebrates by Family Across New Ponds

As the average abundance of marine worms increases, the average abundance of crustaceans decreases (Figure 5.7). The figure shows an exponential line of best fit. Using the TTEST formula on google sheets, the p-value is calculated as 0.068.

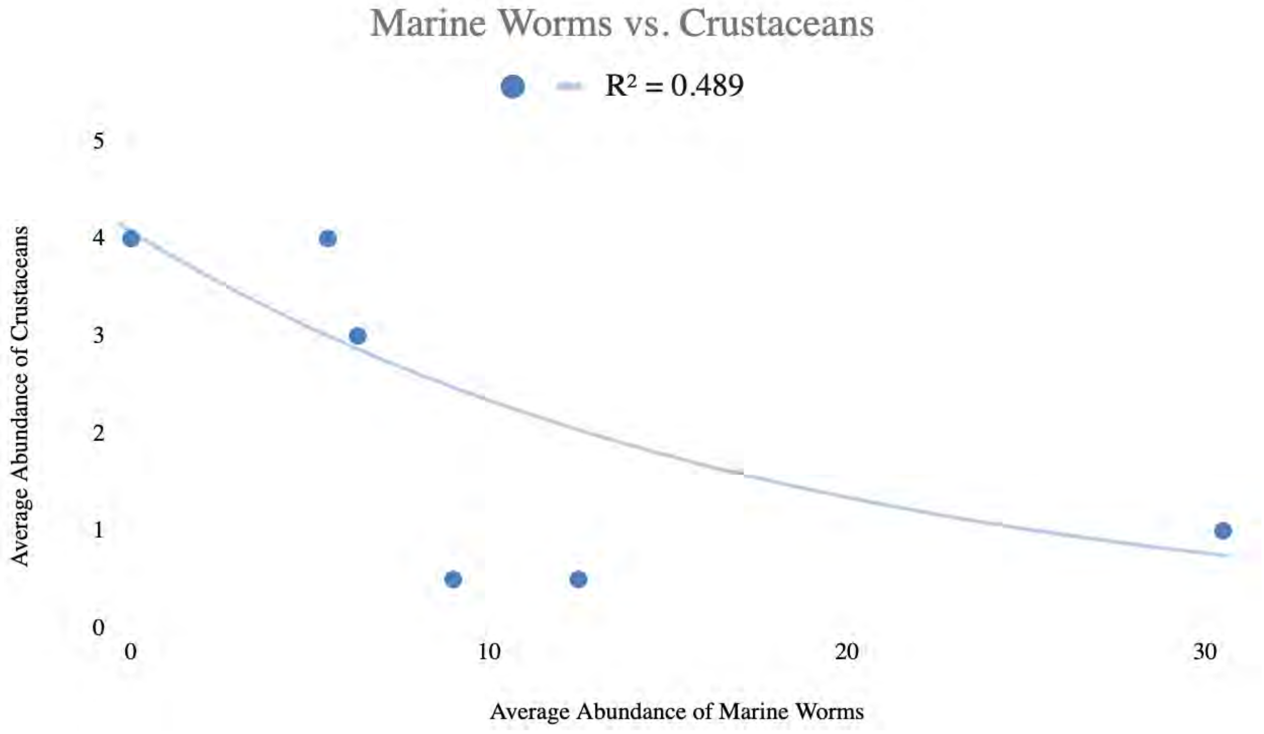


Figure 5.7. Plot of Abundance of Marine Worms versus Abundance of Crustaceans Found within New Ponds

Diversity

New Pond 2 had the lowest diversity index across all the ponds (Figure 5.8). New pond 1 featured the highest diversity index (Figure 5.8). New Ponds 1.5, 2.5, and 3 had relatively similar diversity indices.

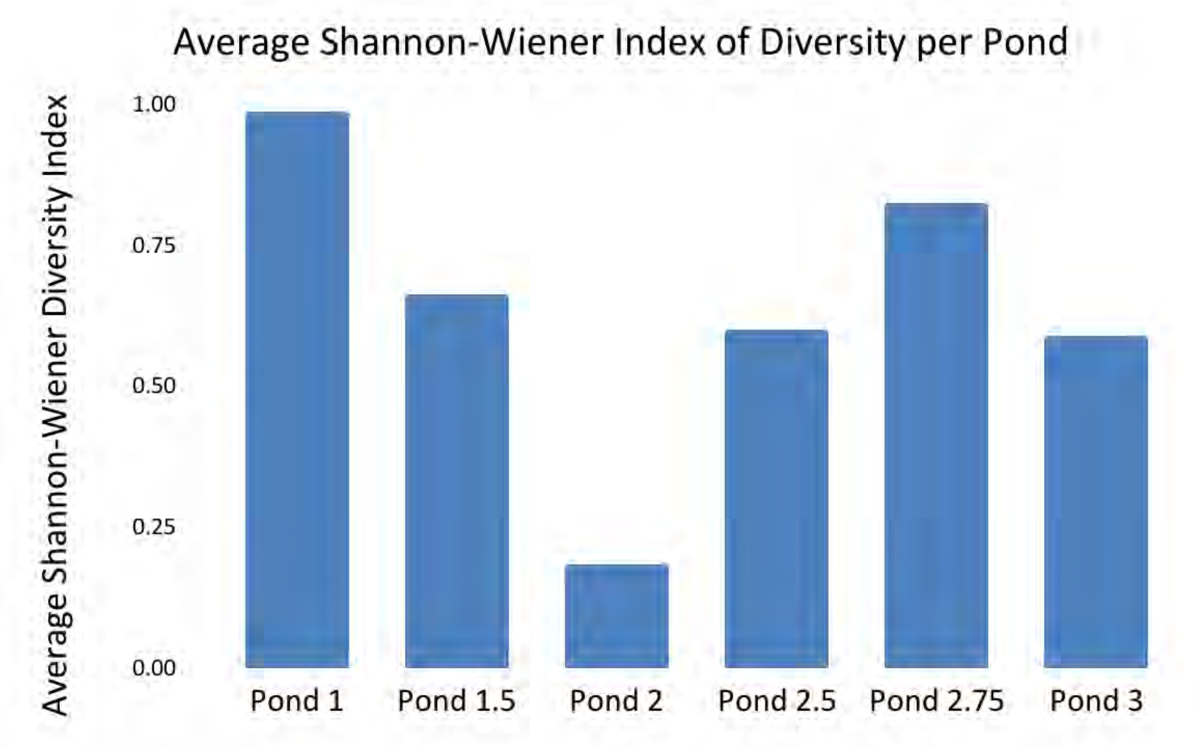


Figure 5.8. Averaged Shannon-Wiener Index of Diversity Across New Ponds

Table 5.2. Average Diversity Metrics across New Ponds

Data type	Pond 1	Pond 1.5	Pond 2	Pond 2.5	Pond 2.75	Pond 3
Average Shannon Wiener Index of Diversity	.99	.67	.19	.60	.83	.59

Diversity and Abundance Related to Environmental Parameters

Comparing averages across new ponds from all sampling days, New Pond 1.5 had the largest area, and New Pond 1 had the second largest area in square meters (Table 5.3.). New Pond 2.5 had the smallest average area in square meters. New Ponds 1 and 1.5 also had the largest perimeters (Table 5.3.).

Table 5.3. Size, depth, salinity, and marsh connectivity measurements for all ponds

Pond	Average Area (m^2)	Perimeter (m)	Average Salinity (psu)	Standard Deviation Salinity (psu)	Marsh Connectivity (m)
1	3870.70	520.61	25.23	2.94	449.61

1.5	5474.10	444.88	19.47	3.41	349.88
2	1884.60	390.00	28.44	7.35	149.67
2.5	1209.90	171.21	21.61	5.10	112.21
2.75	1521.00	395.40	19.95	1.66	307.76
3	1560.80	287.42	21.22	10.49	228.42

Salinity

For salinities, we again averaged all measurements taken in deep and shallow areas of the ponds, as well as accounting for the maximum and minimum salinity measurements taken throughout our sampling days (Figure 5.9).

New Pond 1 had the highest minimum of all ponds and New Pond 2 had the highest maximum salinity (Figure 5.9). New Pond 1.5 had the lowest average salinity while New Pond 2 had the highest average salinity (Table 5.3). New Pond 2.75 had the lowest maximum, and New Pond 3 had the lowest minimum (Figure 5.9). New Pond 2.75 had the least variability in salinity and New Pond 3 had the most variability (Figure 5.9). New Pond 2 also showed a large amount of variability in salinity.

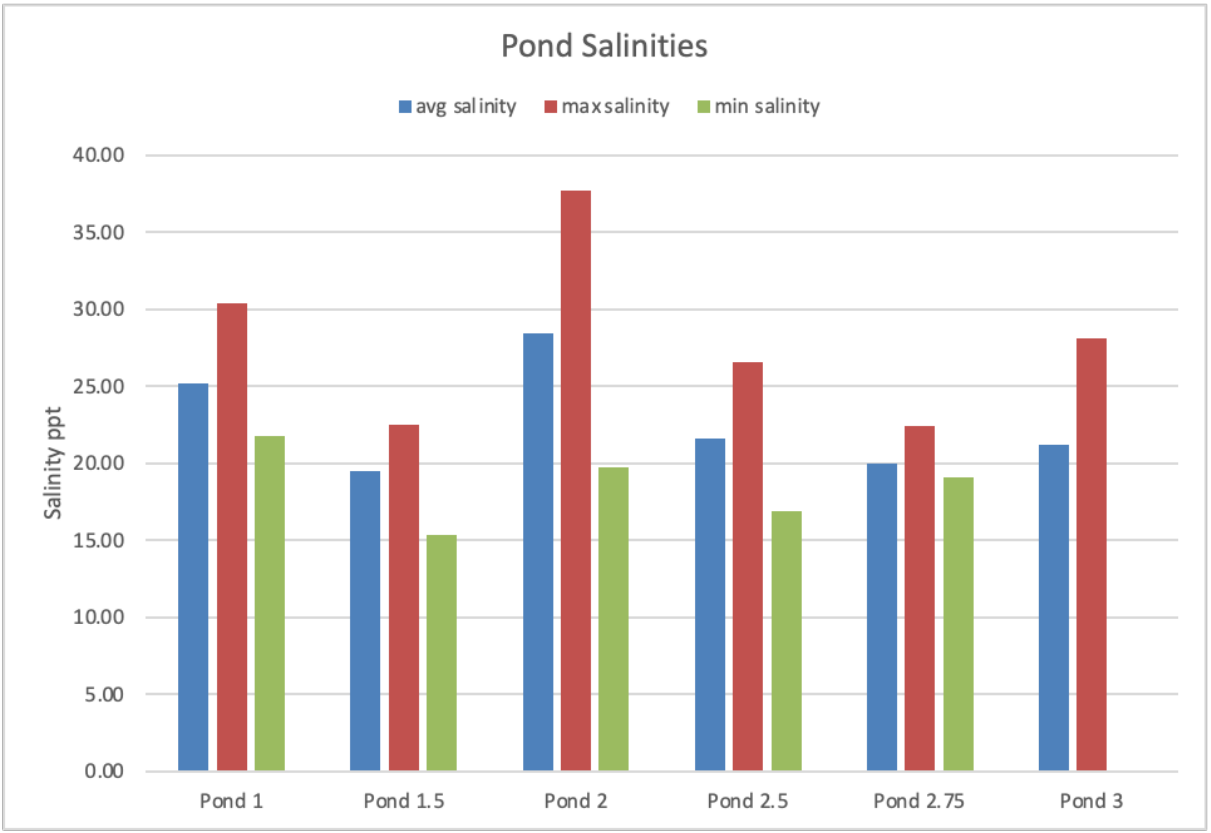


Figure 5.9. Maximum, minimum, and average salinity measurements per pond

The average abundance of worms was not significantly related to salinity (Figure 5.10). The highest average abundance of worms was found at a salinity of 19.05ppt.

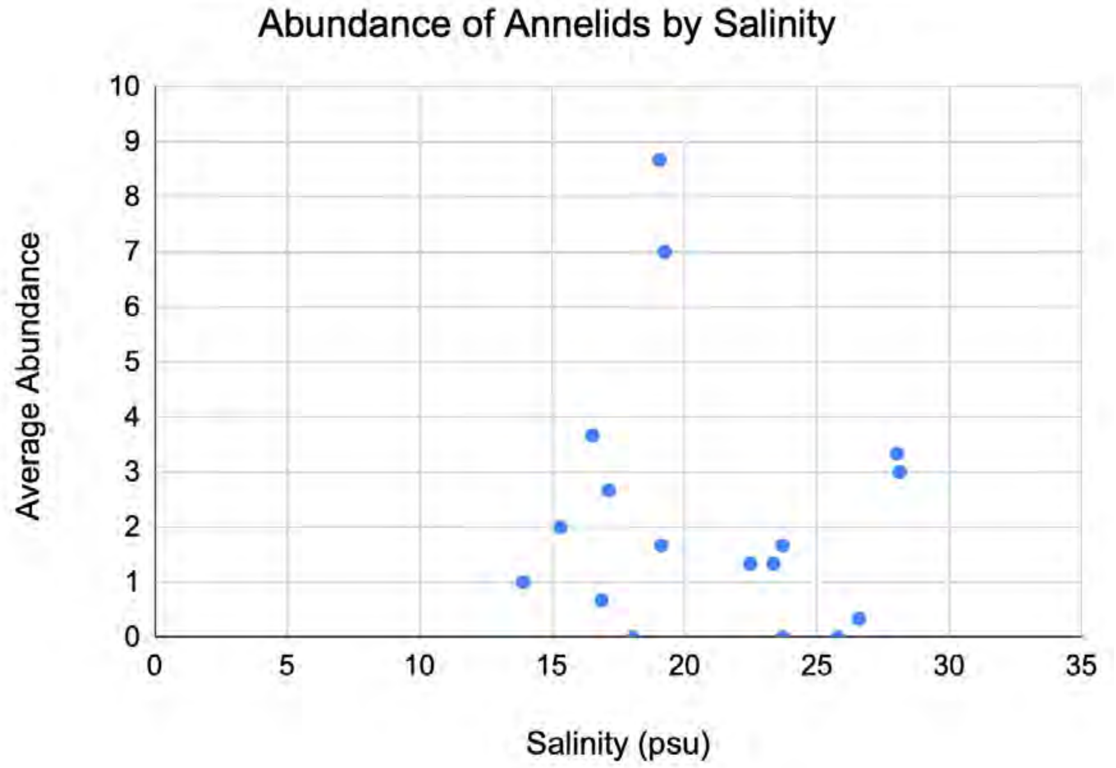


Figure 5.10. Abundance of Worms versus Pond Salinity

The amount of crustaceans found by traps was not related to salinity, as there was only one event where crustaceans were caught by traps. Crustaceans caught by the seine suggested that there was a weak positive relationship between crustacean abundance and salinity, but these relationships were not statistically different (Figure 5.11). All crustaceans were found between 15 and 27 ppt (Figure 5.11).

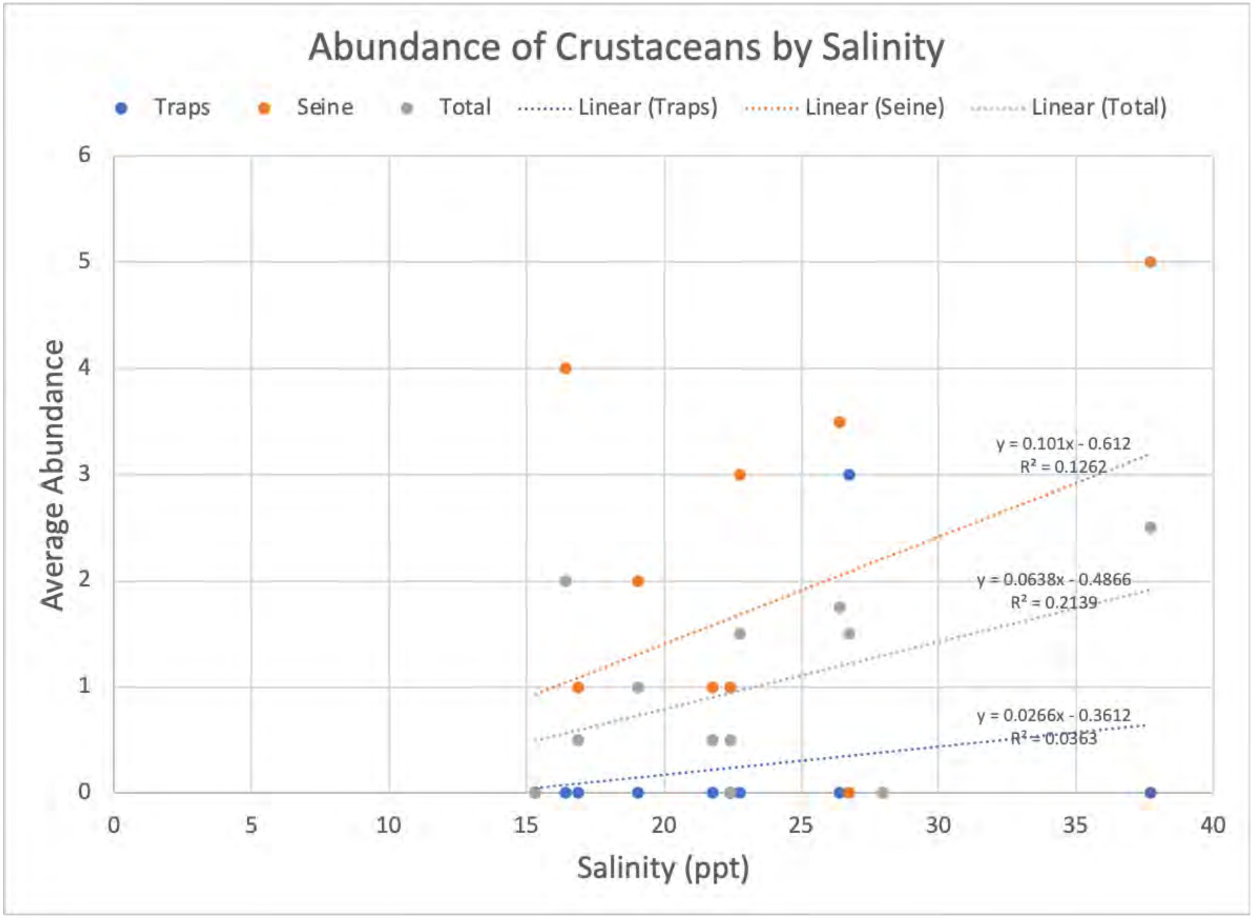


Figure 5.11. Average of Abundance of Crustaceans vs Salinity based on catch method

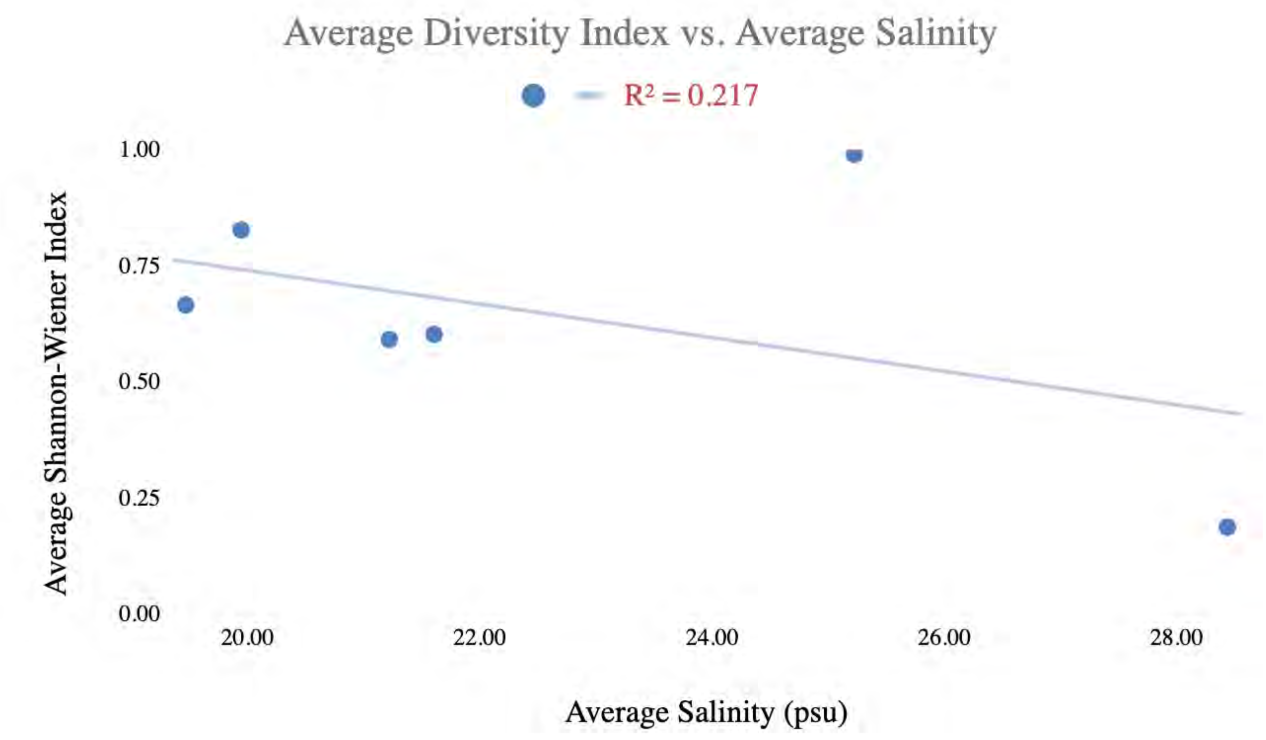
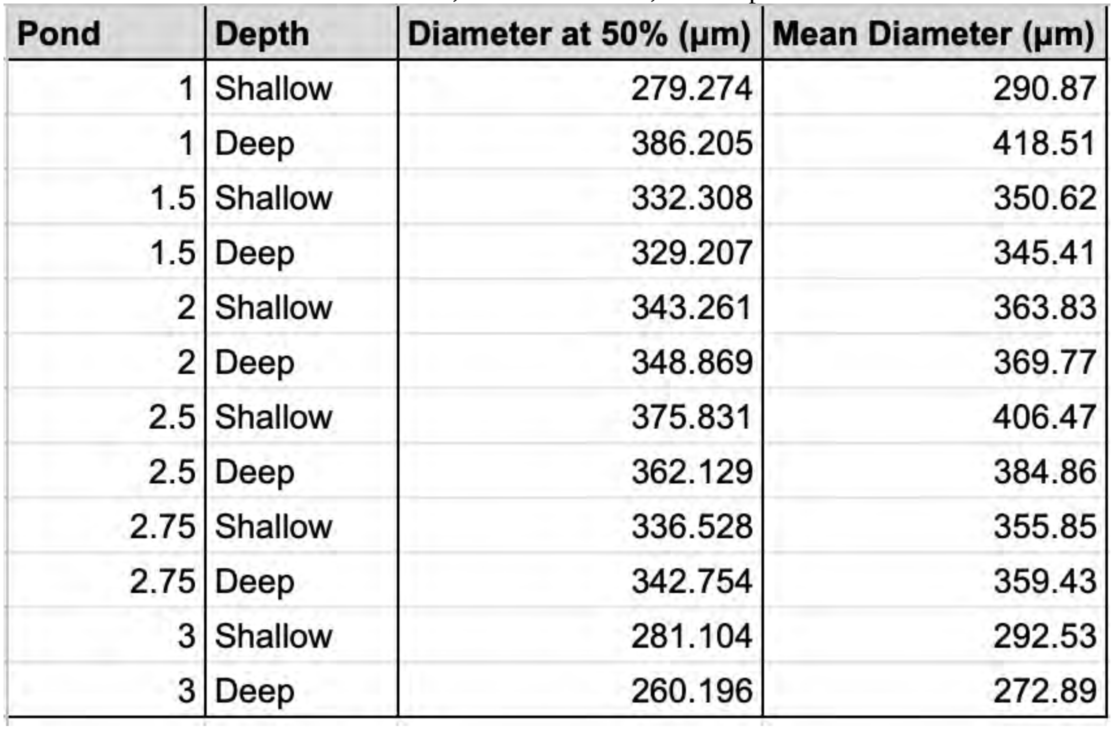


Figure 5.12. Diversity Index vs. Salinity

Sediment Grain Size

Sediment grain sizes are all roughly 300 to 400 microns, falling into the sand size category. The shallow and deep sediment samples at each new pond did not show a significant difference between mean diameter and median diameter (Table 5.4.). Between all ponds, the mean and median diameters were similar. Each pond had relatively similar grain size at the shallow and deep stations by transect (Figure 5.13).

Table 5.4. Sediment Grain Size: Pond, Mean Diameter, and Depth



Pond	Depth	Diameter at 50% (µm)	Mean Diameter (µm)
1	Shallow	279.274	290.87
1	Deep	386.205	418.51
1.5	Shallow	332.308	350.62
1.5	Deep	329.207	345.41
2	Shallow	343.261	363.83
2	Deep	348.869	369.77
2.5	Shallow	375.831	406.47
2.5	Deep	362.129	384.86
2.75	Shallow	336.528	355.85
2.75	Deep	342.754	359.43
3	Shallow	281.104	292.53
3	Deep	260.196	272.89

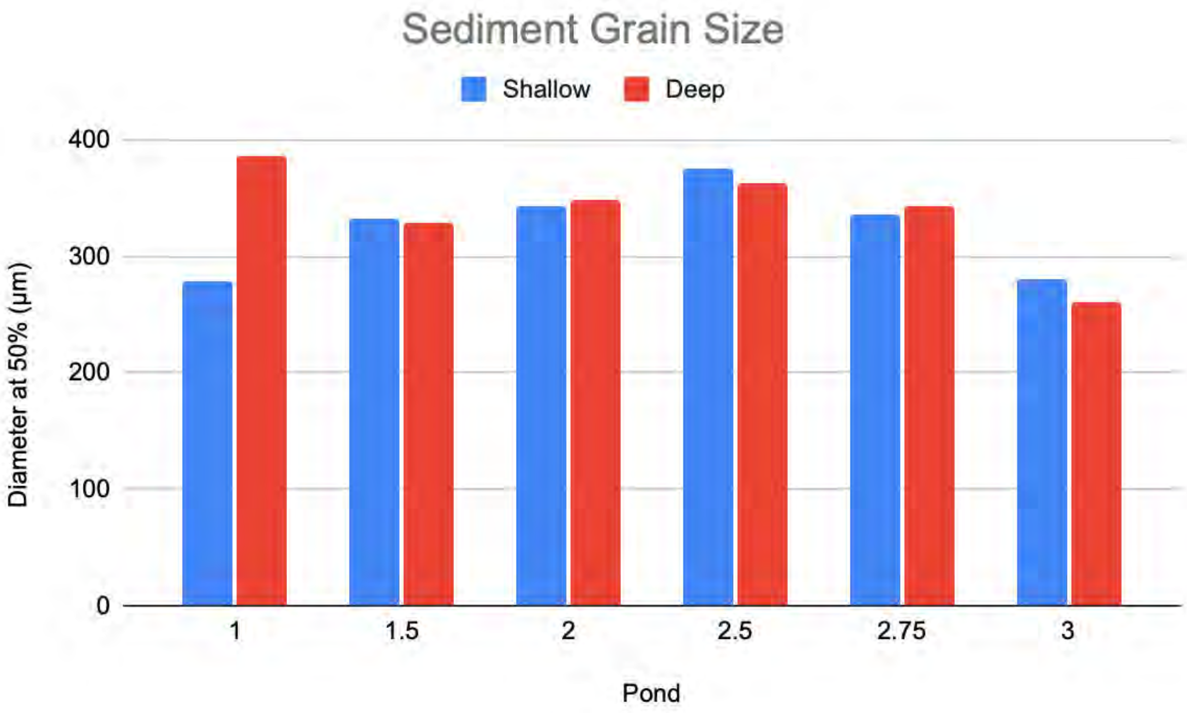


Figure 5.13. Sediment Grain Size in Shallow and Deep Sample Areas

Marsh Connectivity

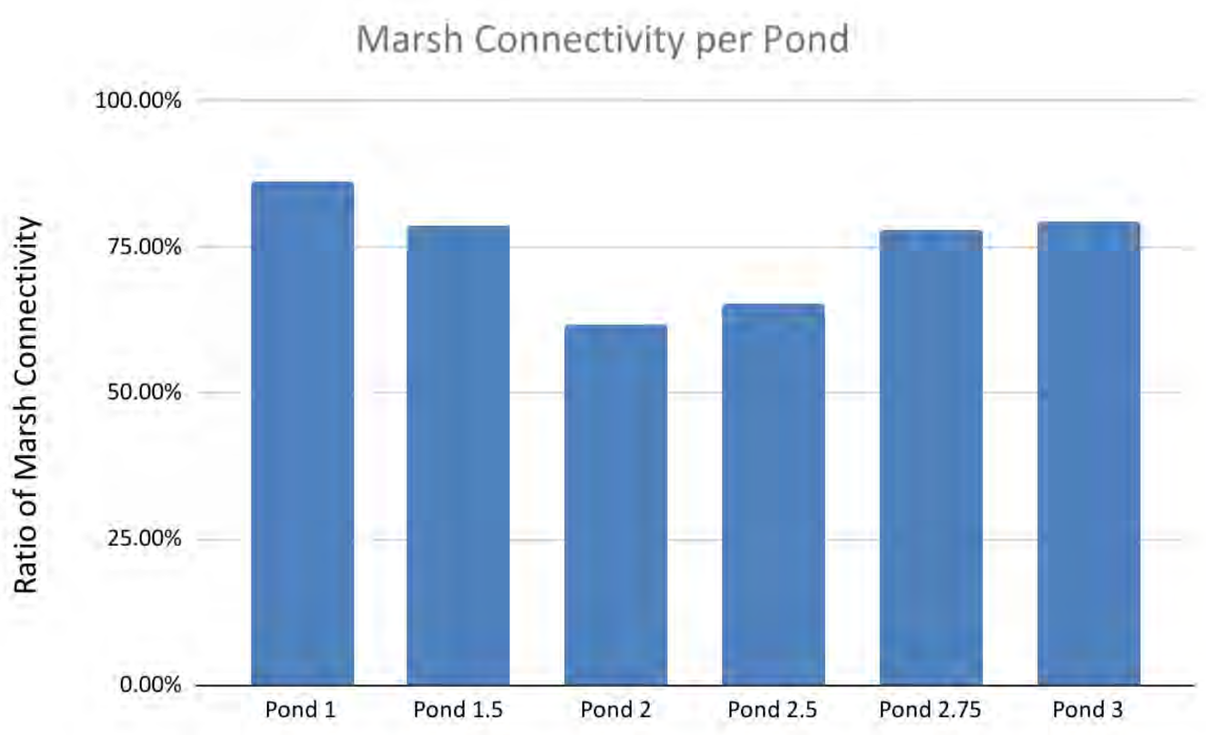


Figure 5.14. Percent Marsh Connectivity per New Pond

New Pond 1 had the highest connectivity to marsh, while New Pond 2 had the lowest. A strong relationship was found between the diversity index and the ratio of marsh connectivity. In addition, the relationship between abundance of individuals and the ratio of marsh connectivity was positive as well, but not as strong (Figure 5.16).

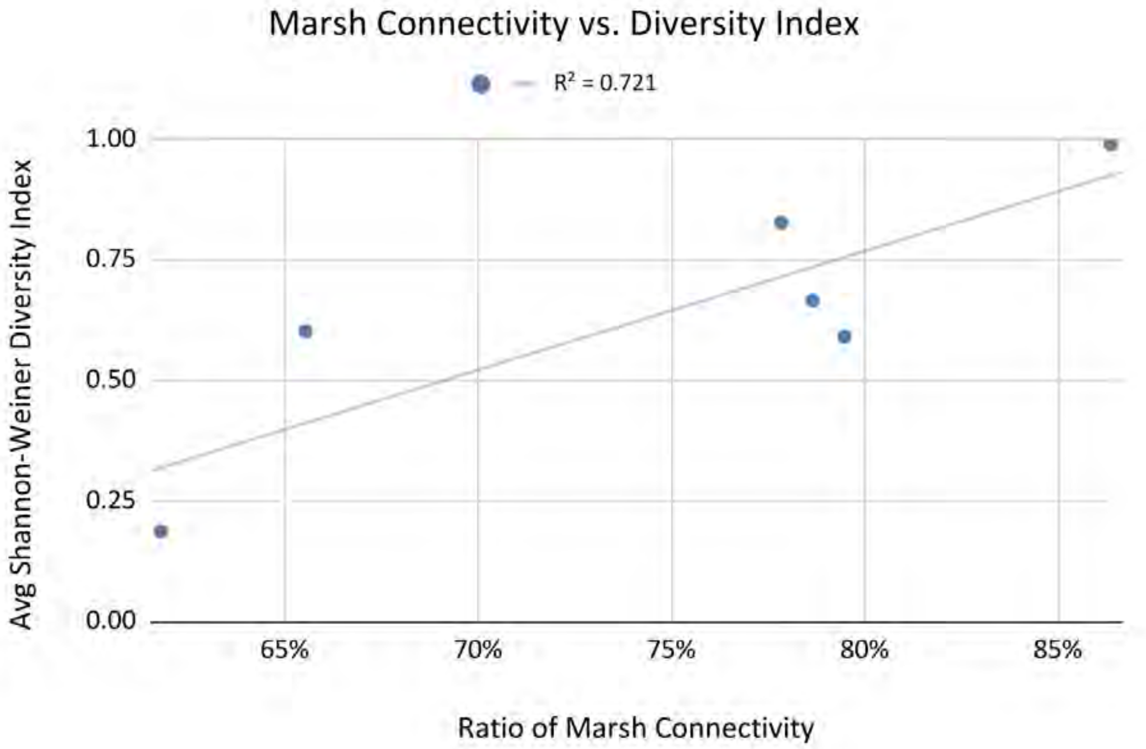


Figure 5.15. Ratio of Marsh Connectivity against Diversity

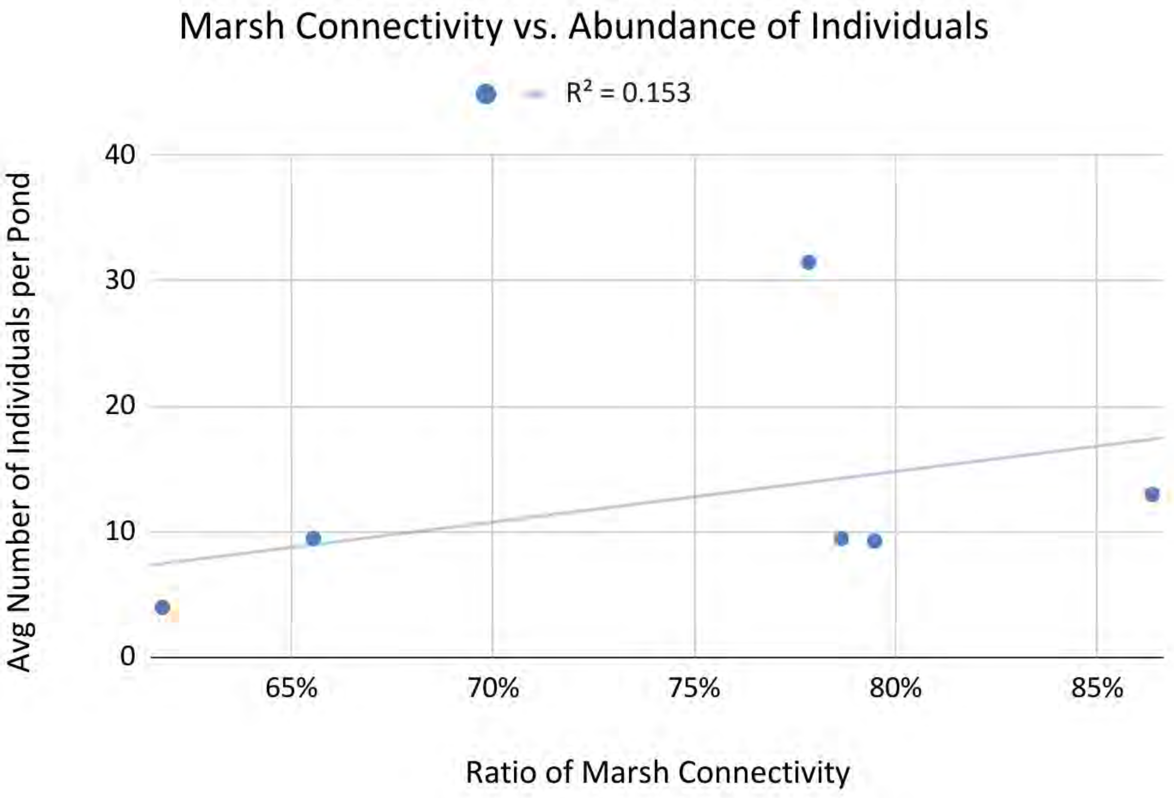


Figure 5.16. Ratio of Marsh Connectivity vs. Abundance

5.4. Discussion

Our results illustrate large variations within new ponds that were not explained by abiotic factors, but rather by marsh connectivity. Consistently, New Pond 2 proved to have the lowest Shannon-Weiner Diversity Index while New Pond 1 and 2.75 had the highest diversity. New pond 2.75 hosted the most individuals on average. It had almost three times as many organisms compared to 4 of the 6 new ponds. In addition, New Pond 2.75 had the second highest Shannon-Wiener index of diversity (0.83). Excitingly, New Pond 2.75 had the highest percent of marsh connectivity. To add to this, New Pond 2 had the lowest diversity, interestingly, since that pond had the lowest abundance as well. There was a strong, positive relation between average Shannon-Wiener Diversity indices and ratio of marsh connectivity. This indicates that marsh connectivity is the most impactful parameter determining benthic invertebrate diversity. The more marsh connectivity a pond in relation to its perimeter, the more likely it is to have a high diversity index.

The family with the overall lowest abundance is *Penaelidae.* All *Penaelidae* and *Portunidae* found in the new ponds were juveniles, which may indicate that the new ponds serve as a nursery habitat.

Across all new ponds, *Naididae* and *Nerididae* had similar abundances, indicating one is not dominating and that competition is likely low. In addition, we found a moderately strong, negative relationship between the abundance of marine worms and the abundance of crustaceans. As more marine worms were found, less crustaceans were found.

Depth had no impact on the abundance of marine worms found. There was a large variability in salinity, but salinity was not strongly related with abundance or diversity of invertebrates. Sediment grain size did not vary greatly between new ponds and can also be ruled out as a factor in the distribution of benthic invertebrates on Core Banks. Sediment grain size did not vary significantly between shallow and deep sites within each new pond and overall was not significantly different across all ponds. All sediment grains were in range with sand grain sizes.

Across each of the new ponds, the abundance of macroinfauna found was very low. Densities of macroinfauna across sediment cores ranged from 0.004 per cm^2 to 0.011 cm^2 per surface area. Compared to similar studies this is extremely low. In a study from salt marsh areas of Tar Landing Bay in Morehead City, densities of macrofauna ranged from 0.026 per cm^2 surface area, which was described as extremely low, to 11.95 per cm^2 (Rader, 1984).

A previous study found that most benthic invertebrates sampled were food in silt-clay fractions of sediment (Johnson et. al, 2007). In addition, a recent study concludes that increasing particle size corresponds with a decreased abundance of benthic invertebrates (Huotari, 2015). Sand is a larger grain size than silt and clay, which indicates the low overall abundance of invertebrates found.

For the new ponds, there was no evidence of carbon sequestration and nutrient biomass in the new ponds (Chapter 3). This lack of nutrients and organic matter would make sense as the new ponds' sediment structure is composed entirely of sand. Sand particles tend to have a large chunky structure and have a small surface area relative to their mass, meaning they do not hold on to nutrients well (Crouse, 2018). A lack of organic material suggests there is little substrate to fuel the benthic food web.

The infaunal invertebrates we did find were mostly small, indicating they may be early colonizing species. Due to the variability of conditions and abiotic factors in these newly formed ponds, it is likely that infaunal populations have not yet been able to strongly establish themselves here. Physical disturbances such as tidal movements, storms, heavy rainfall, and overwash events may dislodge (and transport) species that live on or under the sediment surface (Kneib, 1984). The new ponds were formed from hurricane Dorian and have experienced disturbances such as overwash events and water surface level changes (Chapter 2). These events may mean that benthic invertebrates, particularly infaunal invertebrates, have not yet been able to successfully establish populations in these, indicating why we found such a small abundance of invertebrates.

Confounding factors may have influenced the findings in this study. These include the worms splitting into different pieces when picked up and extracting from the sieve was difficult. However, our abundances were so low that these likely did not heavily influence results. The benthic food web may also be dominated by much smaller meiofauna that we did not sample such as nematodes and ostracods.

5.5. Conclusion

Marsh connectivity is the main abiotic factor correlated with benthic invertebrate diversity. Protecting these areas and ensuring the marsh stays intact will benefit the benthic organisms living in the new ponds. The current diversity and abundance of macrofauna was relatively low. According to other studies cited, the typical number of marine worms we found per unit area is much lower than previously studied productive systems. It is unknown why, but potential reasons could be the new pond's early states of succession. The organisms we did find were largely comprised of juveniles and small individuals, indicating we may be seeing early colonizing species and newly establishing populations. As the new ponds age, we could expect an increase in diversity, abundance and size of benthic invertebrates in a few years. Yet, due to the amount of benthic invertebrates we did find, we expect most new ponds to provide habitat and food for other creatures such as shorebirds, that may prey on marine worms. In addition, the presence of juvenile shrimp and blue crabs lead us to suspect the new ponds serve as nursery habitats for these growing invertebrates. Since only juveniles were accounted for, this is evidence to suggest management efforts to protect these new ponds would be beneficial. In order to understand the scope of the food web within these ponds, future research should sample microinvertebrates, which was beyond the scope of this study.

Chapter 6: Fishes

Fish Diversity and Biomass in Ponds at Core Banks, NC

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Fall 2021

6.1. Introduction

In August and September of 2019, Hurricane Dorian impacted the coast of North Carolina and created a series of saltwater ponds on North Core Banks. Since the hurricane, these ponds have likely become inhabited by both benthic and pelagic life, but these ponds have never been surveyed and their importance to the barrier island ecosystem is unknown.

Each year, there is public pressure for the recreational usage of Core Banks. Each recreational usage group hopes that overdevelopment and misuse will not hinder their use of the island. They enjoy the natural state of the island and hope that it will remain so (Schwartz et al., 1992). Therefore, it has become increasingly important to survey the species assemblage in new ponds located on North Core Banks. This way, recreation of the island can continue without negatively impacting the species that reside there. Aside from recreational concerns, knowing the types of fish that inhabit the pond will allow us to better understand the complexity of food webs and species interactions on North Core Banks. It will also provide insight on which species are most important to the food web and the system's ecological function. This is crucial because it will allow the National Park Service to understand what species are present, which species are necessary to protect in these ponds to maintain a healthy ecosystem, and how recreation on the island may affect these ecosystems.

In the 1980s, Schwartz et al. (1992) sampled ponds on Core Banks, but these ponds either still exist as old ponds on the island, or may have since disappeared. In our study, we looked at the new ponds that were created from overwash events after hurricane Dorian in 2019. Our research is significant because we are sampling different ponds than the ones that Schwartz et al. sampled. This means that the species living in the ponds, the geography of the ponds, and the ponds' abiotic influences will be unique from those studied by Schwartz et al. We also conduct analyses that Schwartz et al. did not include, such as biomass, species diversity, and environmental factors.

Few studies have been conducted looking at populations of fish species in saltwater pond formations making them a unique study subject. However, on Petit Bois Island in Gulf Islands National Seashore in Mississippi, similar pond formations that support a wide variety of fish species have been observed for many years (Beavers et al, 2020). Due to the rare occurrence of research examining such ponds, studies identifying the role of these fish in the food web and the ecosystem are important in order to inform decisions on how to protect the ecosystem and the species that reside there. Additionally, little is known about the populations of fish in these ponds and whether these fish arrived due to overwash events or have been repopulating the ponds on their own for years.

In this study, we investigated six unique ponds on Core Banks that were created by overwash events from Hurricane Dorian in September of 2019. This chapter documents the abundance and biomass of fish species populating many of the overwash ponds created by the hurricane, as well as their respective diversity and evenness. This chapter will also examine the relationship between species diversity and biomass to pond size. Ultimately, this information can be used to help map and predict species distributions between ponds as well as inform the National Park Service and recreational users on how to preserve the biodiversity of these ponds.

Our first research question was how does the size of the ponds affect species diversity? We hypothesized that larger ponds will have higher species diversity because the pond is larger and can therefore support more total number of species. A study by Oertli et al. (2002) supports this hypothesis and found that as the size of the pond increased, species that did not exist in smaller areas were found. Their results showed that a group of small ponds was more diverse than a single large pond. Although pond size did correlate with species diversity, this relationship was not particularly strong. They conclude that a variety of pond sizes should be protected in restoration efforts for the purpose of biodiversity and species richness preservation (Oertli et al. 2002).

Our second research question considered the biomass of these fish in relation to the size of the pond. We hypothesized that when the area of the pond increased, the biomass of the fish residing in it would increase because there are more resources available. Few to no relevant studies have been conducted comparing the size of the pond to the biomass of fish in them. However, species diversity often correlates with the overall ecosystem health, so it is likely that fish biomass will increase with pond size (Burkhard et al. 2008).

6.2. Methods

Study Site

We selected six new ponds on North Core Banks that were created during Hurricane Dorian in September of 2019 (Figure 1). These new ponds were visited in trips of three at a time in order to work as efficiently as possible on each trip to Core Banks. The first set of new ponds comprised of New Ponds 1 (34.90083, -76.25389), 2 (34.910202, -76.241421), and 3 (34.93028, -76.21778). Set two included New Ponds 1.5 (34.9041770, -76.2496240), 2.5 (34.9115960, -76.2402380), and 2.75 (34.9227290, -76.2261600). These ponds periodically experienced water level changes which altered the shape and depth of the ponds over time and throughout the study period. We collected our data between September and October of 2021 on four different occasions. Set one was visited on September 23, 2021 and October 13, 2021. Set two was visited on September 29, 2021 and October 27, 2021.

Figure 6.1. The image above shows the new ponds (ponds created since Hurricane Dorian) that were identified and sampled with their labels along Core Banks. The first set of ponds (New Ponds 1, 2, & 3) are denoted with a wave. The second set of ponds (New Ponds 1.5, 2.5, and 2.75) are denoted with a fish icon.

Data Collection

At each of the ponds, we used two methods for quantifying the abundance and diversity of the fish communities. First, we set four minnow traps around the perimeter of the pond and labeled them traps 1-4. We set two traps in shallow water at each pond and two traps in deep water. After setting the traps we measured and recorded the depths where each trap lay. The traps were left in the water for approximately 40 minutes at each pond. Once the time had elapsed, we identified each individual by species, and then measured their length. We then adjusted the number fish collected at each pond for the time the traps were in the water in order to control for the time that the traps were in the water.

Figure 6.2. The image above shows the in situ data collection process. Here the length and weight of the fish were taken and recorded on a data sheet.

The second method that we used to collect fish was by seining across transects of the ponds. We seined across a section of the pond that was standardized to a distance of 35 meters. Once the net was pulled ashore, we sorted the fish by species and put unidentifiable species into a separate bucket. We then measured length and weight of 50 individuals from each species (unless there were fewer than 50 individuals collected) and then measured the total mass of all individuals within each species. In cases where there were more than 50 individual fish of a single species, we weighed the subsample as well as the total mass of all the fish that were caught using a spring scale to extrapolate total abundance by species. Due to logistical constraints with spring scales, total biomass of some species was not measured if their total mass was less than 5-10 grams leaving us without data for species where very few fish were caught.

When a fish could not be identified in the field, it was collected, put in a cooler, and brought back to the lab to use other resources such as microscopes and the *Early Stages of Atlantic Fishes* Volumes I and II in order to classify it. Additionally, online resources such as fishbase.se were used to assist in fish identification. At each pond, water quality data including temperature, salinity, and dissolved oxygen were recorded as supplementary information in order to explain observed patterns in diversity and biomass that could not be explained by pond area differences. All of this data was recorded in an excel spreadsheet. Additionally, we partnered with colleagues (see Chapter Two) to collect drone data to ensure the area of the ponds.

Data Analysis

The Shannon Wiener Diversity Index was used to calculate species diversity at each of the ponds. The formula for this diversity index is $H = -\Sigma \, p\ln(p)$, where H is the calculated diversity value, and p is the proportion of each species found at each pond. Species evenness was also calculated using $(H/\ln(S))$, where H is the diversity value and S is the number of species present. Species evenness is defined as a measure of the relative abundance of each species at one pond. The more similar the relative abundances of each species are, the higher the species evenness value will be.

We used R version 3.6.2 to analyze our data and formulate figures. Additionally, we used Microsoft Excel and Google Sheets to generate pie charts and bar graphs.

6.3. Results

We found that overall, inland silverside (*Menidia beryllina)* was the most abundant species in each of the ponds. Sheepshead minnow (*Cyprinodon variegatus variegatus*) and rainwater killifish (*Lucania parva*) were also common, particularly in New Ponds 2 and 3 (Figure 3). Rainwater killifish (*Lucania parva*) were also quite abundant at New Pond 2.5.

Although the species found in the ponds were similar between New Ponds 1.5, 1.75, 2, and 2.5, we found that fish populations in New Pond 1 were quite different from the other ponds. In New Pond 1, silversides were still the most abundant fish, however we also found spot (*Leiostomus xanthurus*), pinfish (*Lagodon rhomboides*), white mullet (*Mugil curema*), and black drum (*Pogonias cromis*). These fish were not seen in any of the other ponds. They were also much larger overall than the fish we found in the other ponds.

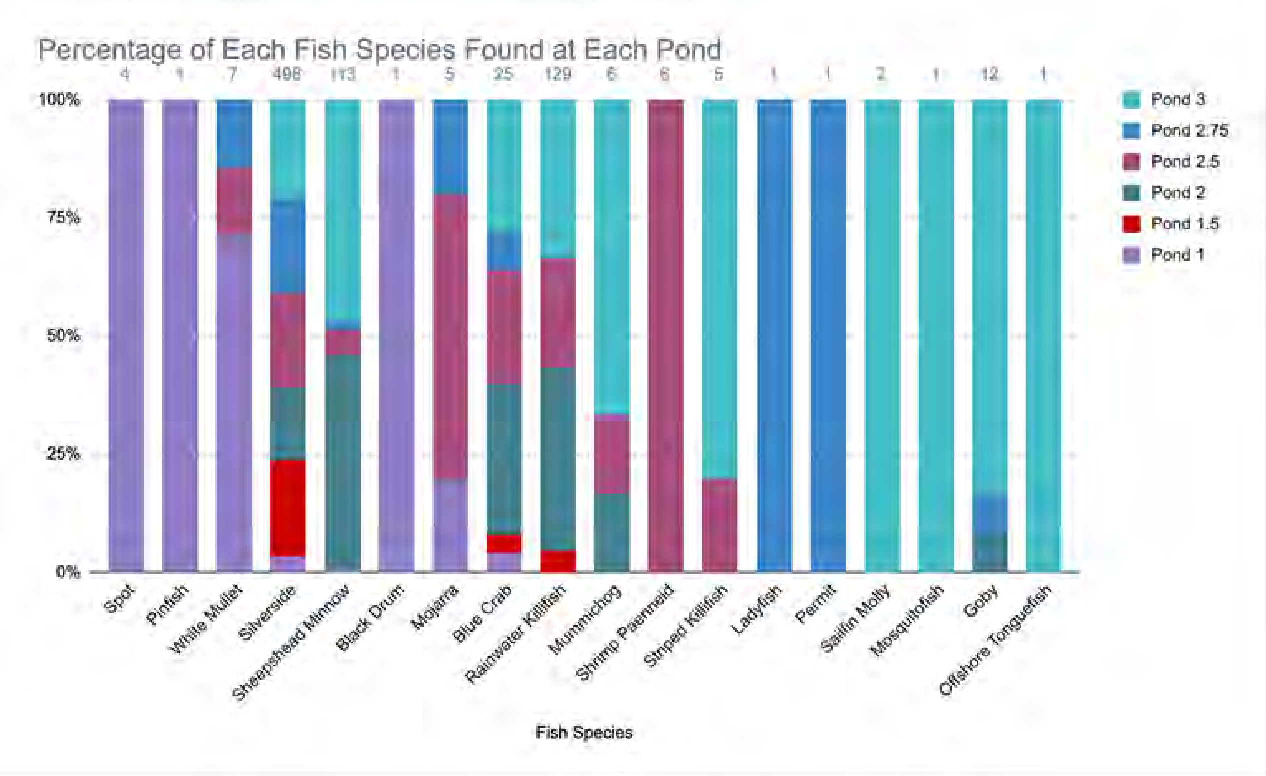


Figure 6.3. The stacked bar chart above shows the percentages of total individuals found at each of the six new ponds that we sampled. As shown in the figure, some species were only found at one pond such as the Spot, but others such as Silversides were found at all of the ponds.

During our analysis, we found that in the population of silversides, some ponds had two distinct peaks representing age groups (example in Figure 6.4a. and 6.4b.). The first peak occurred at one of the smallest length classes, indicating a large population of juvenile fish. This signified that spawning had just occurred and the older generation had likely died off. A second peak existed in the larger length class of fish, showing that a generation had grown up and was about to spawn, although this second peak was more difficult to see in our data (example in Figure 6.4b.). The other group that saw a similar peak in juvenile fish and adult fish were the sheepshead minnows (Figure 6.4c.).

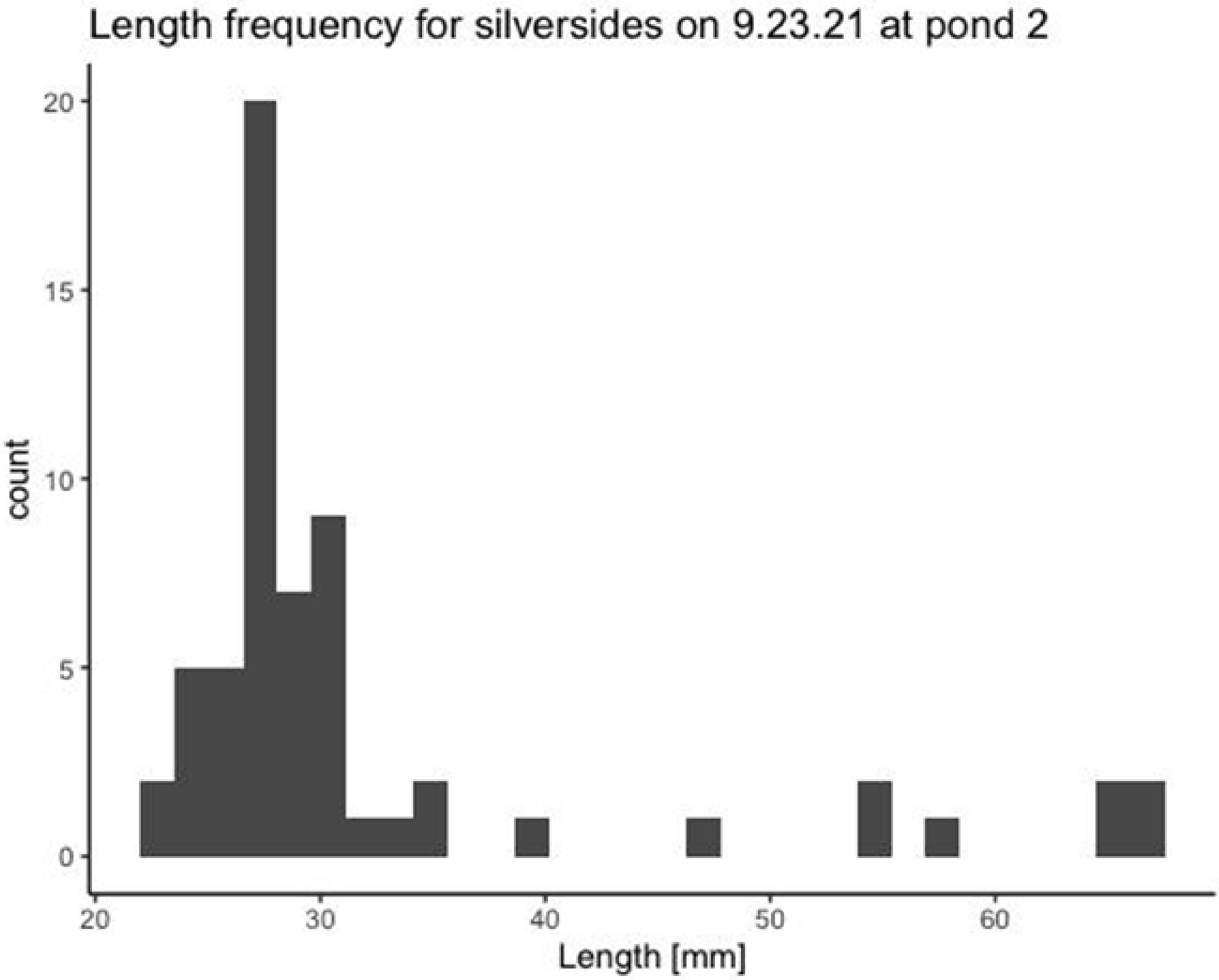


Figure 6.4a. The histogram above show the length frequency of Inland Silversides and a peak where a younger generation of silversides is the most prominent. The silversides at New Pond 2 showed the most clear and prominent peak. While other peaks at other ponds indicated a prominence of a specific generation, this pond showed the clearest peak.

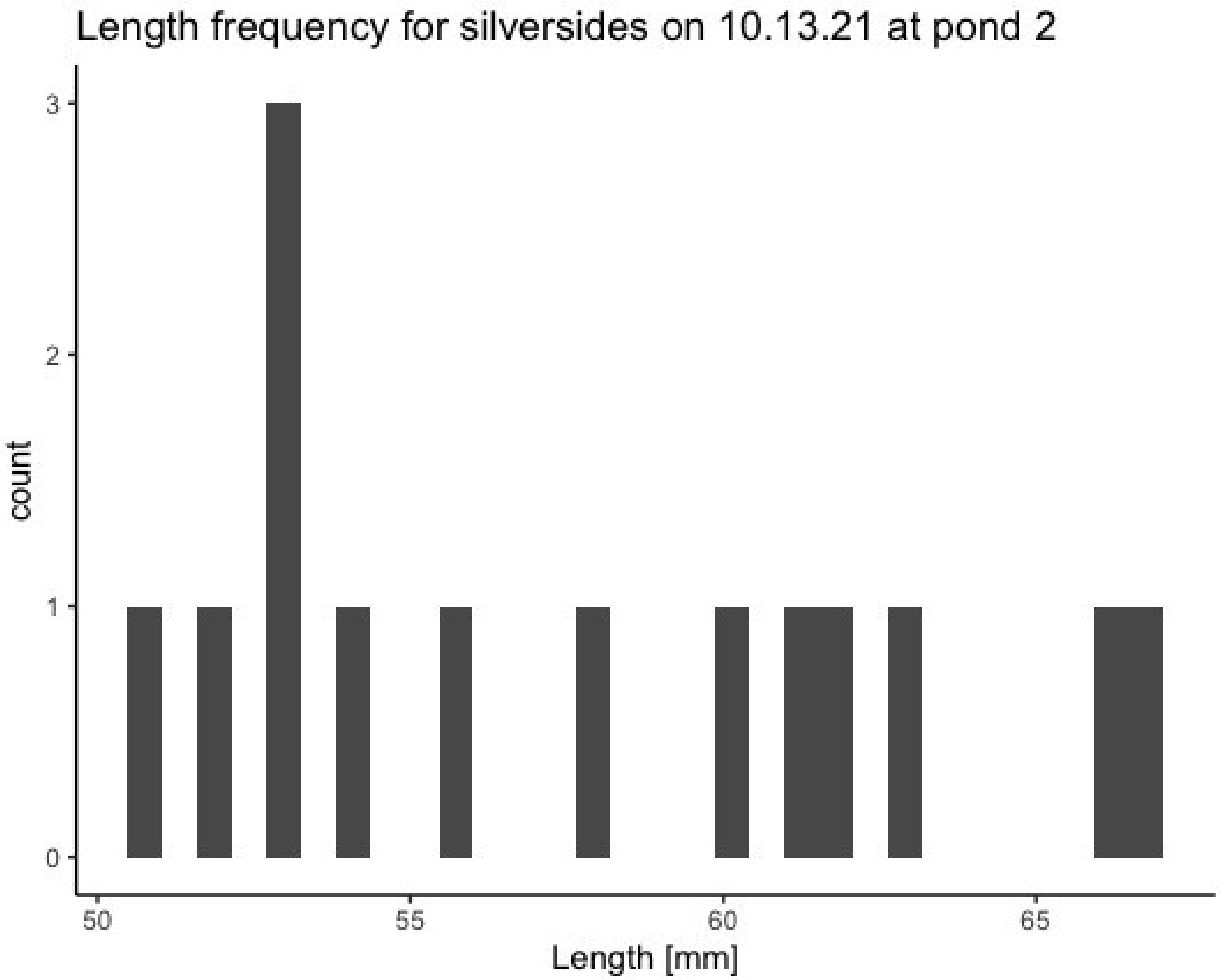


Figure 6.4b. The histogram above shows the length frequency of silversides at new pond two at a later date. This histogram shows a less clustered peak at the lower lengths, and instead shows a peak and more distribution above 50mm.

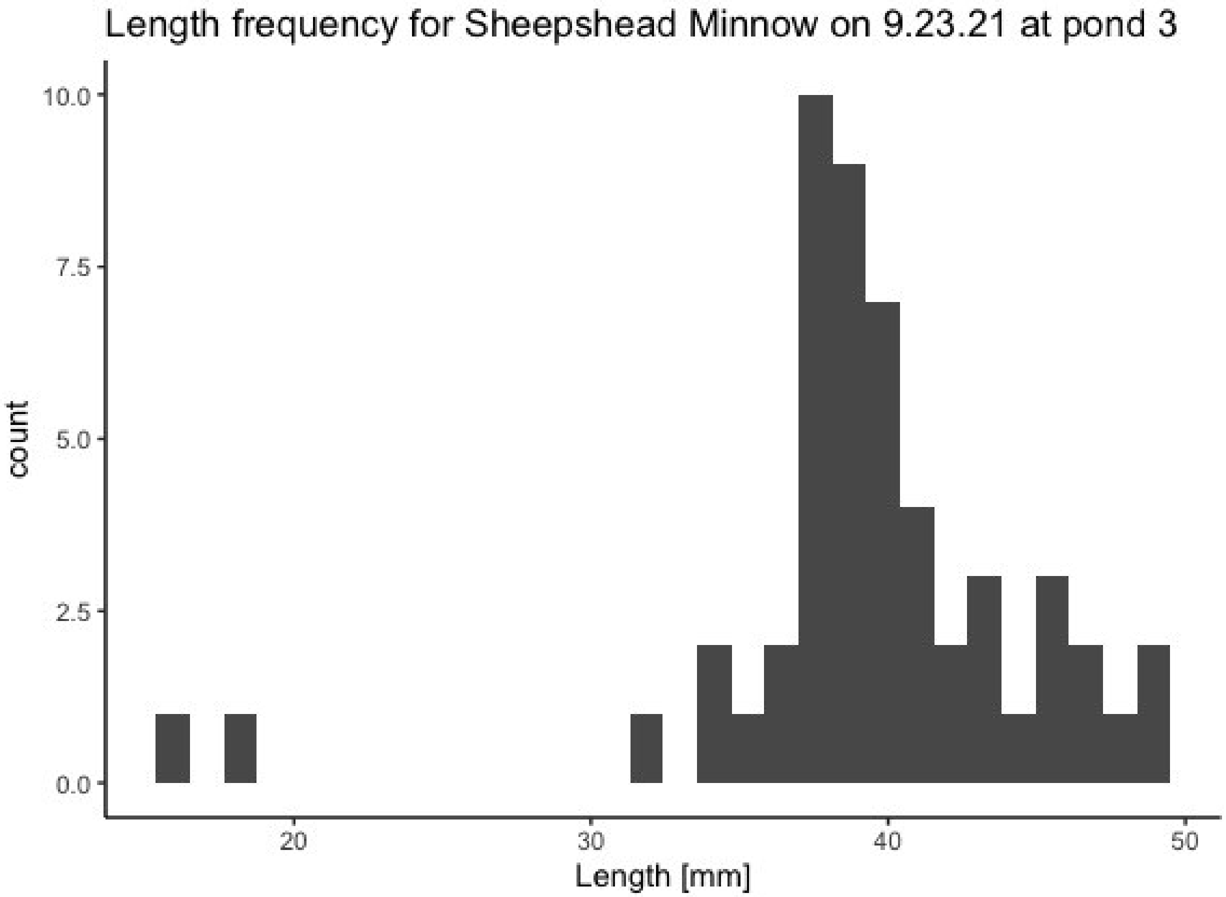


Figure 6.4c. The histogram above shows a similar peak as the ones seen in figures 4a and 4b, but for sheepshead minnows. At the point, there was a clear peak showing a predominantly older generation of sheepshead minnows present.

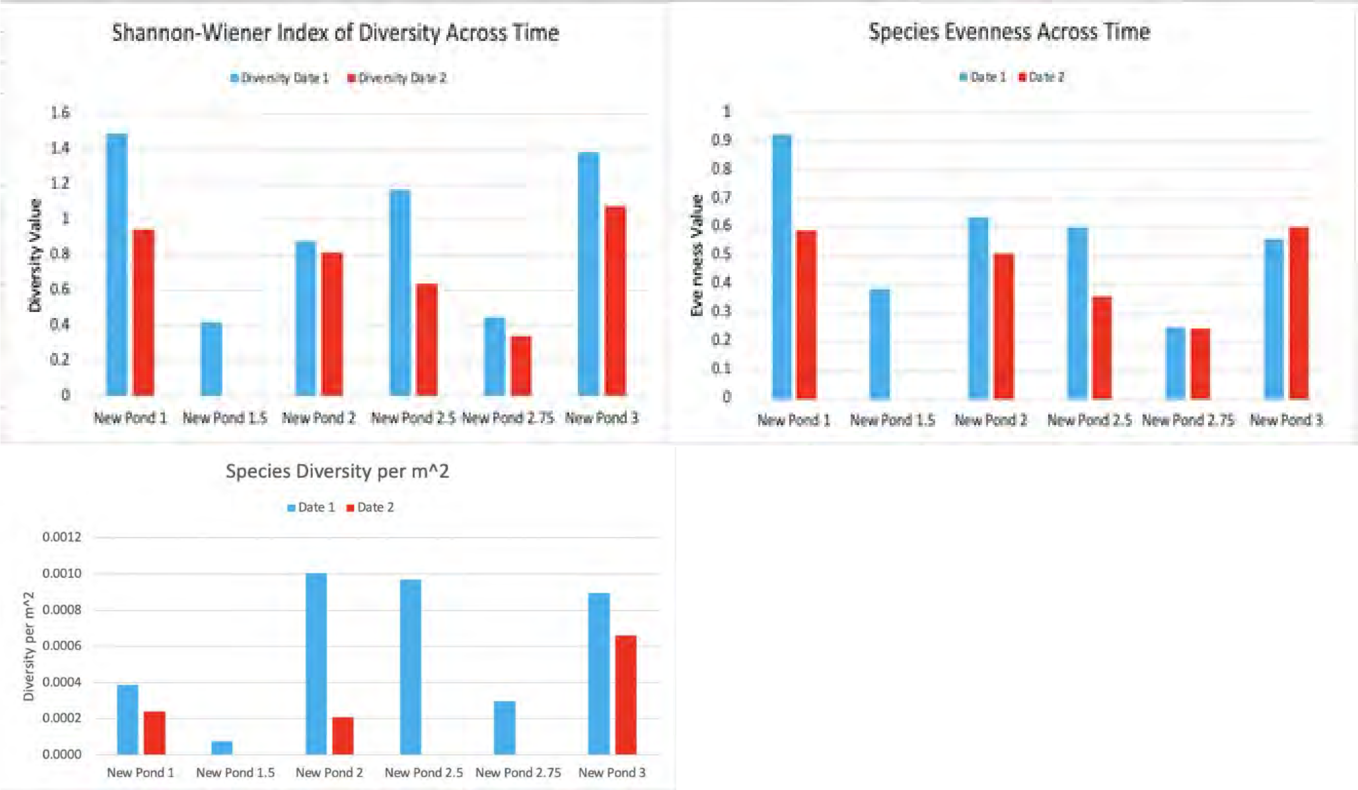


Figure 6.5. The above bar graphs show the distribution of diversity and evenness values within each of the ponds across time, along with the diversity values when pond area was accounted for. The Shannon-Wiener Index of Diversity was used to determine the evenness values in the graph on the top right, then the resulting evenness values were used to calculate diversity for the top left graph. Diversity per m^2 was calculated by dividing the diversity values by the total pond area for the graph on the bottom.

Using species abundance data at each pond, diversity and species evenness values were calculated using the Shannon-Wiener Index of Diversity. Since each pond was sampled twice, the diversity was calculated at both time points to compare the values across time. The diversity at all of the ponds decreased over time, with a value of 0 at the second time point for New Pond 1.5. A diversity value of 0 means that only one species was found, and in this case, we saw this with silversides. Species evenness decreased over time at every pond except for New Pond 3. Based on this data, New Ponds 1 and 3 are the most diverse at both the first and second time points. New Pond 1 has much greater species evenness at the first time point than the other ponds, but New Pond 3 has the greatest evenness at the second time point.

When diversity was normalized by pond surface area, the diversity rankings of the six ponds changed. Based on area, New Ponds 2 and 2.5 had the greatest diversity. New Ponds 1, 2, and 3 were the only ponds that had diversity values at two time points, and diversity decreased over time at all of them. New Pond 2 had the largest decrease in diversity between the two trips to Core Banks, with a drop of approximately 0.0008 from the first time point to the second. New Pond 1 had the smallest drop, with a decrease in diversity value of less than 0.0002. Areas for

New Ponds 1.5, 2.5, and 2.75 were not measured at a second time point, so there are three missing values.

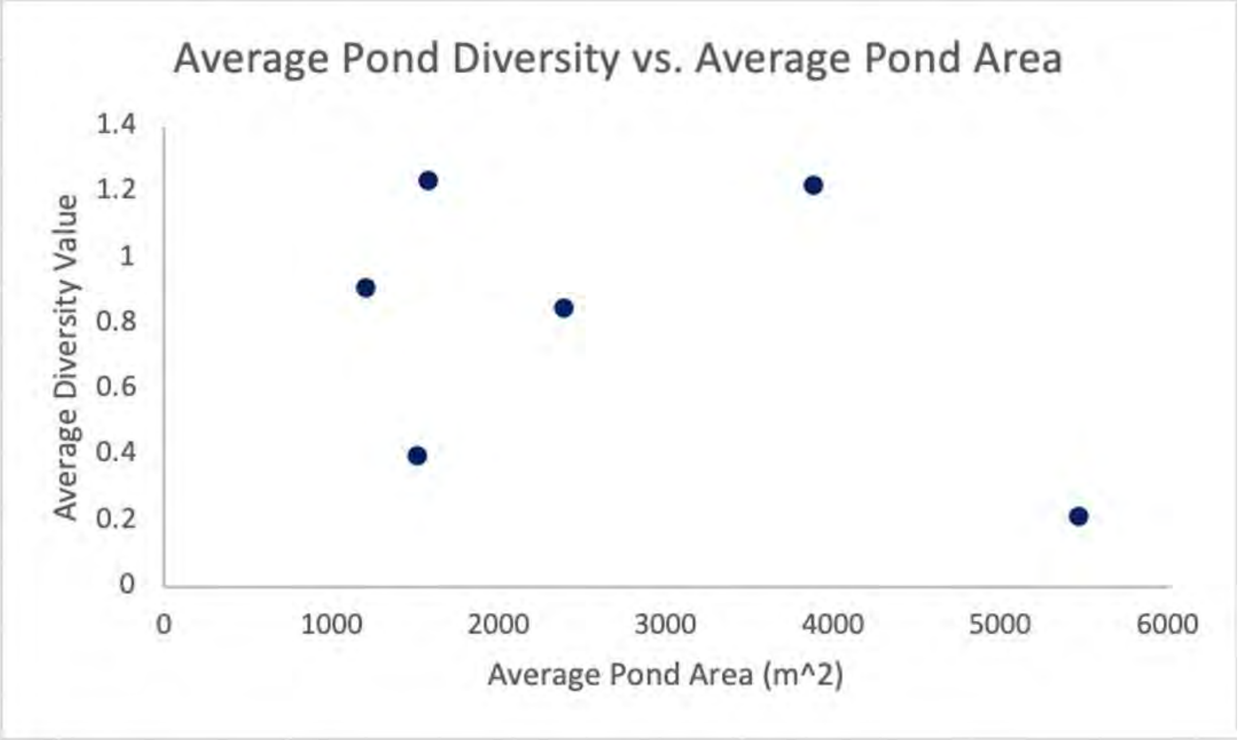


Figure 6.6. Pond areas across two time points and their respective diversity values were averaged and compared. Each point on the scatterplot represents a single new pond. Pond 1.5 is the point with the largest area but smallest diversity value.

In Figure 6.6., diversity and area values were averaged across time and plotted. New Pond 1.5 had the smallest diversity value of approximately 0.2, while having the largest area of around 5500 m^2. New Ponds 1 and 3 had the largest diversity values of approximately 1.22 and 1.23, respectively. New Pond 1 had the second largest area of around 4000 m^2, while New Pond 3 had one of the smallest areas at around 1500 m^2.

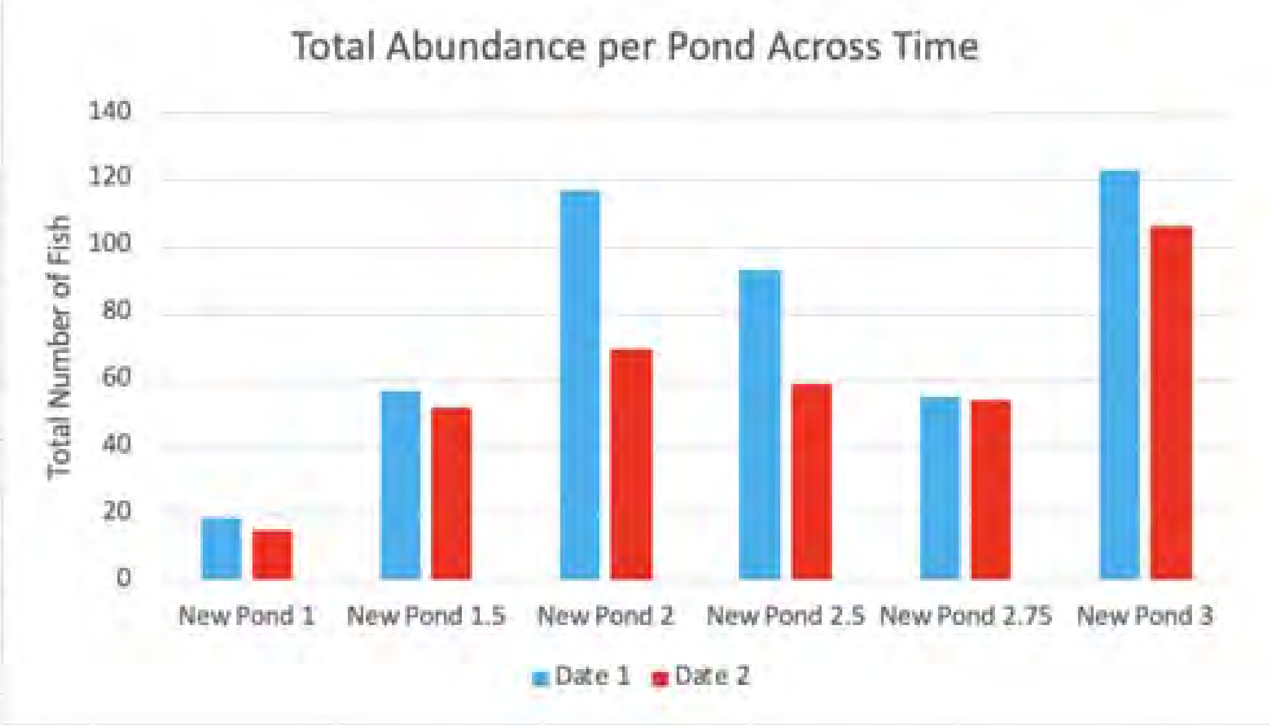


Figure 6.7. The number of each fish caught per pond at each time point and were totaled to generate an abundance value. Fish caught by trapping and seining were added together to measure total abundance at each pond. The graph shows a decrease in abundance over time at each pond, with New Pond 3 having the highest abundance.

Abundance per pond was calculated by adding the total number of fish caught at each pond. Abundance was calculated at both time points and shows a decrease over time at each of the ponds. New Pond 2 showed the greatest decrease in abundance, with almost 50 less fish caught the second day than the first day. New Pond 2.75 showed the smallest decrease in abundance, with only 1 less fish caught between time points.

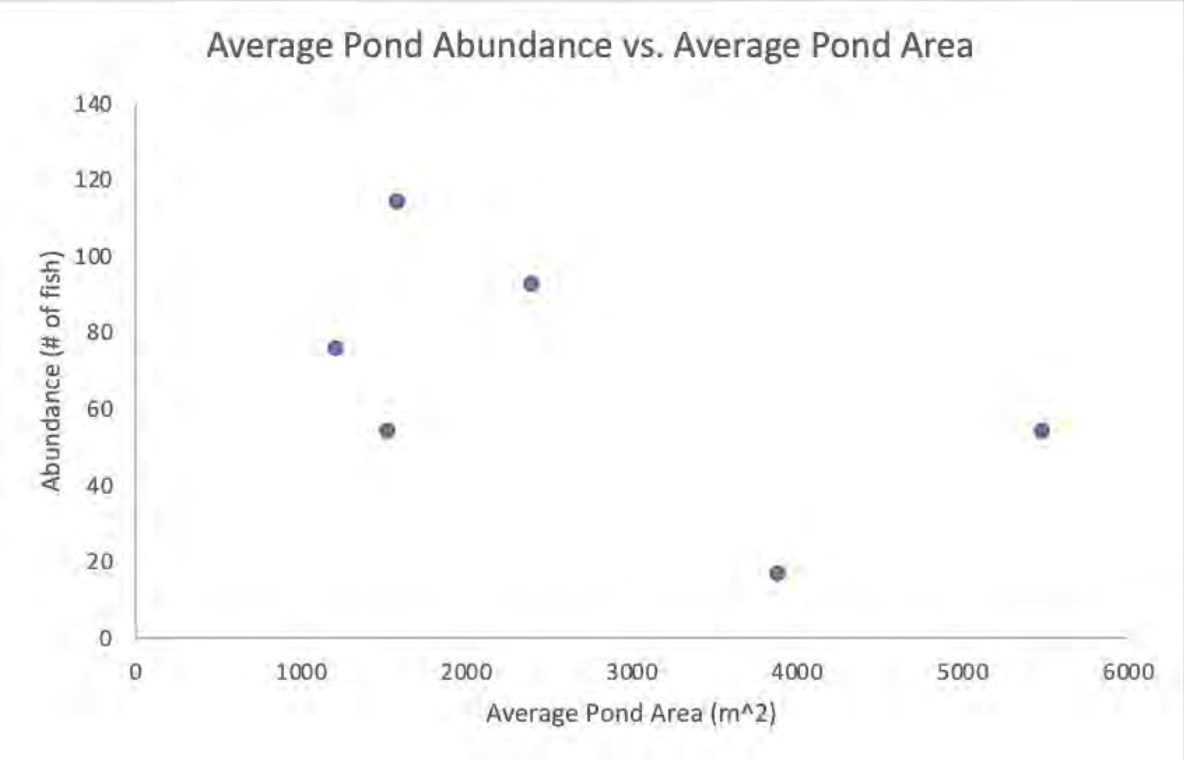


Figure 6.8. Abundances and areas across time points were averaged for one value, then plotted against each other. No clear relationship is seen, but New Ponds 1 and 1.5 with large areas have low abundances. New Pond 3 has high abundance and a small area.

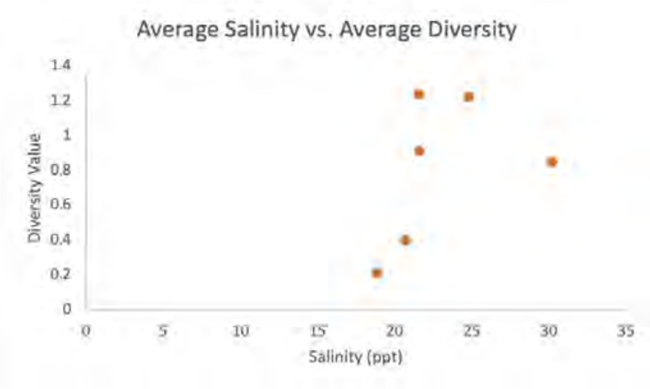
Average abundance versus average pond area was graphed in Figure 6.8. The line showing the areas of the ponds is the same as Figure 6, with New Pond 1.5 having the largest area. New Pond 1 was the least abundant, with an average value of 17 fish caught each day. New Pond 3 was the most abundant with an average of approximately 115 fish caught each day. Figure 6.8. shows that although New Ponds 1 and 1.5 have the greatest areas, they have two of the smallest abundances. New Pond 2.75 is similar in abundance to New Pond 1.5, but it had a much smaller area.

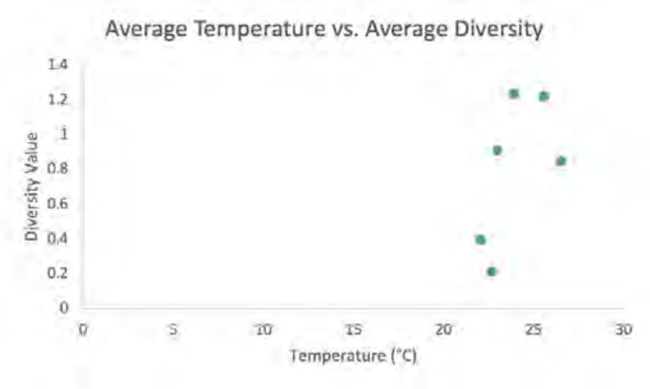
Table 6.1. Temperature, salinity, and dissolved oxygen data taken at two different sampling periods for each pond. Time points were taken from New Ponds 1, 2, and 3 on 9/13/21 and 10/13/21. Time points were taken from New Ponds 1.5, 2.5, and 2.75 on 9/29/21 and 10/27/21.

	Pond Number	**Salinity (ppt)**	**Temperature (°C)**	**DO (mg/L)**
Day 1	**New Pond 1**	27.96	26	7.99
	New Pond 1.5	22.43	26.5	3.96
	New Pond 2	37.7	27.21	11.58
	New Pond 2.5	26.38	26.8	7.71
	New Pond 2.75	22.43	27.3	11.515

	New Pond 3	26.74	25.1	7.64
Day 2	**New Pond 1**	21.75	25.19	7.91
	New Pond 1.5	15.32	18.9	10.09
	New Pond 2	22.75	25.84	9.27
	New Pond 2.5	16.87	19.2	8.3
	New Pond 2.75	19.05	16.8	8.72
	New Pond 3	16.43	22.8	8.38
Averages	**New Pond 1**	24.855	25.595	7.95
	New Pond 1.5	18.875	22.7	7.025
	New Pond 2	30.225	26.525	10.425
	New Pond 2.5	21.625	23	8.005
	New Pond 2.75	20.74	22.05	10.118
	New Pond 3	21.585	23.95	8.01

Temperature, salinity, and dissolved oxygen were similar across most of the ponds (Table 6.1.). Notably, dissolved oxygen in New Pond 1.5 was quite low and salinity in New Pond 3 was quite high at our first sampling period. Temperature and salinity at all of the ponds decreased over time. However, New Pond 1 and 2 consistently had the highest salinity. Dissolved oxygen decreased over time at most of the ponds, except for New Pond 1.5 that increased from 3.96 mg/L to 10.09 mg/L, New Pond 2.5 that increased from 7.71 mg/L to 8.3 mg/L, and New Pond 3 that increased from 7.64 mg/L to 8.38 mg/L.





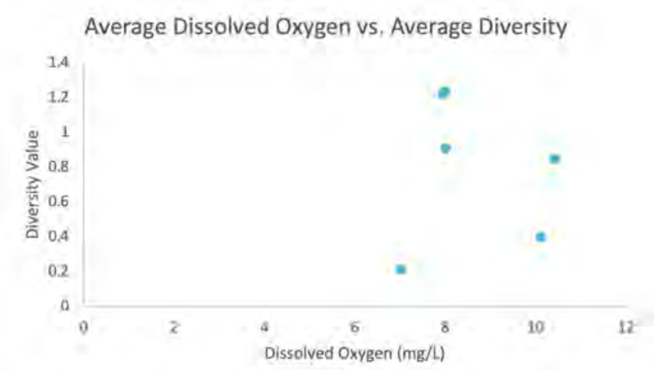


Figure 6.9. These graphs show the plotted relationships between average diversity values of each pond compared to their average salinity, temperature, and dissolved oxygen values.

To determine if there was a relationship between diversity at the ponds and their respective abiotic variable values of temperature, salinity, and dissolved oxygen. The data do not show a clear linear relationship between the variables at for each abiotic factor.

Table 6.2. This table shows the different species found in the 6 surveyed ponds. Stated above are their common names, scientific names, the ponds they were found in, and their total biomass in grams.

Fish	**Scientific Name**	**Number Pond(s) in Which it was Found**	**Pond(s) it was Found in**	**Method Found**	**Total Biomass (g)**
Spot	*Leiostomus xanthurus*	1	1	Seine	160
Pinfish	*Lagodon rhomboides*	1	1	Seine	360
White Mullet	*Mugil curema*	3	1, 2.5, 2.75	Seine	185.0414594
Silverside	*Menidia beryllina*	6	1, 1.5, 2, 2.5, 2.75, 3	Trap and Seine	775.2190909
Sheepshead Minnow	*Cyprinodon variegatus variegatus*	5	1, 2, 2.5, 2.75, 3	Trap and Seine	235.53
Black Drum	*Pogonias cromis*	1	1	Seine	200
Mojarra	*Gerreidae (family)*	3	1, 2.5, 2.75	Seine	30.46
Blue Crab	*Callinectes sapidus*	6	1, 1.5, 2, 2.5, 2.75, 3	Seine	184.745
Rainwater Killifish	*Lucania parva*	4	1.5, 2, 2.5, 3	Trap and Seine	80.86
Mummichog	*Fundulus heteroclitus*	3	2, 2.5, 3	Trap	14.77
Shrimp Paenneid	*Penaeidae (family)*	1	2.5	Seine	7.325
Striped Killifish	*Fundulus majalis*	2	2.5, 3	Trap and Seine	5.96
Ladyfish	*Elops (family)*	1	2.75	Seine	11.97

Permit	*Trachinotus falcatus*	1	2.75	Seine	0.94
Sailfin Molly	*Poecilia latipinna*	1	3	Trap	5.88
Mosquitofish	*Gambusia affinis*	1	3	Trap	0.54
Goby	*Gobiosoma bosc*	3	2, 2.75, 3	Trap and Seine	2.31
Offshore Tonguefish	*Symphurus civitatium*	1	3	Seine	0.12

Silversides provided the greatest biomass, and along with blue crabs were the only species to be found in all 6 ponds (Table 6.2.). In contrast, species such as the offshore tonguefish, mosquitofish, and permit provided the least biomass, and along with others were only found in a single pond. For species in which biomass was not able to be recorded due to being under the initial weight necessary for a spring scale, literature values of length to weight ratios were used. These values were used for blue crabs (Gokce G. et al. 2006), shrimp paenneids (Araneda, M. et al. 2008), mojarra, ladyfish, permit, sailfin molly, mosquitofish, goby, and tonguefish (fishbase.se). Some species did not have reliable literature values for their length to weight ratios, so similar species were used instead. For striped killifish and mummichogs, the length to weight ratio for California killifish was used (fishbase.se).

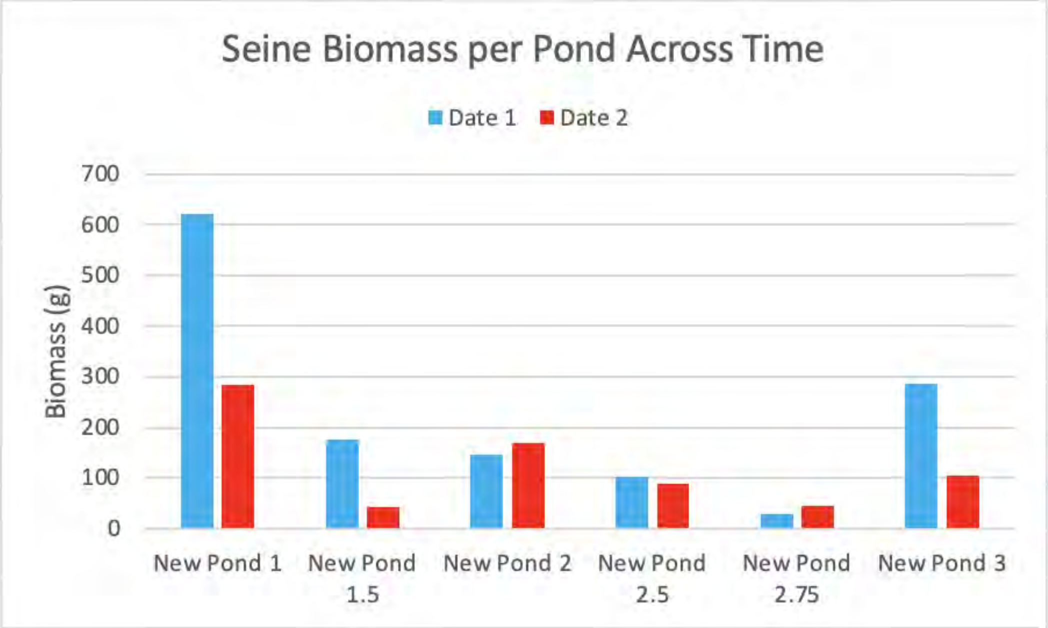


Figure 6.10. Seine biomass was recorded for the two data collection dates at each pond. During each trip to each pond, we seined once at a standardized length of 35 meters. New Pond 1 had the most biomass caught at both time points, while New Pond 2.75 had the least at the first time point and New Pond 1.5 had the least at the second time point.

Figure 6.10. shows the biomass found at each of the ponds between the different times the ponds were sampled. In New Pond 1, larger species such as spot, pinfish, and black drum represented the majority of the biomass due to their large size. This would mean that a single

black drum could drastically change a pond's biomass. In New Pond 1.5, silversides represented the majority of the biomass. In New Pond 2, silversides and blue crabs represented the majority of the biomass. The silversides remained high in population count, while the blue crabs accounted for a greater biomass to individual ratio due to their size in comparison to small fish species such as silversides and sheepshead minnows. In New Pond 2.5, silversides represented the majority of the biomass. Additionally, lesser seen species such as the white mullet and sheepshead minnow represented a fair amount of biomass on the second visit to New Pond 2.5, where less silverside biomass was recorded. In New Pond 2.75, silversides and white mullet represented the majority of the biomass. In New Pond 3, silversides and sheepshead minnows represented the majority of the biomass. Additionally, average biomass was found to be 174.59 grams across all ponds.

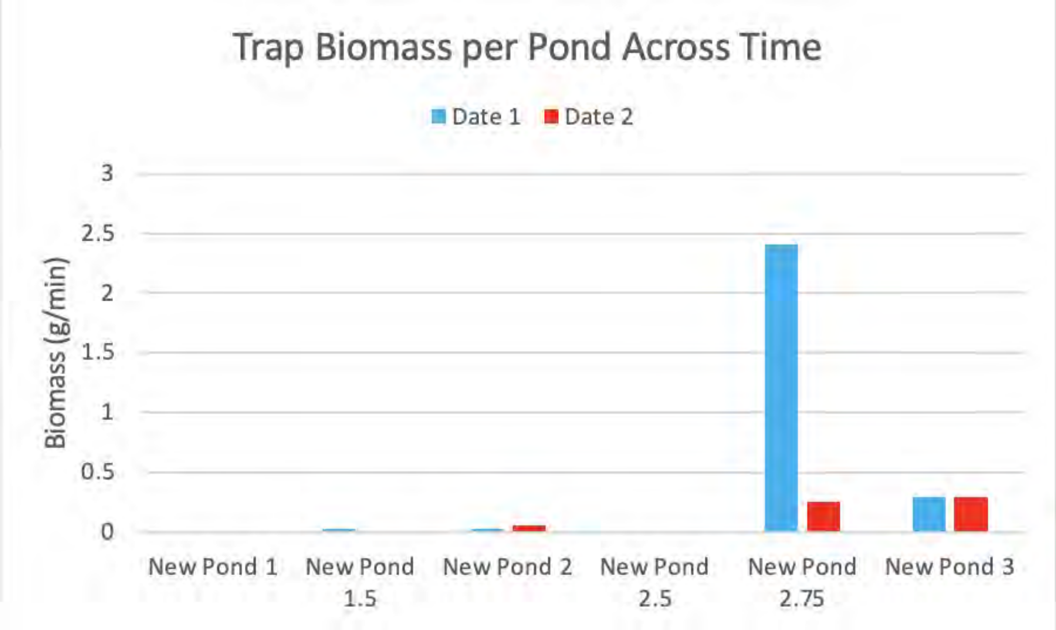


Figure 6.11. This bar chart shows biomass found in each new pond over time. Each set of traps remained in each pond for between 39-44 minutes. These corresponding biomasses were then corrected for time in order to make sure the differing times did not serve as a confounding variable.

Figure 6.11. shows the biomass found at each new pond through the use of minnow traps. New Pond 2.75 had the highest total biomass per minute by a large margin due to a single trap catching an entire school of sheepshead minnows. Most new ponds, however, had very little luck catching fish species overall with the trapping method. Because of this, we based our discussion off of biomass only caught through seining. Fish caught through minnow traps were only used for identification of any unique species caught, as seen in Table 2.

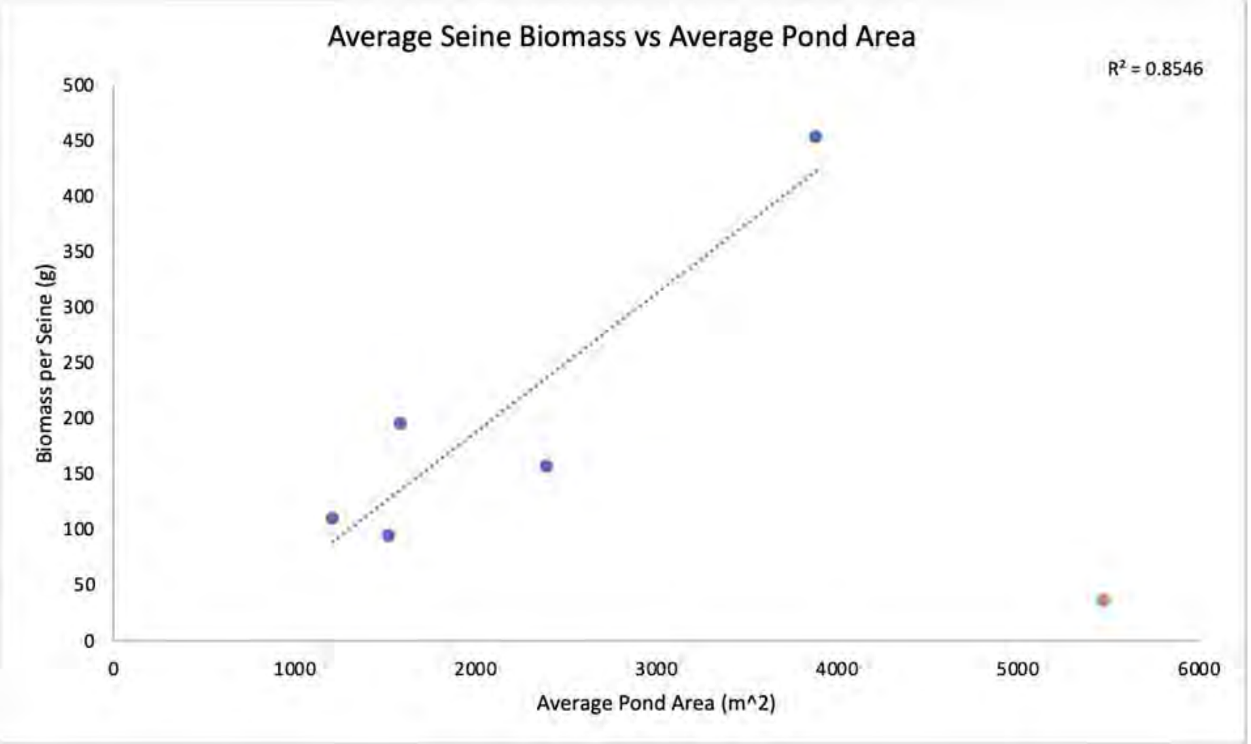


Figure 6.12. This chart shows the biomass averaged over time compared to average pond area over time. New Pond 1 had the most average biomass, with approximately 250 g more than the pond with the second highest biomass, New Pond 3.

Figure 6.12. shows the average biomass found in each pond over two trips compared with average pond area over the same two time points. New Pond 1 had the largest amount of total biomass, while New Ponds 1.5, 2.5, and 2.75 had comparatively low total biomass measurements.

6.4. Discussion

We documented for the first time the species assemblage, diversity and biomass of fish in six ponds that were created after overwash events during Hurricane Dorian. The distribution of fish species was quite similar across the board with the exception of New Pond 1, where many oceanic species were caught. This could be due to a variety of reasons. One possible explanation for the unique fish assemblage in New Pond 1 could be because fishermen are placing bait fish or fish they catch in the ocean in this pond as it is the closest pond to the dock where visitors come in. A second possible explanation could be because of the marsh connectivity found by the benthic invertebrates team (see Chapter 5).

In our results, we found clear evidence that silverside and sheepshead populations were reproducing, but we did not find evidence of this for many of the other fish species. Though this may be partially due to the mesh size of the seine, only rare evidence of both juveniles and adults of a species was observed. It is possible that for some fish species, the juveniles are so small that they cannot be caught in the seine net or the minnow traps. We hypothesize that this may be due to potential overwash events long ago that brought new fish into the ponds that were closest to the ocean such as New Pond 1. However, during the study period, no evidence of overwash was found. So, there must have been adult fish in the ponds that have been reproducing since the last overwash event.

Additionally, we found that many different species that were not observed by Schwartz et al. (2002) including white mullet (*Mugil curema),* pinfish (*Lagodon rhomboides),* black drum (*Pogonias cromis),* mojarra (*Gerreidae),* blue crab (*Callinectes sapidus),* shrimp paenneid (*Penaeidae),* permit (*Trachinotus falcatus),* sailfin molly (*Poecilia latipinna),* mosquitofish (*Gambusia affinis),* goby (*Gobiosoma bosc),* or offshore tonguefish (*Symphurus civitatium).* This is particularly significant because it shows the unique nature of the ponds that we surveyed in comparison with the ones that Schwartz et al. studied. This could be because the locations of the ponds of the two datasets were different, the ponds were formed differently, the time difference of the two studies and what was carried into the ponds from overwash at those times, or other potential factors such as the chemistry of the ponds. Schwartz et al. (2002) characterized the ponds sampled on Portsmouth Island in the vicinity of our study area (See Table 1) as having mud or silt substrate and the color of tea so it is likely that he sampled ponds more similar to the "old" ponds sampled by other groups in this study (See Chapters 2,3,4, and 7). Schwartz et al (2002) also sampled in May and may explain why we saw some species that he did not. However, the most likely difference between the species he observed is because these are ponds from overwash events, whereas the ponds that he sampled were likely more established marsh ponds.

Hypothesis 1

The Shannon-Wiener Index of Diversity was used to calculate species evenness and species diversity at two time points for each pond. Species evenness is used to calculate species diversity, so the two are interrelated. As seen in Figure 4, species diversity and evenness decreased overtime at every pond, except for evenness at New Pond 3. Some had more dramatic decreases in diversity (New Pond 1 decreased by approximately 0.6), while others had comparatively small decreases (New Pond 2 decreased by approximately 0.1). The overall trend of a decrease in diversity and evenness indicates the possibility that an event occurred that could have impacted fish populations or high predation levels. This could include rainfall from a storm changing the salinities of the ponds, or temperature fluctuations that some of the fish couldn't acclimate to. Another possibility is that some fish species, such as silversides, experience low mortality rates during the summer. After spawning, adult silversides die. This could be the case for some of the other fish species observed in these ponds. The increase in evenness over time at New Pond 3 might be attributed to a change in predation levels. A decrease in predation could allow populations to reach equal levels if one species was preyed on more than others. An increase in predation also could have caused this if there was one species in particular that was far more abundant than others until predation increased.

It is likely that the larger ponds have greater diversity because of the abundance of resources. However, this might not always be the case if the pond has low productivity levels. A pond with a large area but low productivity might have low diversity because there may be more substrate and less crowding. By taking area into account, it allows for a better comparison of the diversity values between ponds with largely different areas. When area is factored in, New Pond 2 is the most diverse with New Ponds 2.5 and 3 not far behind. Although only New Ponds 1, 2, and 3 have area values at both time points, the general trend in these shows that diversity still decreases over time. There are no area values for the second time points for New Ponds 1.5, 2.5, and 2.75, but based on diversity values from Figure 4, it is reasonable to conclude that diversity would still decrease over time. Diversity in New Pond 2 dropped dramatically over time, and this pond also had the greatest variations in area each time we visited. The changing physical features of the pond could be a reason for the decrease in diversity. Few studies exist that show this trend in other cases, however, in the fisheries industry, the size of the pond is very important to the reproduction capabilities of the species living there (Virginia DWR). In this case, a pond that is continuously changing in size would make it difficult for fish species to maintain their populations in such a variable environment. New Ponds 2 and 2.5 could be the most diverse per meter squared because of their geographic location on the island. These ponds could be in an optimal zone where conditions are the most ideal for fish population growth and reproduction. On Day 1, New Pond 2 had the highest salinity at 37.7 ppt, the second highest temperature at 27.21°C, and the highest DO at 11.58 mg/L. New Pond 1 also has the highest diversity value at the first time point. This suggests that higher values of each of these abiotic factors might provide the most suitable environment to sustain diversity. As previously mentioned, another possible explanation for the difference in diversity levels when compared to area might be productivity levels. If a large pond has low productivity levels while a small pond has high productivity level, then the smaller pond will likely have greater diversity.

As for the overall trend in diversity compared to area, our data show that there is no clear positive or negative correlation between diversity and pond area. The pond with the largest area, New Pond 1.5, also is the least diverse. However, New Pond 1 has the second largest area and is one of the most diverse. New Ponds 2.75 and 3 have similar small areas but New Pond 3 is over two times more diverse than New Pond 2.75. This does not provide sufficient evidence to support our hypothesis that larger ponds have greater levels of diversity.

Graphs of diversity compared to salinity, temperature, and dissolved oxygen at each pond were created to determine if there was a relationship between any of these variables. Based on the data, there is no evidence of a relationship between diversity and the three abiotic factors.

Although abundance is not a part of our hypotheses, it’s information that could be vital to the National Park Service when making decisions about the recreational usages of the ponds. In general, abundance decreased over time at all of the ponds. New Ponds 2 and 3 are the most abundant at each time point.

Hypothesis 2

As seen in Figure 9, New Pond 1 has the largest biomass by a large margin. This is due to the large size of the species found in New Pond 1, including pinfish, black drum, and spot. New Pond 1 also has one of the highest average areas, with an area of 3384 m². This supports the notion that ponds with increasing areas are likely to have higher total biomass. New Ponds 1.5, 2.5, and 2.75, by comparison, have the lowest biomass values. Though New Pond 1.5 has the highest recorded area at 5474 m², New Ponds 2.5 and 2.75 have the two lowest, with areas of 1210 m² and 1521 m², respectively. In general, the New Ponds' areas did accurately coincide with their recorded biomasses, where only New Pond 1.5 skewed from this trend. This may be because volume could not be taken into account. New Pond 1.5 had an extremely large area, but there is a good chance that it may not have been nearly as deep as some other new ponds. This would lead to a comparatively large pond area, while having a comparatively small pond volume. Because of this, volume is likely a much better measure in relation to diversity and abundance, as it takes into account more confounding variables such as pond depth.

Additionally, recorded pond biomass generally decreased over the course of the two trips taken. Many of the biomass readings decreased greatly, with New Ponds 1, 1.5, 2.75, and 3 seeing a decrease of over 50%. Only New Pond 2 saw a slight increase in biomass. New Pond 2 also had the greatest fluctuation in area over the two trips. The massive upswing in pond area could help explain the increase in biomass. An exponential increase in pond size could be the reason why New Pond 2 did not fit this trend. This increase in pond size over a short time could have been due to a number of reasons, including a rainfall or overwash event. In the event of high levels of overwash, individuals could have been brought into New Pond 2, leading to a large increase in biomass.

Our hypothesis stated that we would expect to see a trend of higher biomass in ponds with larger areas. This trend was accurately represented in every pond aside from New Pond 1.5. In general, all of the ponds aside from New Pond 1.5 support the hypothesis and further represent the theory that a larger pond area allows for increased biomass. New Pond 1.5 may not follow this trend because it was one of the deepest ponds we surveyed, and it had the lowest average salinity and dissolved oxygen. Even though most of the new ponds increased in size while decreasing in biomass over time, this can be explained through potential changes in environmental conditions, including but not limited to, heavy rainfall, overwash, or a heat wave. Additionally, different levels of predation could have occurred in each pond. A wide variety of waterfowl and terrestrial animals including otters and plovers (see Chapter Seven) were found in and around the ponds, and their presence and differing levels of hunting in each pond could have led to data discrepancies found in this study.

Lastly, in this study we found that the average biomass in each pond seine was 174.59 grams. In comparison, Schwartz et al. found an average pond biomass of 1916.19g. When adjusting for and removing the 72 ponds that Schwartz et al. found to be void of biomass, they had found an average pond biomass of 3105.72g. However, Schwartz et al. seined each pond between 1 and 20 times in order to maintain catch composition. Even if the average pond was seined 10 times, each singular seine contained 310.57g of biomass. When compared to the biomass data for our one seine per pond, it is still clear that the ponds seined by Schwartz et al. contained much more average biomass. This is logical because the older ponds surveyed by Schwartz et al. had a much more stable ecosystem in comparison to the ever-changing ponds used for this study. The biomass found in the older ponds surveyed in other chapters are more likely to have biomass similar to those surveyed by Schwartz et al. due to their more stable ecosystems.

Additionally, biomass did not share any significant relationship with abundance. Though both biomass and abundance dropped between each of the trips to each pond, biomass has a general positive trend with pond area. Conversely, abundance does not have a strong correlation to pond area. Because biomass tends to be skewed by a few large fish, as seen in New Pond 1, it is likely that abundance would be a better indication of ecosystem function in these ponds than biomass. Abundance is much less likely to be skewed due to a few outlying datapoints, and would therefore be more accurate overall. Additionally, it is safe to assume that in the context of this experiment, biomass does not have any clear relationship with abundance.

In conclusion, there was not sufficient evidence to support our first hypothesis that fish diversity increased with increasing pond area. However, our second hypothesis that fish health would increase with increasing pond area was supported. Although Pond 1.5 does not fit this trend, the remaining ponds provide evidence that fish health and pond area are directly related.

Chapter 7: Terrestrial Animals

Terrestrial Wildlife of Old and New Ponds on Core Banks, NC

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Fall 2021

7.1 Introduction

Predators play an important role in the ecosystem by controlling the populations of their prey, structuring the food web, and even causing trophic cascades. However, many predators are highly mobile and have large ranges, making it difficult to assess their population size, foraging habits and thus, their impact on food webs. To our knowledge, there has been no comprehensive assessment of terrestrial predators that use ponds on North Core Banks. The National Park Service exists to protect vulnerable species, as well as provide recreational opportunities for its visitors, so it is critical to document the assemblage of terrestrial species and assess their use of ponds on the Cape Lookout National Seashore (U.S. Department of the Interior, 2021). Gaining insight into how terrestrial animals, such as birds and mammals, interact with the barrier island ponds on North Core Banks will allow for the National Park Service to determine the ponds' significance and recreational relevance.

Terrestrial animals need freshwater to survive. On North Core Banks, a possible source of freshwater comes in the form of the ponds formed by Hurricane Dorian, which are often filled during rain events. Along with being a potential drinking source, birds and mammals come to coastal areas and ponds based on food types and foraging opportunities. Studies have shown that intertidal flats, such as the marshes on North Core Banks, are important feeding and roosting habitats, especially as stopovers for migrating birds. Food availability at a pond may predispose each species to select different habitats where their preferred food may be found. Herons, plovers, and sandpipers have been known to use shrimp ponds in China for foraging, and they often use the pond banks for high-tide roosts (Zou et al. 2006). Food preferences are also dependent on age; for example, an adult white ibis typically feeds on saltwater prey, while a juvenile requires freshwater prey to develop normally (Chavez-Ramirez et al., 1995). Along with shorebirds, the habitat of mammals is also dependent on food type and plant composition. For example, nutria (*Myocaster coypus*) have been found to aggregate towards ponds with *Spartina patens*, *Sagittaria lancifolia, Schoenoplectus americanus,* and *S. californicus,* as they offer cover and protection from the elements. These plants also allow for the construction of dry resting platforms above the water's surface for grooming and feeding, and an abundant food source of roots and rhizomes (Nolfo-Clements, 2012). Previous studies have found that otter (*Lontra canadensis*) activity is positively related to pond size and vegetation cover. This could be attributed to the correlation of pond size and vegetation cover with mean higher prey availability, better prey quality, and shelter availability (LeBlanc et al., 2007). Similarly, a recent study found that pond area, percent of area covered by grassland, and distance to pond inhabited by beavers were important variables in where other beavers chose to construct dams (Hood, 2020).

It is important to understand how different species respond to environmental conditions to accurately influence conservation and management regulations, such as mitigating human disturbance in necessary areas. Human disturbances may require animals to be more vigilant, and subsequently decrease time spent foraging. One example is seen with cranes; they will tolerate moderate human disturbance close to their nesting site but will only choose areas with minimal human disturbance if they must travel far in search of food (Nilsson et al., 2020). This indicates animals will be pickier about their foraging sites if they have already expended a lot of energy to get there. As the North Carolina Coast is a popular resting site for migrating birds, human interactions on North Core Banks may influence animal’s pond usage. In this study we assume seabirds and mammals to be central place foragers, which means the probability of occurrence of the animal decreases with distance from their central location, while others exhibit unrestricted mobility and foraging behavior (Hood, 2020; Nilsson et al., 2020; Massardier-Galatà et al., 2017). This assumption is necessary to make inferences about animal activity from camera trap surveillance and is reasonable especially for terrestrial mammals given the small size of North Core Banks.

It is nearly impossible to obtain an exact count of animals in the wild (Romani et al., 2018). An accurate count is imperative in order to make decisions about habitat management and restrictions against human activities. The use of camera traps for wildlife surveillance and to estimate populations size is a practice that has been gaining popularity as technological advancements are made. However, there are a few issues with camera traps, one of which is imperfect detection. Imperfect detection is where individuals present within a sampling area are not always detected, whether that be from a technology error, camera angle discrepancies, or lighting issues (Burton et al., 2015). One way to avoid these errors is to set cameras to take time-lapse photos at specific intervals so animals outside of the camera's trigger range may still be seen. With this method you can also record how many of each species are present in a shot without having to distinguish between individuals. There are a few different methods for estimating density with camera traps. Some studies have used camera trapping rates as an estimation for animal density, but they often do not account for the probability of detection. A camera’s probability of detection is influenced by factors such as detection zone, placement, sensitivity, ambient and animal temperatures, sampling timing and duration, and animal density and behaviors (Rowcliffe et al., 2008).

We aimed to discover how the terrestrial animal community diversity differs between ponds on North Core Banks, as well as how the diversity and abundance of fish and invertebrates influence the diversity and abundance of terrestrial animals. We examined the role landscape plays in terrestrial animal diversity, specifically how pond area, foliage diversity and human interaction affects the amount and duration of time animals spent at the ponds. We expected to see a positive relationship between plant, fish, benthic invertebrates, and terrestrial animal diversity and abundance. As for abiotic factors, we expect to see a positive trend between animal diversity and pond area and salinity. A negative relationship between human interactions and terrestrial animal activity is also expected. We hypothesize that ponds created by Hurricane Dorian will be frequented more by mammals and birds as compared to pre-Dorian ponds, due to the open landscape and easy accessibility of the younger ponds. The intentions and hypothesis were tested by capturing terrestrial animal activity using an array of wildlife cameras, deployed at both types of barrier island ponds over the same period. The activity was compared to landscape cover adjacent to the ponds and pond morphology.

7.2. Materials and Methods

Study Area

We selected a total of five ponds on North Core Banks for our study, two of which are old ponds and three that are new ponds (Figure 7.1, Table 7.1). The old ponds had denser foliage and were not easily accessible on foot when compared to new ponds. For the purposes of this study, we define terrestrial animals as any land-based mammal or avian species. We excluded insects from the study as their small size would not trigger the cameras.

Figure 7.1. Map showing the locations of the ponds studied on North Core Banks, North Carolina. Ponds investigated in this chapter are indicated with a red arrow.

Table 7.1. Coordinates of ponds studied in this chapter at North Core Banks, North Carolina.

	Latitude	*Longitude*
NP1	34° 54’ 3.87” N	76° 15’ 14.99” W
NP1.5	34° 54’ 14.9” N	76° 14’ 58.8” W
NP2	34° 55’ 22.5” N	76° 13’ 35.06” W
OP2	34° 56’ 37.74” N	76° 12’ 9.87” W
OP3	34° 56’ 50.96” N	76° 11’ 57.81” W

Camera Settings and Parameters

Surveys were conducted over a span of ten weeks from September 15th, 2021, to October 27th, 2021. A total of eight cameras were in use by the end of the sampling period (Table 7.2). Each camera recorded air temperature, date, and time, during daylight and nighttime hours. North Core Banks was visited every one to two weeks after initial camera deployment, at which time researchers checked camera functionality, battery, and memory while downloading the camera data. Each camera was affixed to a metal garden stake at a height of 0.6 - 1m above the ground. Care was taken to ensure the cameras were facing the banks of the ponds, where there was little to no foliage or where animal activity was evident--based on tracks and scat--and they were hidden as best as possible to avoid detection by animals. Three cameras were set to capture three consecutive photos in one second intervals when motion was sensed, with no quiet period during either day or night. Five cameras were set to time-lapse mode where pictures were taken every minute day and night and set to motion sensing or trigger mode.

Reconyx HC500 Hyperfire Semi-Covert IR cameras (Reconyx), were placed at New Pond 1 (NP1), New Pond 2 (NP2), and Old Pond 2 (OP2) on September 15th and 16th. The Reconyx cameras were set to time-lapse mode in addition to motion sense in order to estimate the length of consecutive time animals spent interacting with ponds. These cameras took photos every minute from 5 AM – 9 PM each day. Programming the cameras for time-lapse capture helped ensure an accurate representation of time animals spent at the pond outside of the camera's trigger area of 15.24 meters (*Hyperfire*, 2017). On September 23rd, a Moultrie A-series Game camera (Moultrie) was placed at Old Pond 3 (OP3), and a Tasco Trail Camera (Tasco) was placed at NP2. On September 29th, a Moultrie was placed at NP1 and a GardePro A3 Trail camera (GardePro) at New Pond 1.5 (NP1.5) and OP2. All cameras, other than Reconyx, were only set for motion-activation, not time-lapse. By September 29th, eight cameras were deployed on North Core Banks, five of which were motion-activated (Moultrie, Tasco, and GardePro) and three of which were both time-lapse and motion activated (Reconyx).

The use of camera traps for wildlife surveillance is a practice that has been gaining popularity as technological advancements are made. However, there are a few issues with camera traps, one of which is imperfect detection. Imperfect detection is where individuals present within a sampling area are not always detected, whether that be from a technology error, camera angle discrepancies, or lighting issues (Burton et al., 2015). We experienced a few technological issues during the study. Cameras that were set up at ponds but did not record any photos due to malfunction or corrupted SD cards were not included in this description nor the data analyses. NP1 and OP2 had cameras for the shortest length of time among all ponds due to technological malfunctions. Because of this, New Pond 3, originally chosen to be studied, was not used, as we were not able to capture images for the first four weeks of the study. Therefore, NP1.5 was chosen as researchers observed a higher abundance of animal activities.

Table 7.2. Camera deployment information at each pond chosen for this chapter. Asterisks are placed next to dates when camera malfunctions are suspected.

Pond	Camera	Capture Mode	Week of Study	Dates	Photos Taken
NP1	Reconyx	Time-lapse	4	9/13 - 9/19	8314
NP2	Reconyx	Time-lapse	4	9/13 - 9/19	7898

OP2	Reconyx	Time-lapse	4	9/13 - 9/19	7301
NP1	Reconyx	Time-lapse	5	9/20 – 9/26	6339
NP1	Moultrie	Trigger	5	9/20 – 9/26	12
NP2	Reconyx	Time-lapse	5	9/20 – 9/26	630
NP2	Tasco	Trigger	5	9/20 – 9/26	36
OP2	Reconyx	Time-lapse	5	9/20 – 9/26	6123
OP3	Moultrie	Trigger	5	9/20 – 9/26	1991
NP1	Reconyx	Time-lapse	6 and 7	9/27 – 10/10	0*
NP1	Moultrie	Trigger	6 and 7	9/27 – 10/10	211
NP2	Reconyx	Time-lapse	6 and 7	9/27 – 10/10	13294
NP2	Tasco	Trigger	6 and 7	9/27 – 10/10	120
OP2	Reconyx	Time-lapse	6 and 7	9/27 – 10/10	14153
OP2	GardePro	Trigger	6 and 7	9/27 – 10/10	342
OP3	Moultrie	Trigger	6 and 7	9/27 – 10/10	52
NP1	Reconyx	Time-lapse	8 and 9	10/11 – 10/27	805
NP1	Moultrie	Trigger	8 and 9	10/11 – 10/27	0*
NP1.5	GardePro	Trigger	8 and 9	10/11 – 10/27	7056
NP2	Reconyx	Time-lapse	8 and 9	10/11 – 10/27	17*
NP2	Tasco	Trigger	8 and 9	10/11 – 10/27	116
OP2	Reconyx	Time-lapse	8 and 9	10/11 – 10/27	46*
OP2	GardePro	Trigger	8 and 9	10/11 – 10/27	12677
OP3	Moultrie	Trigger	8 and 9	10/11 – 10/27	713

Analysis of Terrestrial Animal Community Composition

Individuals that were in consecutive photos or left and returned within a 5-minute period were recorded as a single individual and counted and grouped with their species. We define this as an occurrence. We classified animals to the lowest taxonomic level possible given the lighting, angles, and distance shot by the camera. To differentiate between species of birds present, we used the Bird ID Guide by Cornell University's Lab of Ornithology (*All About Birds*, 2019). For every animal seen, we recorded the time of the first sighting, the duration of the animal was present (using Reconyx cameras), and how many members of the same species were present in the frame. We also recorded camera specifications such as temperature and camera type. As the Reconyx cameras were recording on a time-lapse, the duration of time each animal spent at the ponds can be estimated for ponds NP1, NP2, and OP2. Measuring the duration of time animals spent using the ponds is important to infer how the animals could be interacting with the ponds differently. Taking note of how many of each species were present at one time helps determine how numerous those species may be on North Core Banks.

In addition to wildlife cameras, we investigated a 15-meter perimeter in the unvegetated areas in front of the ponds recording evidence of terrestrial animals. This includes tracks, scat, carcasses, paths, and foraging. This allowed for a better understanding and visualization of terrestrial animal interactions with the ponds, and it furthered our ability to infer what kinds of organisms used the ponds apart from those captured on camera.

For each pond studied, salinity, pond area, and relative abundances of different plant, fish, and benthic organisms were measured (see Chapters 4-6). These factors were considered in our analyses to infer if there is a correlation between them and the type and abundance of terrestrial animals present. We also examined these metrics in response to terrestrial animal diversity using either the Shannon-Weiner or the Simpson Diversity Index, whichever was more fitting for the data.

7.3. Results

Wildlife Community at Different Ponds

A total of 95,618 photos were examined and the type and number of each species, either human or animal, present in each frame was recorded. We identified a total of 32 species encompassing 22 families on North Core Banks through visual identification of wildlife camera photos. The most common families were Scolopacidae, or sandpipers, at 21.7% of all species recorded, followed by Laridae (seagulls, 13.8%) and Echimyidae (nutria, 10.4%). Ponds across North Core Banks had a higher percentage of avian occurrences compared to mammalian and reptilian; 78.6% of all animal sightings were avian, 21.0% mammalian, and 0.4% reptilian. New ponds saw 22% more birds compared to old ponds, but conversely, old ponds had 97% more mammals sighted than new ponds (Table 7.3).

Table 7.3. Percentages of each species recorded.

Class	Family	Scientific name	N	% of Total
Aves	Unknown	Unknown	39	3.83%
	Alcedinidae	Ceryle alcyon	31	3.04%
	Anatidae	Anas discors	1	0.10%
		Anas rubripes	8	0.79%
	Areidae	Unknown	12	1.18%
		Ardea alba	1	0.10%
		Ardea herodias	64	6.28%
		Butorides virescens	1	0.10%
		Egretta thula	22	2.16%
		Egretta tricolor	17	1.67%
		Nycticorax nycticorax	2	0.20%
	Canidae	Unknown	1	0.10%
	Charadriidae	Unknown	4	0.39%
		Charadrius semipalmatus	21	2.06%
		Pluvialis squatarola	11	1.08%
	Columbidae	Zenaida macroura	3	0.29%

	Corvidae	Unknown	10	0.98%
	Gaviidae	Gavia immer	1	0.10%
	Icteridae	Unknown	3	0.29%
		Quiscalus major	47	4.61%
	Laridae	Unknown	209	20.51%
		Larus argentatus	1	0.10%
	Mimdae	Dumetella carolinensis	3	0.29%
	Pelecanidae	Pelecanus occidentalis	1	0.10%
	Phalacrocoracidae	Phalacrocorax auritus	6	0.59%
	Rallidae	Rallus longirostris	11	1.08%
	Scolopacidae	Unknown	193	18.94%
		Calidris alba	6	0.59%
		Calidris fuscicollis	6	0.59%
		Calidris himantopus	2	0.20%
		Calidris minutilla	2	0.20%
		Calidris pusilla	24	2.36%
		Scolopacidae rafinesque	1	0.10%
		Tringa semipalmata	36	3.53%
		Tringa totanus	1	0.10%
	Threskiornothida	Eudocimus albus	17	1.67%
Mammalia	Canidae	Unknown	12	1.18%
	Leporidae	Unknown	28	2.75%
	Mustelidae	Lontra canadensis	22	2.16%
	Myocastoridae	Myocastor coypus	88	8.64%
	Procyoidae	Procyon lotor	48	4.71%
Reptilia	Colubridae	Unknown	1	0.10%
		Lampropeltis getula	2	0.20%

To quantify the number of organisms, we recorded the total occurrence and averaged them over the entire 10-week study. We found a high Simpson Diversity index among all ponds 0.994 ± 0.009 except for NP1, which could be attributed to the fact that seagulls and sandpipers made up 34.1% and 11.8% of all animal sightings there (Figure 7.3). There were no clear trends regarding Shannon-Weiner diversity. Reptiles were rarely seen, possibly due to the positioning, distance, and type of cameras used. Birds were the most abundant animal at all ponds, both old and new, except for OP2 where the most mammals occurred (59.0%). However, no mammals were sited at OP3. Despite OP3 having no mammal sightings, old ponds combined saw 9% more mammals than new ponds combined. While new ponds saw 22% more birds than old ponds, four species were seen only at old ponds (grey catbirds, common redshanks, American black ducks, and blue-winged teal ducks) (Figure 7.4, Table 7.3). We recorded fewer total terrestrial animals at older ponds, with 60% of all animal sightings occurring at new ponds (Figure 7.2, Table 7.3).

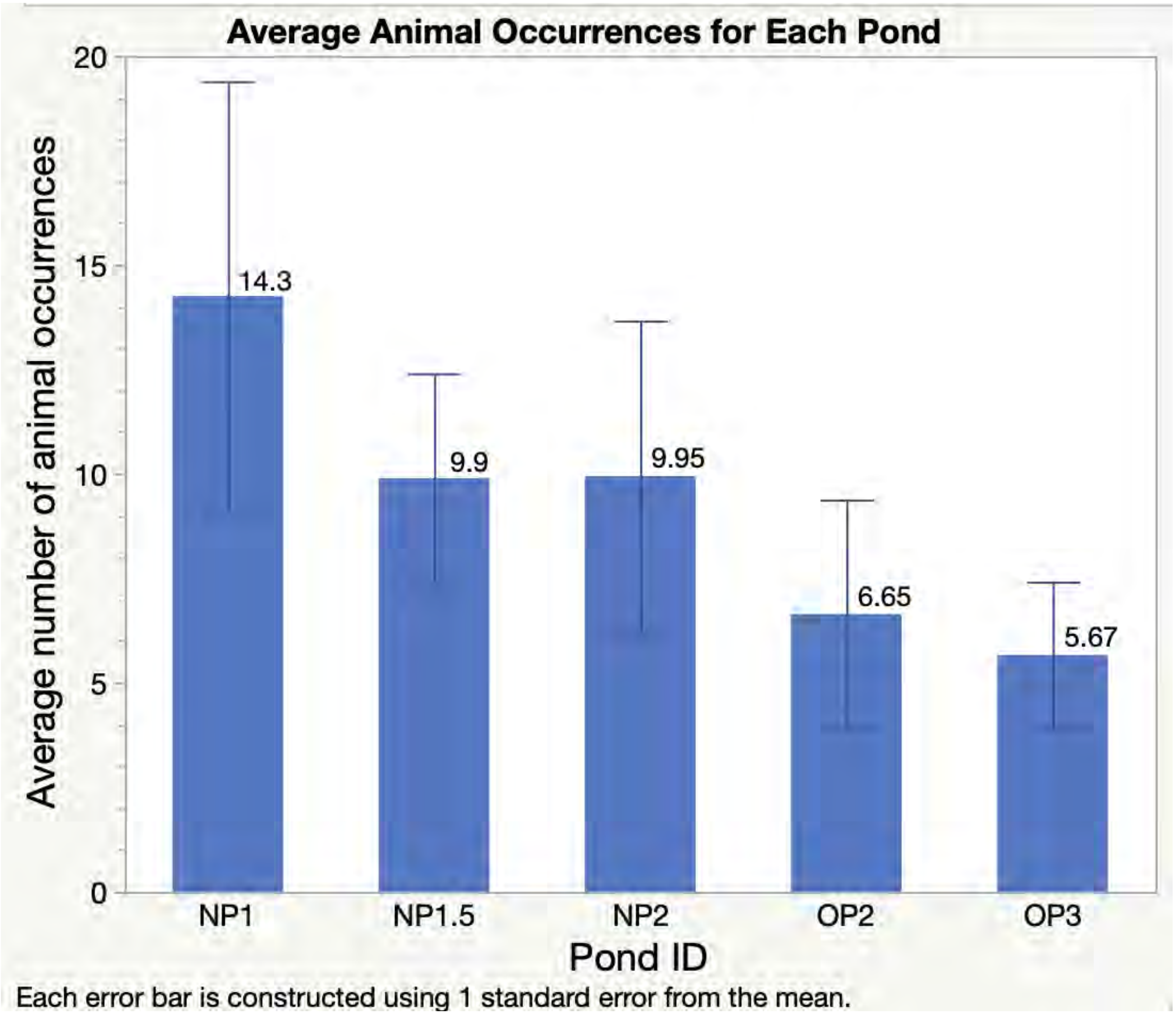


Figure 7.2. Average terrestrial animal occurrence for each pond.

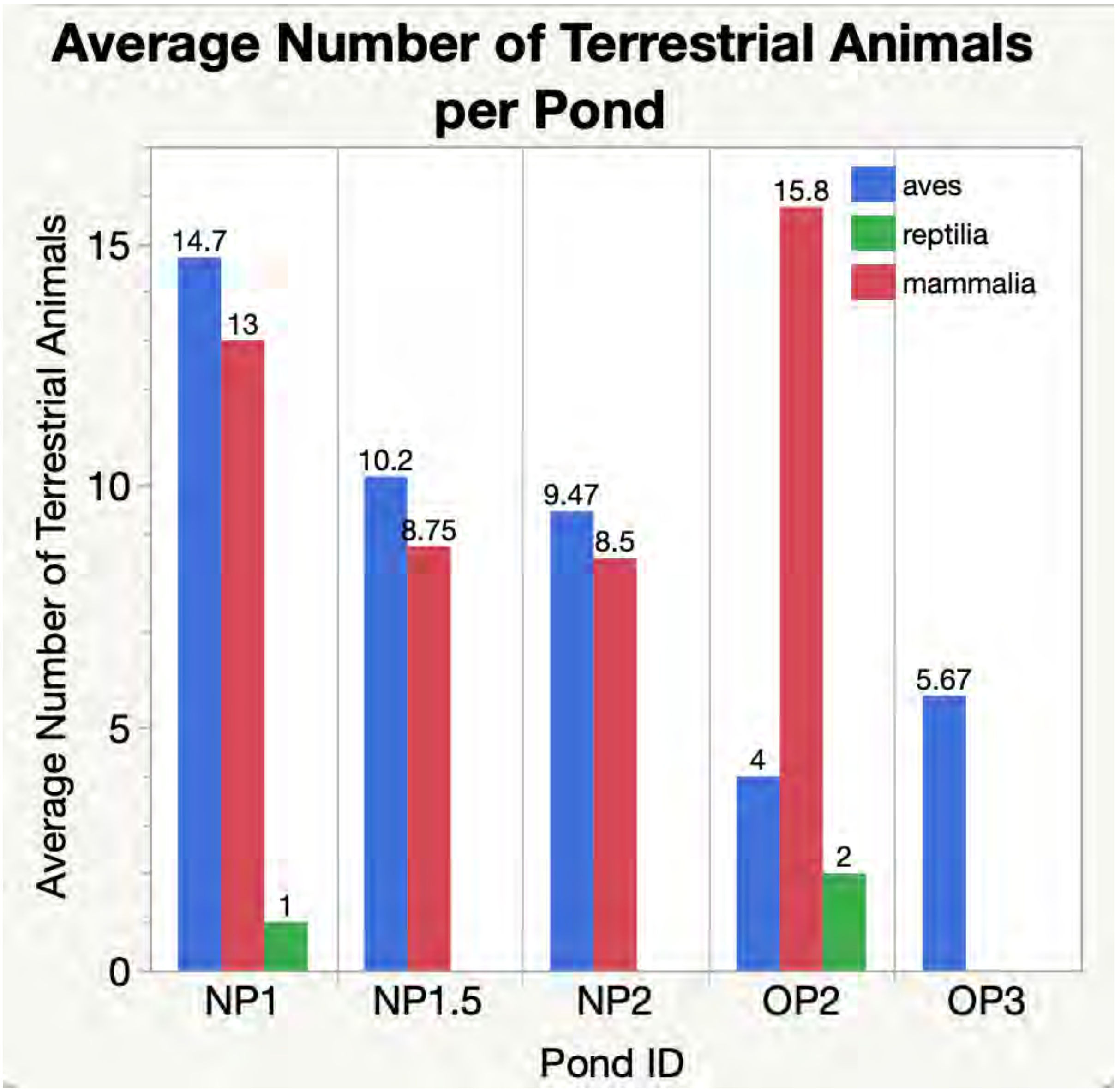
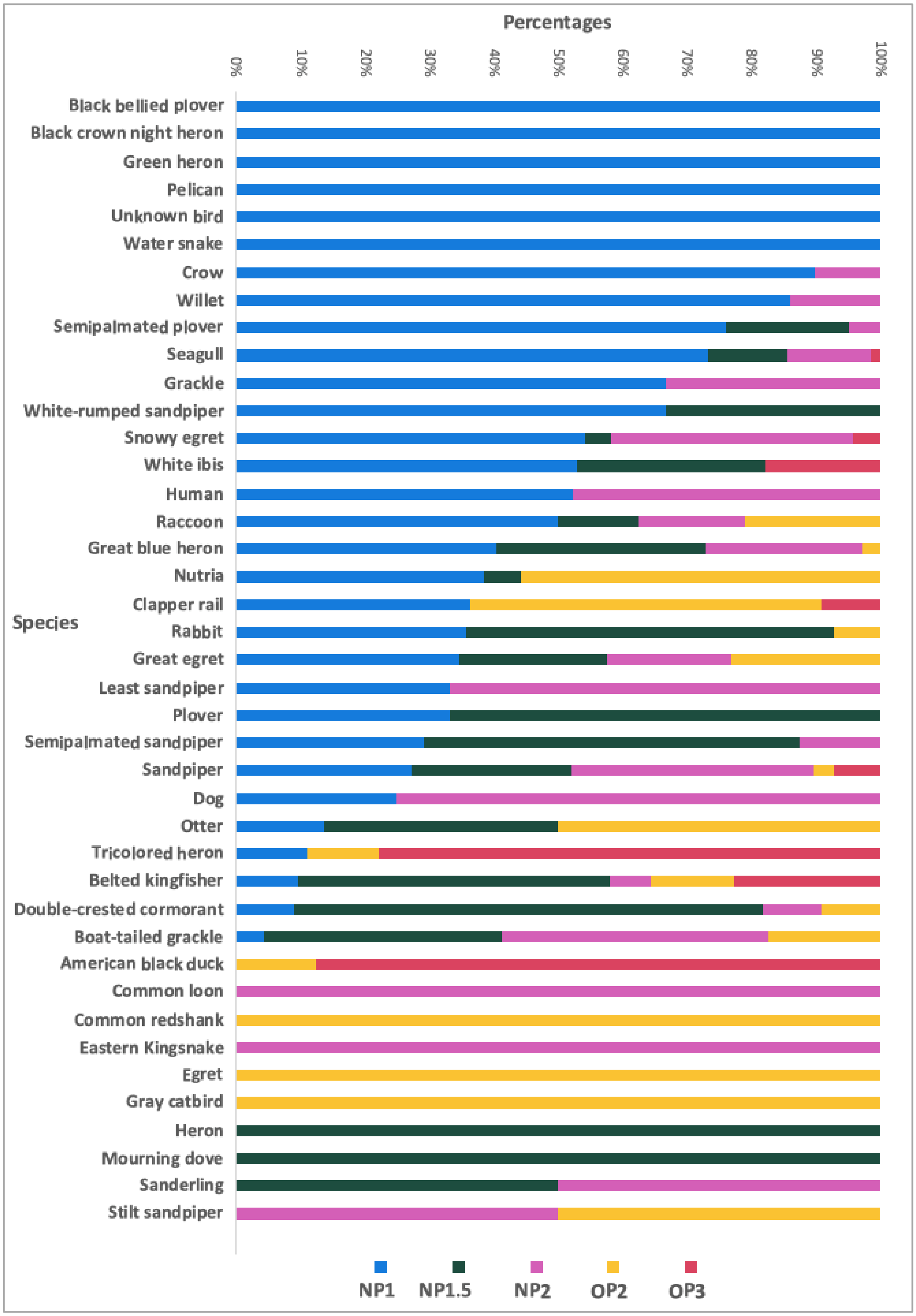


Figure 7.3. Average number of each class of terrestrial animal occurrences for each pond

Figure 7.4.



Percentages of pond distribution for each species observed.

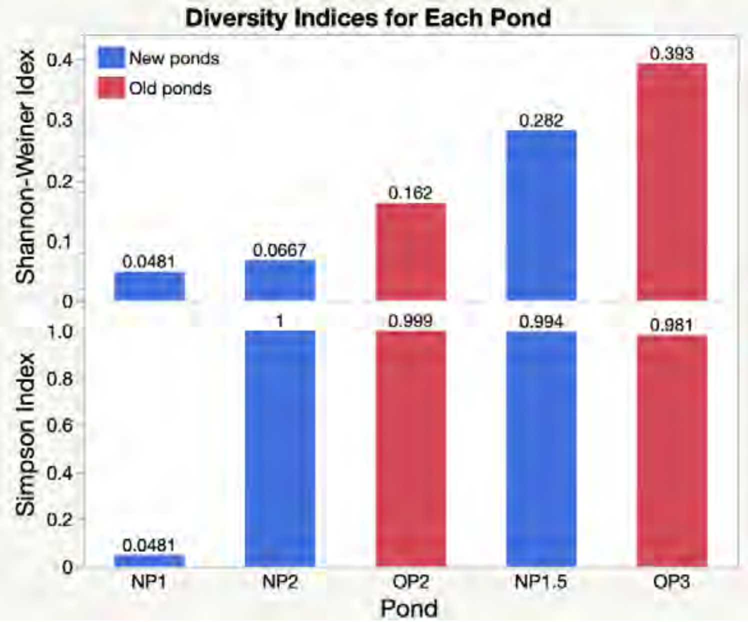


Figure 7.5. Simpson and Shannon-Weiner diversity indices for each pond.

Some photos show animals actively using the ponds to fish, wade, swim, or interact with the plants around the ponds (Figure 7.6). Families Canidae (dogs) and Anatidae (ducks) spent the longest amount of time at ponds. However, there is no major trend by classes for the longest period of time a class spent at a pond; there is an almost even distribution of mammalian and avian families (Figure 7.7). At the new ponds, birds spent the longest, an average of 5.36 minutes, while at OP2, mammals spent the most time at 6.2 minutes. Reptiles spent an equal amount of time (1 minute) at both the old and new ponds. Birds often spent an average of 10.45 minutes at NP1, 3.48 minutes at NP2, and 2.1 minutes at OP2 (Figure 7.8).

Figure 7.6. Two American black ducks feeding in Old Pond 3

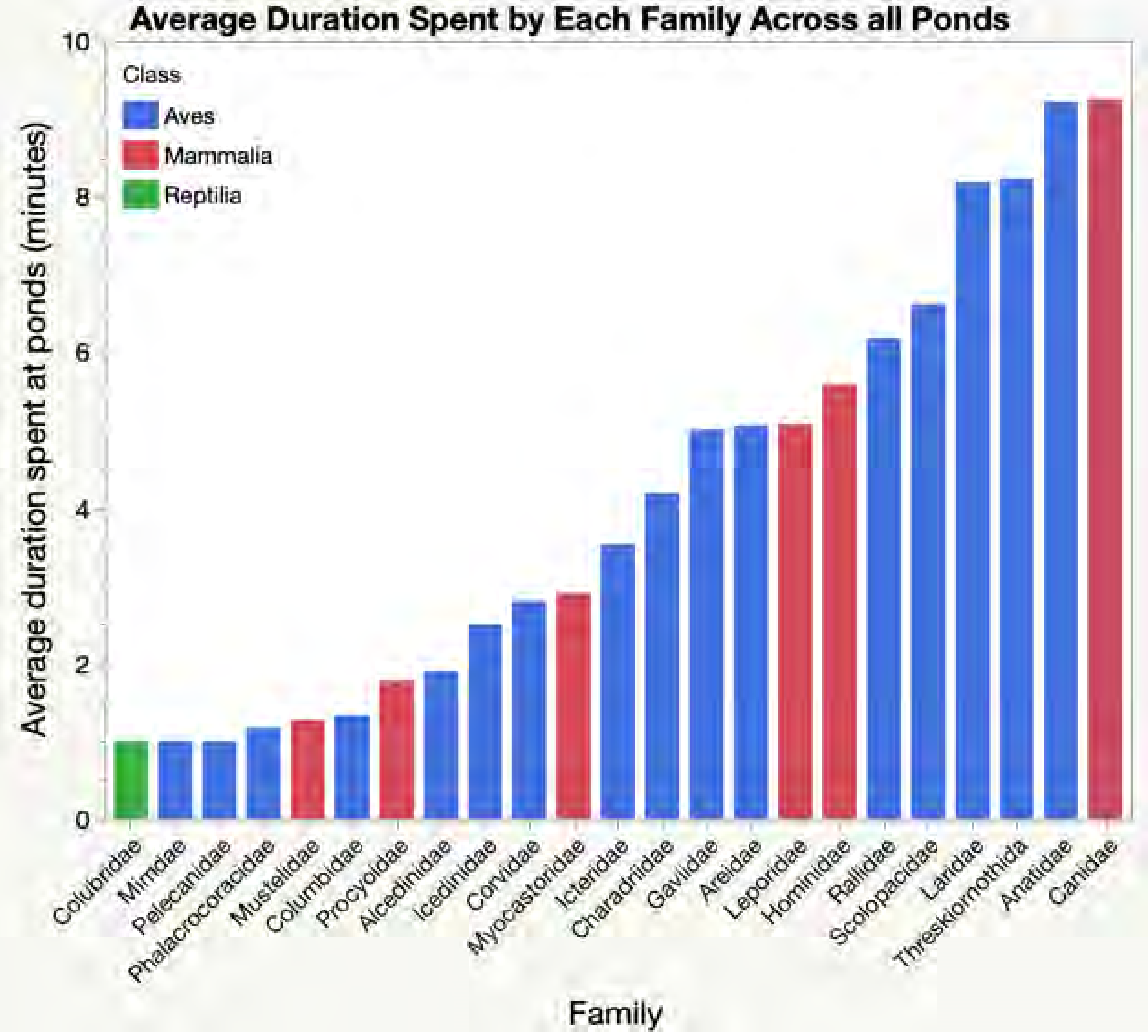


Figure 7.7. Average duration spent by each family across all ponds. See appendix for species distribution by pond.

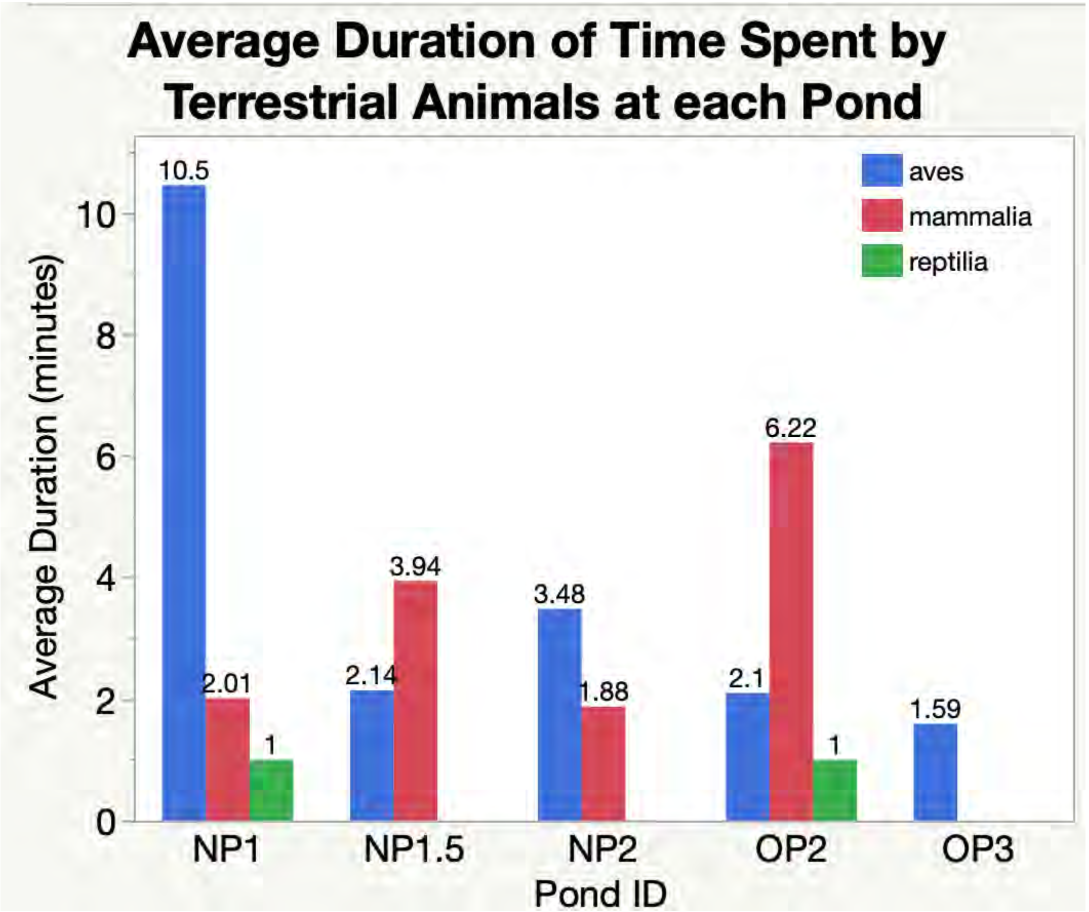


Figure 7.8. Average duration spent by each class of animal at each pond.

In order to determine the relationship between human and terrestrial animal activity across all ponds, the averages were taken of the counts of humans and animals observed over time. Humans refers to the people camping, fishing, cast netting, and more on North Core Banks, that were caught by the cameras. Researchers were not recorded. Some peaks in terrestrial animal occurrence occurred when human occurrence peaked, after September 21, not at all, a little before October 11, or were mismatched around October 21. Therefore, within the 10-week study period, there was no significant trend but a possible correlation between human activity and terrestrial animal occurrence (Figure 7.9).

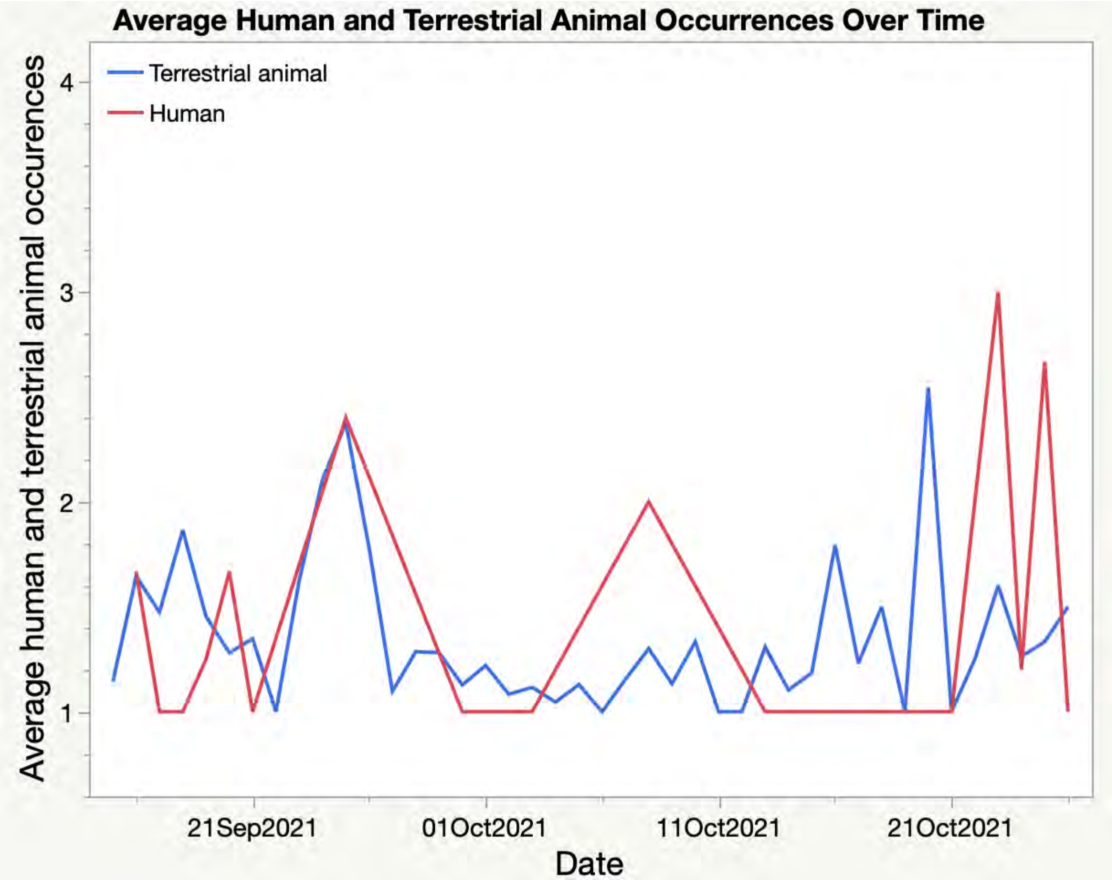


Figure 7.9. Average human and terrestrial animal occurrences over the course of the study.

As far as fish species, Silversides were the most abundant overall, especially at NP1.5, which correlates with an abundance of Scolopacidae (sandpipers) at NP1.5 and 2, and Laridae, (seagulls) at NP1, although all new ponds show a high number of sightings of both families (see Chapter 6). Rainwater killifish and sheepshead minnows are found in high amounts at NP2 where there is also a lower diversity of terrestrial species. In general, we see an insignificant (p value = 0.3417) trend, yet negative correlation between fish and terrestrial animal diversity across the ponds (Figure 7.10). We do see the highest terrestrial animal abundance at the pond with the lowest fish abundance, NP1 (Figure 7.11). This is also the pond with the highest benthic invertebrate abundance. The number of invertebrates at each pond varies significantly with Nereididae being the most prolific at NP1 and NP1.5, and Portunidae at NP2 (see Chapter 5). We do not see any clear correlation between terrestrial animal and benthic invertebrate diversity or abundance (Figure 7.12).

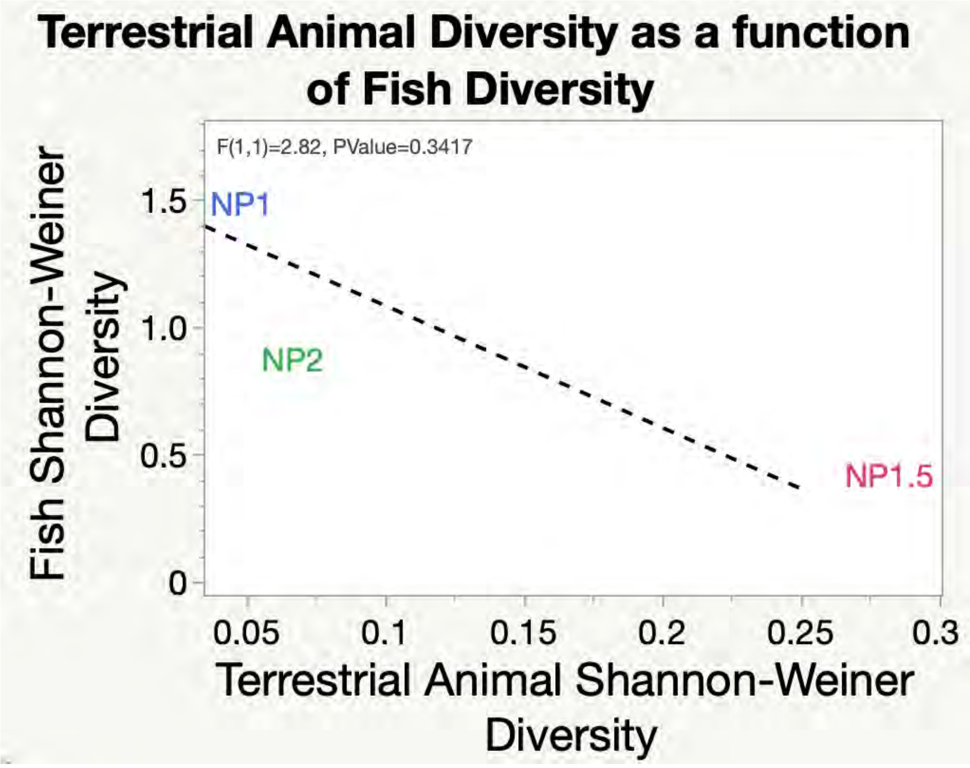


Figure 7.10. Shannon-Weiner Diversity of fish and terrestrial animal species.

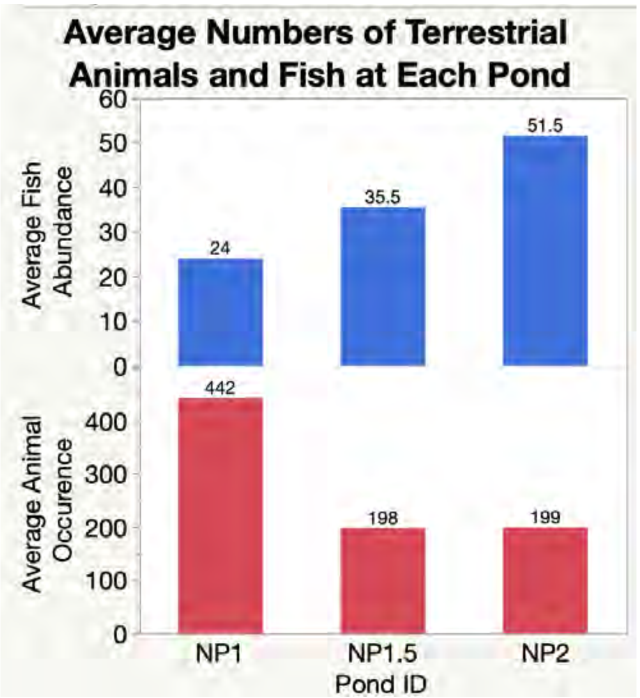


Figure 7.11. Average total animal occurrence for the entire 10-week study and average fish abundance recorded for each pond.

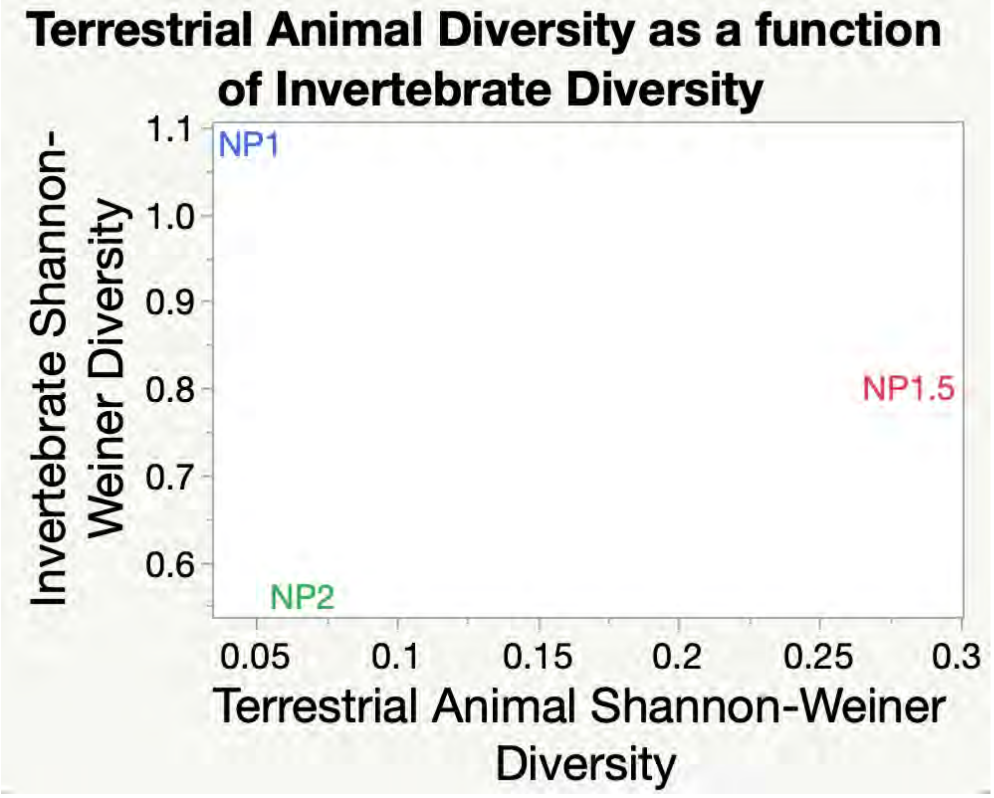


Figure 7.12. Shannon-Weiner Diversity of invertebrates and terrestrial animal species.

Landscape and Location Effects on Wildlife

To quantify if landscape of the ponds affects wildlife communities, floral abundance and diversity, pond area, and salinity were examined (see Chapters 2-5). Older ponds generally have fresher water, around 4.4-5.9 ppt, while new ponds are saline, ranging from 19.5-28.5 ppt. At the more saline new ponds, the largest number of animals was recorded, especially at NP1, but the old ponds have the smallest number, especially OP3 (Table 7.4). Shannon-Weiner's diversity index indicates a negative correlation with salinity, with diversity decreasing as ponds get more saline (Figure 7.13).

Table 7.4. The average salinity and sum of occurrences of animals at each pond over all the dates the ponds were visited

Pond	Average Salinity (ppt)	Sum of Animals Recorded at Each Pond
NP1	25.09	444
NP1.5	19.47	187
NP2	28.50	197
OP2	5.89	112
OP3	4.39	51

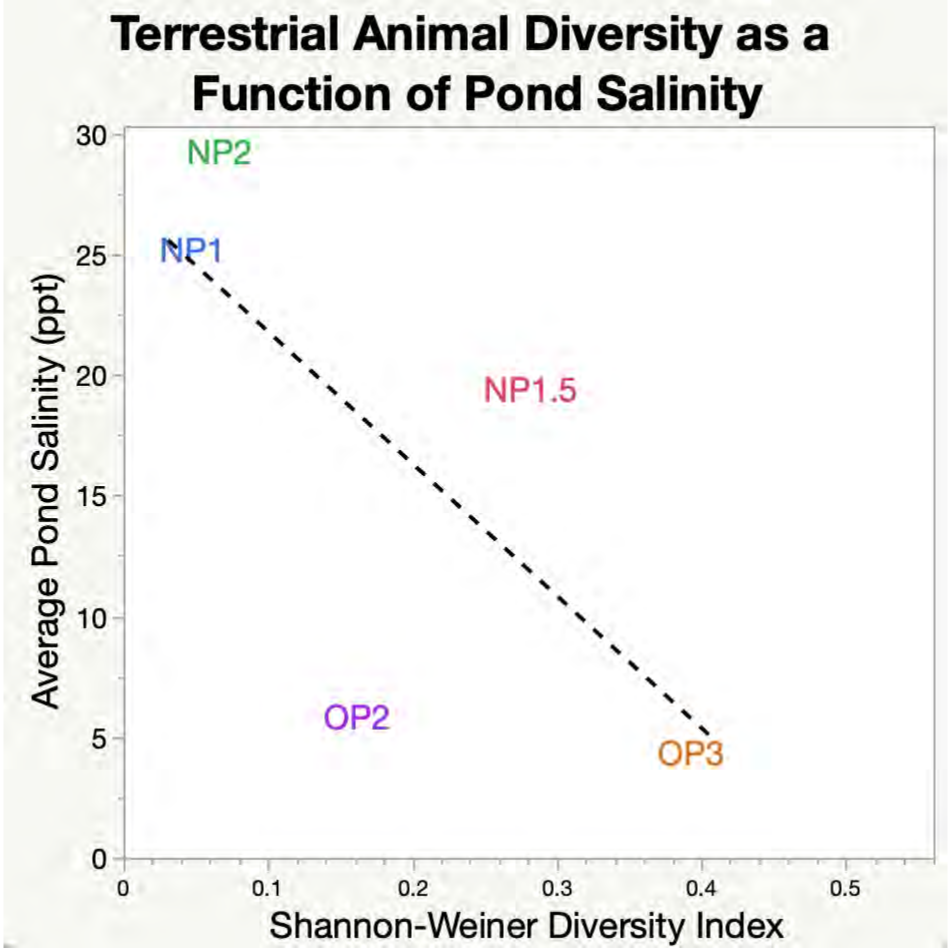


Figure 7.13. A comparison of salinity and terrestrial animal diversity

Total foliage is denser at old ponds, ranging from 502 to 605 m^{-2} at old ponds and 223 to 267 m^{-2} at new ponds (see Chapter 4). *Spartina patens* is the densest plant at each pond, with OP2 and OP3 having the highest densities (437 and 389 m^{-2}) compared to the NP1 and NP2 (95 and 162 m^{-2}). While there is no significant correlation between plant and terrestrial animal diversity, the plant diversity and density may explain animal activity at different ponds. NP1 has the highest diversity and widest variety of plant species at 12 species; NP1 also had the highest occurrence of terrestrial animals (444) but the lowest diversity. The rest of the ponds have high terrestrial animal diversity but low plant diversity (Figure 7.14, Table 7.3).

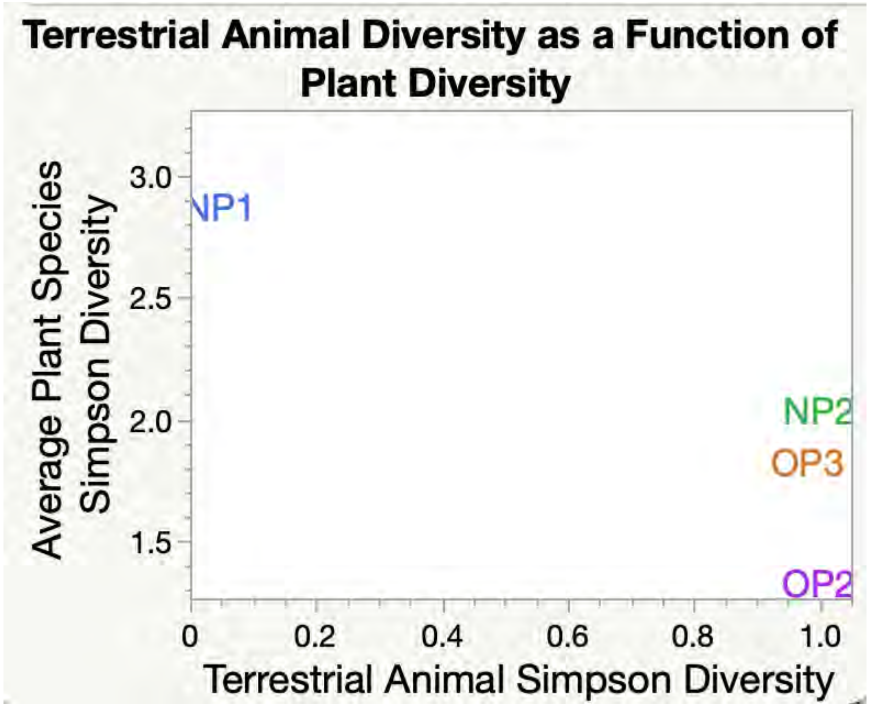


Figure 7.14. Comparing plant and terrestrial animal Simpson's Diversity indices for all ponds.

The pond area is also of importance as the size of the pond could affect what is living in it, therefore, changing what animals would visit and use the pond. NP1, NP1.5, and NP2 are all larger than OP2 and OP3 (3870m^2, 5474 m^2, 1885 m^2, and 510 m^2, 733 m^2 respectively). There is no significant trend as far as terrestrial animal diversity and pond area (Figure 7.15).

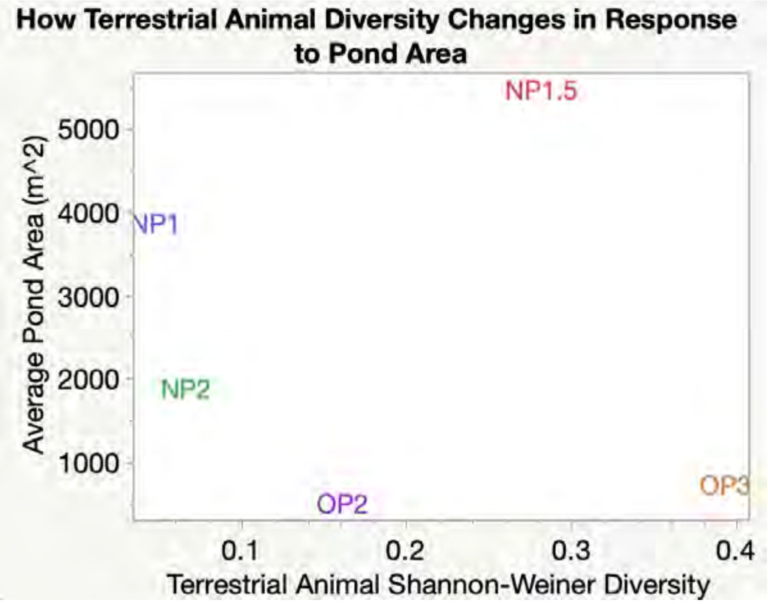


Figure 7.15. Average pond area (m^2) compared to terrestrial animal diversity.

Walking around the 15-meter perimeter of each pond studied, we found an assortment of nutria and otter tracks, as well as rabbit feces, nutria feces, and evidence of foraging activities like unearthed foliage and digging for roots. We also observed numerous canine tracks, but we were unable to dictate whether they were from wild animals, specifically coyotes, or from campers' dogs. This indicates there could have been many terrestrial animals present but not captured on camera.

7.4. Discussion

4.1. Wildlife Community at Each Pond

Wildlife cameras recorded a higher number of animal sightings at new ponds rather than old ponds, which supports the hypothesis that post-Dorian ponds will see more terrestrial animal interaction than pre-Dorian ponds. We found a high Simpson Diversity index among all ponds except for NP1, which could be attributed to the fact that seagulls and sandpipers made up the majority of animal sightings there. When measuring Shannon-Weiner diversity index, we found that NP1.5 and OP3 were the most diverse with NP1 again being the least diverse.

Excluding researchers, there were no human occurrences recorded at old ponds. We attribute this to the fact that old ponds were not easily accessible on foot, whereas new ponds were accessible right from the beach. While we expected human presence to negatively impact animals' pond usage, we did not see a clear or significant trend indicating so. Despite this, human interaction may still be a factor in the increase in mammal sightings at old ponds compared to new ponds, as mammals may be more likely to be deterred by human presence (which only occurred at new ponds). As previously mentioned, avian species have a possible fleeing advantage over land-based mammals, so human interaction we would be less likely to deter avian species from utilizing the new ponds.

Time spent at each pond is also an indicator of how animals utilize ponds. Longer times spent may indicate the animal is more actively using the pond rather than passively. Active meaning the individuals are foraging, swimming, or nesting at the pond rather than just being seen there, and passive meaning spending very little time there. The high abundance and long duration spent by birds at new ponds correlates with the finding that new ponds, specifically NP1, had the lowest fish abundance (see Chapter 6). This indicates birds, specifically sandpipers, plovers, herons, and egrets may rely on these ponds for food. Interestingly, NP1 had low fish diversity, inferring an interesting relationship between terrestrial animals' preference for ponds and whether it correlates to the species present rather than the sheer number. Species have been known to favor ponds with desirable foods, such as herons, plovers, and sandpipers foraging in shrimp ponds more so than other ponds in the area (Zou et al. 2006). It is important to note that fish and benthic invertebrates were only studied at new ponds, so we do not have any old pond data to compare with for these metrics.

Landscape Effects on Wildlife Communities

Avian species were the most common terrestrial animals among all ponds; however, mammals were far more common at OP2 compared to all 3 new ponds. This could be attributed to the fact that new ponds offer little coverage for mammals to hide in the case of predators or humans approaching, whereas birds can exit easily. This is supported by the fact that higher densities of plant species were recorded at old ponds, along with more occurrences of mammals. We also saw high terrestrial animal diversity at ponds with low plant species diversity. This may indicate animals prefer the ponds that are composed primarily of a single species they like to eat. On North Core Banks, *Spartina patens* comprised the majority of each ponds' perimeter, especially at old ponds which were frequented by mammals. Certain plants observed, such as the *Spartina patens*, *Sagittaria lancifolia*, *Schoenoplectus americanus,* and *S. californicus,* provide a nice material to make beds, good protection from the elements, and are a nice food source. Nutria, one of the mammalian species commonly seen at OP2, have been known to seek out ponds with Spartina spp. (Nolfo-Clements, 2012). The high abundance of *Spartina patens* helps to explain the large amount of nutria and other mammals observed because of the resources the plant provides.

We also observed changes in terrestrial animal diversity depending on abiotic factors like pond area and salinity. As far as pond area, we observed a higher Shannon-Weiner diversity index for both small and large ponds, with a decrease in diversity at mid-sized ponds. It is important to note that both old ponds we studied were smaller than any of the three new ponds. The small size and dense foliage at old ponds may explain why we observe a higher number of mammal occurrences, because as previously mentioned these characteristics lend themselves to safe areas for foraging. In addition, old ponds had relatively low salinity compared to new ponds which saw substantially more terrestrial animals. This indicates the animals may be favoring these ponds for a specific food type, rather than for drinking water. For example, adult white ibises are known to feed on saltwater fish, which correlates with our results of seeing more white ibises at new ponds (Chavez-Ramirez et al. 1995).

Significance

This study was conducted on behalf of the National Park Service, to determine the ecological functions and ecosystem services the ponds on North Core Banks may provide, in part, for terrestrial animals. We found a wide assortment of species, including different shorebirds, wading birds, waterfowl, and a mixture of mammals were using the ponds for food, water, bathing, or just a place to play. Many of the birds we recorded were migratory species--such as the common loon, the stilt sandpiper, and the semipalmated plover and sandpiper--and were presumably using the ponds on North Core Banks as a stopover. Ornithologists and recreational birdwatchers may want to know this information to better understand the avian communities, as well as witness different birds.

This study helped us gain a deeper understanding of the terrestrial animal diversity of North Core Banks and allowed us to keep a lookout for rare/endangered species, such as North Carolina's piping plover, or even invasive species, like nutria. While we did not identify any piping plovers, we recommend the National Park Service continue regular monitoring to ensure the future wellbeing of this species. Meanwhile, we recorded activity of an invasive species to the area: nutria. Through future monitoring, a better understanding of their populations and effects on other species can be achieved. Even though we saw a difference between animal communities at new ponds verses old ponds, we still saw animals at all the ponds observed. We also highlighted the key relationship between trophic levels on North Core Banks; the ponds with the most benthic invertebrates had the least fish and most terrestrial animals. This predator-prey relationship is important to understand to inform species conservation and management practices. No matter the age, all the ponds serve an important, unique resource for the animals on North Core Banks.

Considerations

Our study had temporal limitations, but future studies could be extended beyond 10 weeks, in order to observe possible migration patterns and other seasonal changes of the community. The number and variety of ponds studied could also be increased, to gain a broader perspective on the different community structures. Researchers could also investigate more biotic and abiotic factors, beyond fish, benthic invertebrates, plants, pond area and salinity, to figure out why they are different. More cameras could be placed at each pond, and at different heights or angles, as well as different areas. This will ensure they capture a more extensive range of animals, including more low-lying or elusive organisms, such as reptiles or coyotes. By placing cameras near a path of canine tracks, there is a chance to clarify whether we observed just dog tracks coming from campsites or coyote tracks.

Appendix

Table A.1. Break down by pond id of the percentages and number of animals by scientific name, family, and class

Pond ID	Class	Family	Scientific Name	N	% of Total
NP1	Aves	Unknown	Unknown	2	0.20%
		Alcedinidae	Ceryle alcyon	3	0.29%
		Areidae	Unknown	2	0.20%
			Ardea herodias	24	2.36%
			Butorides virescens	1	0.10%
			Egretta thula	11	1.08%
			Egretta tricolor	2	0.20%
			Nycticorax nycticorax	2	0.20%
		Canidae	Unknown	1	0.10%
		Charadriidae	Unknown	1	0.10%
			Charadrius semipalmatus	16	1.57%
			Pluvialis squatarola	11	1.08%
		Corvidae	Unknown	9	0.88%
		Icteridae	Unknown	2	0.20%
			Quiscalus major	2	0.20%
		Laridae	Unknown	153	15.01%
			Larus argentatus	1	0.10%
		Pelecanidae	Pelecanus occidentalis	1	0.10%
		Phalacrocoracidae	Phalacrocorax auritus	1	0.10%
		Rallidae	Rallus longirostris	4	0.39%
		Scolopacidae	Unknown	52	5.10%
			Calidris fuscicollis	4	0.39%
			Calidris minutilla	1	0.10%
			Calidris pusilla	7	0.69%
			Scolopacidae rafinesque	1	0.10%
			Tringa semipalmata	31	3.04%
		Threskiornothida	Eudocimus albus	9	0.88%
	Mammalia	Canidae	Unknown	2	0.20%
		Leporidae	Unknown	10	0.98%
		Mustelidae	Lontra canadensis	3	0.29%
		Myocastoridae	Myocastor coypus	34	3.34%
		Procyoidae	Procyon lotor	24	2.36%
	Reptilia	Colubridae	Unknown	1	0.10%
NP1.5	Aves	Unknown	Unknown	4	0.39%
		Alcedinidae	Ceryle alcyon	15	1.47%

		Areidae	Ardea herodias	18	1.77%
			Egretta thula	1	0.10%
		Charadriidae	Unknown	2	0.20%
			Charadrius semipalmatus	4	0.39%
		Columbidae	Zenaida macroura	1	0.10%
		Icteridae	Quiscalus major	17	1.67%
		Laridae	Unknown	26	2.55%
		Phalacrocoracidae	Phalacrocorax auritus	3	0.29%
		Scolopacidae	Unknown	48	4.71%
			Calidris alba	6	0.59%
			Calidris fuscicollis	2	0.20%
			Calidris himantopus	1	0.10%
			Calidris pusilla	14	1.37%
		Threskiornothida	Eudocimus albus	5	0.49%
	Mammalia	Leporidae	Unknown	16	1.57%
		Mustelidae	Lontra canadensis	8	0.79%
		Myocastoridae	Myocastor coypus	5	0.49%
		Procyoidae	Procyon lotor	6	0.59%
NP2	Aves	Unknown	Unknown	9	0.88%
		Alcedinidae	Ceryle alcyon	2	0.20%
		Areidae	Ardea herodias	15	1.47%
			Egretta thula	9	0.88%
		Charadriidae	Unknown	1	0.10%
			Charadrius semipalmatus	1	0.10%
		Columbidae	Zenaida macroura	1	0.10%
		Corvidae	Unknown	1	0.10%
		Gaviidae	Gavia immer	1	0.10%
		Icteridae	Unknown	1	0.10%
			Quiscalus major	19	1.86%
		Laridae	Unknown	27	2.65%
		Phalacrocoracidae	Phalacrocorax auritus	1	0.10%
		Scolopacidae	Unknown	73	7.16%
			Calidris himantopus	1	0.10%
			Calidris minutilla	1	0.10%
			Calidris pusilla	3	0.29%
			Tringa semipalmata	5	0.49%

			Tringa totanus	1	0.10%
	Mammalia	Canidae	Unknown	10	0.98%
		Procyoidae	Procyon lotor	8	0.79%
OP2	Aves	Unknown	Unknown	21	2.06%
		Alcedinidae	Ceryle alcyon	4	0.39%
		Anatidae	Anas rubripes	1	0.10%
		Areidae	Unknown	10	0.98%
			Ardea herodias	7	0.69%
			Egretta tricolor	2	0.20%
		Columbidae	Zenaida macroura	1	0.10%
		Icteridae	Quiscalus major	9	0.88%
		Mimdae	Dumetella carolinensis	3	0.29%
		Phalacrocoracidae	Phalacrocorax auritus	1	0.10%
		Rallidae	Rallus longirostris	6	0.59%
		Scolopacidae	Unknown	6	0.59%
	Mammalia	Leporidae	Unknown	2	0.20%
		Mustelidae	Lontra canadensis	11	1.08%
		Myocastoridae	Myocastor coypus	49	4.81%
		Procyoidae	Procyon lotor	10	0.98%
	Reptilia	Colubridae	Lampropeltis getula	2	0.20%
OP3	Aves	Unknown	Unknown	3	0.29%
		Alcedinidae	Ceryle alcyon	7	0.69%
		Anatidae	Anas discors	1	0.10%
			Anas rubripes	7	0.69%
		Areidae	Ardea alba	1	0.10%
			Egretta thula	1	0.10%
			Egretta tricolor	13	1.28%
		Laridae	Unknown	3	0.29%
		Rallidae	Rallus longirostris	1	0.10%
		Scolopacidae	Unknown	14	1.37%
		Threskiornothida	Eudocimus albus	3	0.29%

Chapter 8: Synthesis

To evaluate the ecological function and ecosystem services of overwash ponds found at North Core Banks we collected data on the geomorphology, hydrology, biogeochemistry, primary productivity, and biodiversity at selected ponds of various ages. While we identified many functions and services of these habitats, the main ones include providing habitat for a diverse assemblage of marine and terrestrial organisms, carbon sequestration, and supporting multiple trophic levels through food web interactions. We hypothesized that the characteristics and dynamics of the new ponds would differ significantly from older, pre-existing ponds on the island. While we did find the water quality to be distinctly different between the old and new ponds, the biodiversity and productivity within each pond were found to be unique regardless of age. The new ponds especially were found to vary extensively in both species diversity and richness. These differences may be caused by the varying degrees of connectivity of each pond to the ocean and surrounding marsh.

We found that the landward sloping washover platform in front of the newer ponds is starting to level out, will be conducive for dune formation in the near future, and is on a trajectory of morphologic change towards what existed prior to Hurricane Dorian. If this trend continues, in time, the ponds formed by Hurricane Dorian are either going to disappear or transition into old ponds. If the ponds disappear, the fishes and benthic invertebrates that serve as convenient food resources for birds and other terrestrial animals will no longer be available. However, if dunes were to form in front of the newer ponds, the ponds would be more likely to persist as the dunes are able to protect them from additional flattening and from filling in. If the ponds were to remain, the characteristics of these ponds may shift to be more similar to those of the old ponds. This shift will likely cause changes in community structure as well as increase the rate of carbon sequestration. Thus, there are many potential futures for the ponds at North Core Banks, but all potential outcomes will have a substantial impact on the dynamics of the island.

Based on our findings regarding the future of the ponds on North Core Banks, we recommend that no permanent infrastructure be built on the seaward side of these ponds where protection from dunes is not available. The geomorphology group (Chapter 1) found that the sediment in these areas is shifting at extremely fast rates. We saw changes in topography of ~0.5 m in the three short months that we were studying this area. With these rapid rates of change in topography, it will be difficult to support infrastructure long term. It is also important to regularly manage and maintain proper functioning of all septic systems on the island to prevent possible contamination and nutrient loading to nearby ponds. Water quality should also be tested regularly and, if necessary, health advisories should be posted at these ponds when harmful bacteria are found to be present. It is also important to continue monitoring these ponds for the abundance of both primary producers as well as aquatic and terrestrial animals. It is critical to continue this monitoring because, while we have collected data and observations, this was only for a brief period within a single season. How the ecological function and ecosystem services of these ponds change seasonally and interannually is not yet understood. Longer-term monitoring of these ponds and their food webs presents a unique opportunity to contribute to our understanding of geological and ecological processes. Our results coupled with further analysis can provide well-rounded synopsis on the ecological functions and ecosystem services of the ponds that the National Park Service can utilize to not only keep the public safe, but to also to preserve these habitats for their intrinsic natural value and for the enjoyment of current and future generations.

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