# EFFECTS OF OYSTER REEF SANCTUARIES ON FISH ABUNDANCE IN PAMLICO SOUND

by

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## Abstract

The value of restoring oysters to enhance natural water filtration is well-established, but the habitat value of restored reef habitat for the estuarine fish community has been less studied. Further, while artificial reef structures in the ocean, like shipwrecks, have been shown to provide valuable feeding and breeding habitat for fish, the function of artificial reefs in estuaries are poorly studied. In estuaries, the availability of adequate shelter and feeding habitat is particularly important due to the abundance of juvenile fishes. The state of North Carolina has established 14 oyster sanctuaries within Pamlico Sound as part of its shellfish restoration efforts. These oyster sanctuaries are considered artificial reef and are established with a variety of substrates including natural oyster cultch, concrete reef balls, and rip-rap marl. Acoustic imaging sonar sampling of oyster sanctuaries in Pamlico Sound was undertaken from June of 2022 to November of 2023 with the goal of comparing fish abundance on these reefs to adjacent areas without artificial substrate. The acoustic samples were analyzed to measure fish abundance and length at 26 sample points per sanctuary, which included areas outside of the sanctuary to act as a control. Fish abundance varied by a factor of 29.16 across the eight sanctuaries that were sampled. Fish abundance observed on the reef was generally higher than in the area without reef substrate, which is consistent with the belief that these reefs provide useful habit. The fish observed within the reef were also, on average, larger than fish observed outside the reef sanctuary in the control area. Understanding the value and use of oyster sanctuaries to fish in estuarine ecosystems like Pamlico Sound would provide justification for the funding and implementation of further restoration efforts.

## Introduction

An oyster reef sanctuary is defined as an area where the take or harvest of oysters is prohibited (*Oyster Sanctuaries*). The primary reason for the establishment of oyster reef sanctuaries is for shellfish restoration through providing a protected brood stock to enhance natural recruitment in the estuary. The state of North Carolina has an extensive shellfish restoration program and has established 14 restoration oyster reef sanctuaries in Pamlico Sound, which is the focus of this study. These completely subtidal restoration oyster reefs are composed of a variety of materials including natural oyster shell, rip-rap marl, reef balls, and crushed concrete. The material is typically arranged in a grid-like formation within the sanctuary boundaries, and the distance of these sanctuaries from shore varies from several hundred meters to several kilometers from shore. The most ideal locations for these sanctuaries were identified as being in the northeast and southwest portions of Pamlico Sound (Puckett et al, 2018).

The oyster reef sanctuaries sampled over the course of this study are located in North Carolina's Pamlico Sound. In 1989, the regional administrator for the United States Environmental Protection Agency Region IV, Greer C. Tidewell, wrote, "The national significance of the Albemarle-Pamlico estuarine system can not be emphasized enough. As the second largest estuary in the nation and having a principal importance of the fisheries of the eastern seaboard, this system must command our priority protective efforts," (Tursi & Okun, 1989). Pamlico Sound is an estuarine ecosystem high in ecological diversity (Settlage, 2017). It functions as important nursery habitat for a variety of species, including for a number of commercially important fish, blue crabs, and shrimp. Two rivers, the Tar-Pamlico and Neuse, feed freshwater into Pamlico Sound and saltwater flows into the sound via the Ocracoke, Hatteras, and Oregon Inlets (*The Albemarle Pamlico Region*). North of Oregon Inlet, the

Albemarle Sound is connected to Pamlico Sound, and is also a source of low salinity water to this system.

Pamlico Sound is the focus of North Carolina's Oyster Sanctuary Program. This program, started in 1996, aims to restore subtidal oyster reef habitat for the eastern oyster or *Crassostrea virginica* (North Carolina Department of Marine Fisheries Habitat & Enhancement, 2023). The state of North Carolina invests significant funds into the installation of oyster sanctuaries and enhancement of natural oyster populations in the state's coastal waters as part of its shellfish restoration program (Grabowski et al, 2011). The sanctuaries established as part of this program range in size from 10 to 80 acres (North Carolina Department of Marine Fisheries Habitat & Enhancement, 2023). These oyster reef sanctuaries provide a large and healthy natural brood stock of adult oysters that will produce oyster larvae that will distribute throughout the sound. In addition, they provide reef substrate for other oyster larvae to settle on. According to the North Carolina Department of Marine Fisheries, these oyster reef sanctuaries also improve water quality in Pamlico Sound. Oysters, as bivalves and filter feeders, are able to significantly reduce the prevalence of phytoplankton and decrease the incidence of eutrophication in waterways (Breitburg et al, 2000).

The oyster reef sanctuaries in Pamlico Sound are also considered part of the state's artificial reef system. One important way to promote the formation and maintenance of healthy habitat is through the establishment of artificial habitats (Atlantic States Marine Fisheries Commission, n.d.). Fish abundance and species richness in the broader estuarine ecosystem have both been shown to increase with the presence or establishment of artificial reefs (Folpp et. Al, 2020). Although, few studies have attempted to link artificial reefs with nearby patterns in biodiversity. The oyster reef sanctuaries in North Carolina might be important for the commercial fishing industry, as approximately one in every four oyster larvae that settle on a commercially harvested oyster reef in Pamlico Sound come from an oyster sanctuary (Peters et al, 2017). It was estimated that the state of North Carolina saw an $8.2 million increase in revenue from commercial fishing as a direct result of the establishment of these oyster reef sanctuaries (Callihan et al, 2016). Recreational fishing opportunities are increased by oyster reef sanctuaries as well, as they are open to hook-and-line recreational fishing.

My study was focused on observing the artificial reef character of Pamlico Sound's oyster reef sanctuaries. The following hypotheses were tested:

1) Fish abundance will be higher within oyster sanctuaries as compared to control areas outside the reef

2) Size structure of fish within the oyster sanctuary will be different inside versus outside the sanctuary. For example, we might see larger fish inside the sanctuary than outside.

My approach used SONAR acoustic imaging methods. Acoustic imaging was the ideal method of sampling for this project, as it does not disturb the habitat and is better able to capture the characteristics of the marine life below, existing on the often dense arrangement of structures that make up the oyster reef sanctuaries, compared to a more traditional net based sampling method. Acoustic imaging is often used to study the underwater physical environment, including both the seafloor itself and the structure, habitat, and marine life higher in the water column (Ruppel et al, 2022). Using a sampling method like acoustic imaging can also help to remove sampling length-bias that can be inherent in more commonly used sampling methods such as seines and trawls (Olson et al, 2023).

**Methods**

Field Methods for Acoustic Sampling

ARIS acoustic imaging SONAR technology was used to study eight oyster sanctuaries and surrounding empty bottom in Pamlico Sound. The ARIS is a high frequency array of 96 SONAR beams, with lenses that focus each beam to create a complete series of images saved as a video. The ARIS samples a discrete volume of water, that expands in size as distance from the instrument increases (29° horizontally and 4° vertically). The frequency of sound the ARIS uses is higher than that of most normal SONAR, which makes the sound waves more sensitive and more likely to bounce off objects in the water column. This acute sensitivity is what allows the ARIS to render the oyster sanctuary reef structures and fish in great detail, even fish smaller than 10 cm. Though the ARIS renders the reef structure and water column in great detail, it is unable to provide enough detail of the specific anatomy of the fish to use the videos to conclusively identify the species of the fish observed. Therefore, the sampling was focused on fish density and the length or size structure of the fish community present in the samples.

Each sanctuary ($N = 8$) was sampled once between June 2022 and October of 2023 (Table 1). Samples were taken at previously determined-fixed positions along two transects bisecting each reef (Figure 1). Each transect contained 13 fixed positions, with one transect running approximately north to south and the other from east to west. Each transect had the following sampling positions: 200, 150, 100, 10, edge, first-quarter, middle, third-quarter, edge, 10, 100, 150, 200; where values indicate locations outside of the sanctuary (in meters from sanctuary edge) and text-positions describe relative points within the sanctuary boundaries. The points that formed each transect were plotted in advance using Google Earth GPS software and then inputted into the boat's navigation system to ensure precise positioning of the ARIS acoustic imaging device. The sampling period for each sample position was approximately two minutes. During the sampling time, the vessel was held in position using a GPS-locked trolling motor mounted to the port-side bow. The ARIS was fixed to an aluminum pole-mount on the starboard-side stern of the vessel (Figure 2). The ARIS was set to a vertical orientation (i.e., aimed straight down), which improved detection ability of fish near the bottom and adjacent to reef structures (Figure 3). ARIS sampling frequency was 1.8 MHz, and typically sampled at 15 frames per second, with a typical minimum target resolution between 3 – 4 mm.

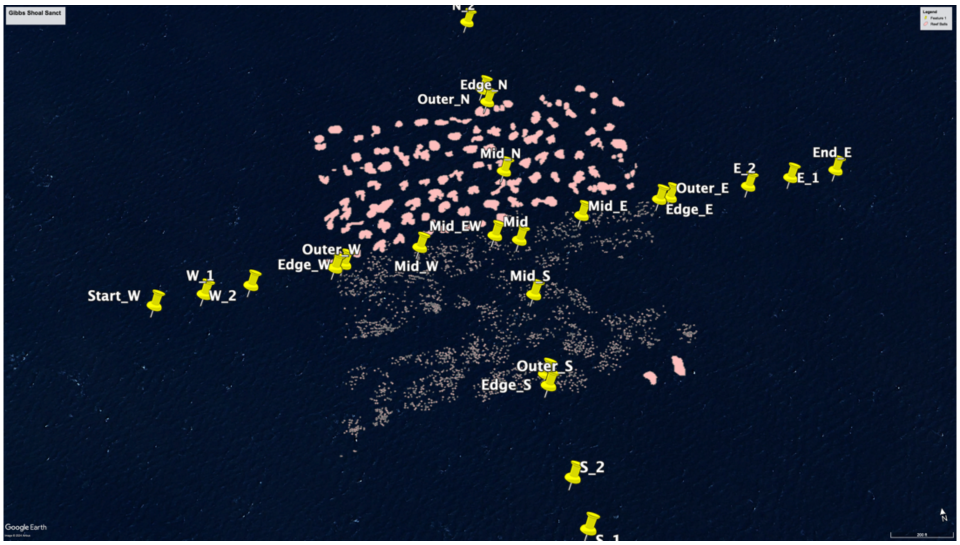

Figure 1: Google Earth image of the transects of Gibbs Shoal Oyster Sanctuary. Each pin represents a sampling point.

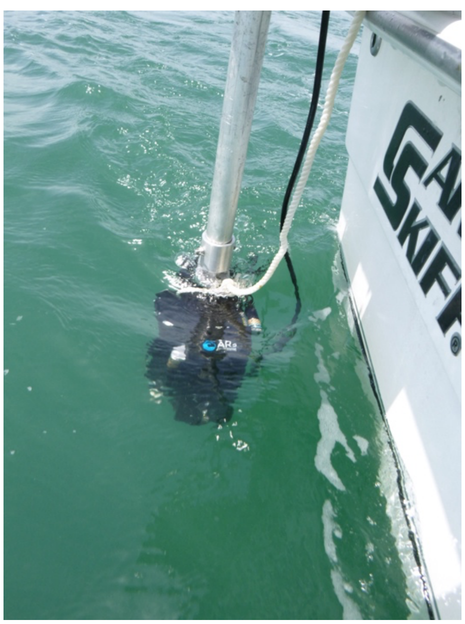
Figure 2: ARIS SONAR device deployed from research vessel.

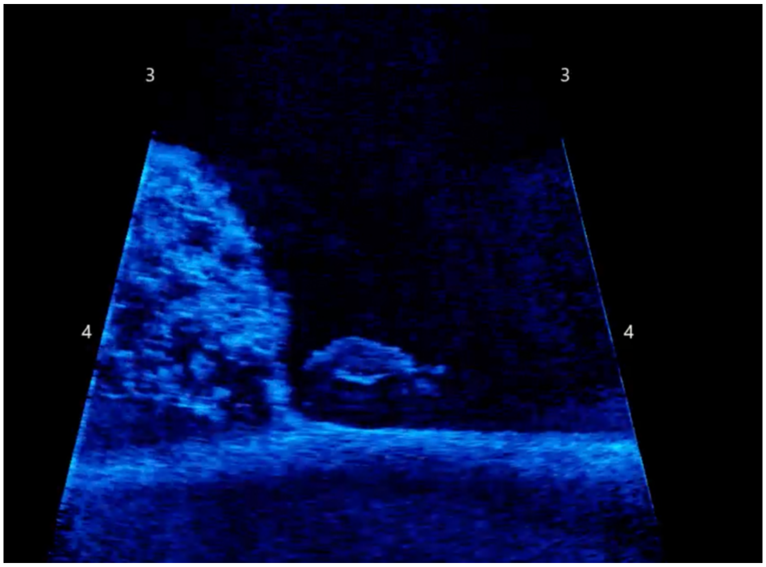

Figure 3: Image taken from ARIS video of Long Shoal Oyster Sanctuary. Contains a reef ball and what is likely a sheepshead.

Water quality samples were taken at each fixed sampling position at both surface (~0.5 m depth) and bottom (within 1 m of bottom) depths using a YSI meter equipped with temperature, salinity, dissolved oxygen, turbidity and depth sensors. Surface turbidity was also measured with a Secchi Disk. The recording of water quality varied over time (Table 2). During 2022, water quality was only taken 3-4 times at the two sanctuaries sampled that year. At the first two sanctuaries sampled during 2023, YSI measurements were taken at every sampling position. However, once it became clear that there was little variability between adjacent sampling points, a transition was made to collecting water quality measurements at every other sampling point. A stratification index was calculated for each sanctuary for both dissolved oxygen and turbidity (mg) by dividing the bottom value by the surface value measured (Table 2). In addition to the outlined water quality measurements, observations of wave height, boat heading, and the timing of sampling positions were recorded.

Data Processing

The acoustic imaging technology created a digital video file and echogram of the reef, which was later analyzed using ARISFish computer software to determine mean fish abundance and approximate size. Ten random frame subsamples were generated using R software (R Core Team 2020) for each file (i.e., file equivalent to sampling position); random frames were not allowed to be within 50 frames of each other (~3.5 seconds), which was intended to increase independence among subsamples. ARIS Fish software was used to analyze each random frame subsample for two quantitative pieces of data: fish abundance and lengths. Fish length was measured from tip to tail for consistency, and all fish were measured (see below for exception). In order to get the most accurate measurement of length, a buffer region of 10 frames on either side of the designated frame was examined and the clearest of those frames was chosen for measurement of a given fish. However, it is important to note that only the fish visible in the designated random frame were counted, even if additional fish came into view within the buffer region. In addition to counting and measuring the fish in each frame sample, notes were made about the type of habitat or bottom substrate present at each sampling position. All information from the field data sheets for each sampling position at each sanctuary was reviewed and imported into a master Excel file.

On rare occasions, if there were large numbers of small prey fish (> ~50) in a given frame, a subsampling protocol was used. A grid system was laid over the frame, which is an option within ARISFish software, and a random number generator was used to select a cell from each row of the grid containing fish. The fish in those cells were then counted and the total number was extrapolated as appropriate. Additionally, ten random fish were measured from the prey school and the mean length from those ten was used to represent the entire school (range in mean length was 4.1 to 8.7 cm). This subsampling protocol was used for 21 frames, from 10 different files (i.e., multiple files had more than one frame requiring subsampling) and three different sanctuaries (Deep Bay, Long Shoal, and Gibbs Shoal).

Data Analysis

Once all fish count and length data were collected into spreadsheets using ARISFish, a master file was compiled using R. For estimating mean fish abundance per sampling position, the fish count in each frame was natural-log transformed (first adding 1 to allow log transformation of zero values) and then the ten random subsamples were averaged.  For this analysis, the fish abundance data was log transformed because the data were not normally distributed. By log transforming the data, it better meets the assumptions of a linear model where the data are normally distributed around the regression line. Fish length was treated in a similar manner as the count data. First, if multiple fish were present on a randomly selected subsample frame, then these lengths were averaged together. Next, all subsample frames where fish were present within a file were averaged in order to get a mean length value per sampling position For this approach, frames with no fish were disregarded. Both mean fish abundance (logged) and mean fish length were analyzed against distance from the sanctuary edge using a random effects regression model, where the differences in mean values between sanctuaries was included as the random effect. This allowed me to examine the effect of the sanctuary on fish abundance and length, while standardizing among the sanctuaries, which had differences among them.

In addition to examining large-scale patterns in abundance and length across the sanctuary boundaries, the data were also narrowed to include only the sampling positions within the reef. With this data subset, mean fish abundance (logged) and mean length were analyzed against the specific habitat types present across samples. These habitat types included reef balls, rocky rip-rap marl, and bare area. Analysis of variance of mean fish abundance and mean length versus artificial substrate type was conducted.

## Results

The random effects regression model, which standardized the differences among sanctuaries, for the mean fish abundance versus distance from reef edge resulted in a *P*-value of 0.0013 and a $r^2$ of 0.132. This result indicates that mean fish abundance showed a significant decline from the center of the sanctuary to the outermost "control" areas of the transects (Figure 4. Indeed, fish abundance was found to be higher inside versus outside the oyster reef sanctuaries by a factor of approximately 2.6 when averaged across all sanctuaries..

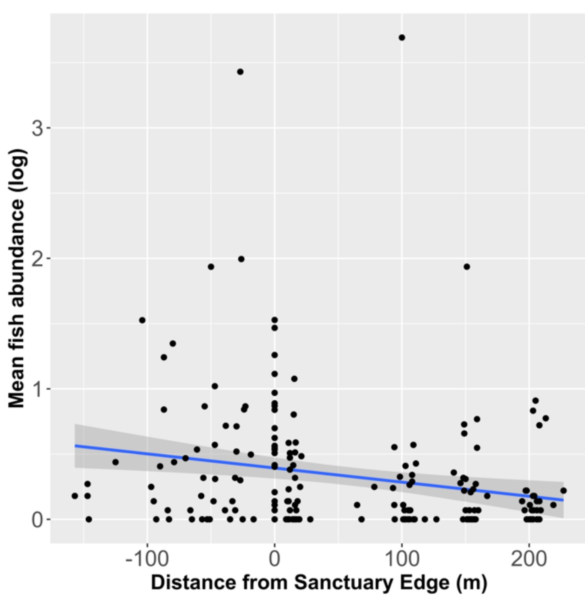


Figure 4: Mean fish abundance (log) vs. distance from sanctuary edge. Zero indicates the edge of the reef sanctuary, negative numbers indicate area inside the reef, and positive numbers indicate control area outside the reef. The *P*-value is 2.6 and the $r^2$ is 0.132.

Similar to the abundance results, the random effects model carried out on mean fish length versus distance from sanctuary edge resulted in a *P*-value of 0.022 and a $r^2$ of 0.275, which also indicates a significant result. The average fish length was found to be 1.3 times higher inside the oyster reef sanctuaries as compared to the bare control area outside the reef edge, averaged across all sanctuaries (Figure 5).

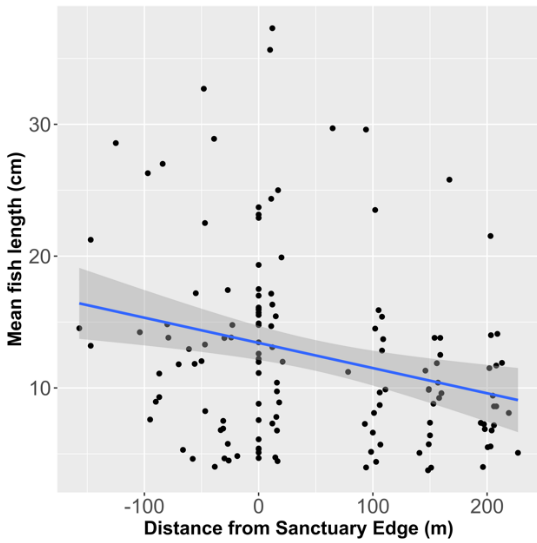


Figure 5: Mean fish length vs. distance from sanctuary edge. Zero indicates the edge of the reef sanctuary, negative numbers indicate area inside the reef, and positive numbers indicate control area outside the reef. The *P*-value is 0.022 and the $r^2$ is 0.275.

The mean fish abundance and length data was also analyzed against the habitat types inside the sanctuary boundaries, disregarding the bare control area outside the reef. The analysis of variance of mean fish abundance versus habitat resulted in a *P*-value of 0.779. This indicates that there is no significant difference in mean fish abundance among the three different substrate types. Though this result was not technically significant, there was a general trend of a higher mean fish abundance over rocky substrate (Figure 6). The analysis of variance for mean fish length versus habitat type resulted in a significant *P*-value of 0.0106. A general trend of smaller fish lengths over the rocky habitat as compared to the reef balls or bare habitat within the sanctuary boundaries was observed (Figure 7).

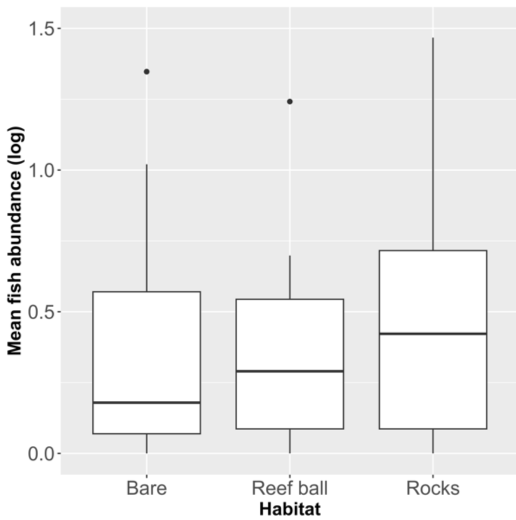

Figure 6: Mean fish abundance vs. habitat type within the oyster sanctuary boundaries. *P*-value of 0.779

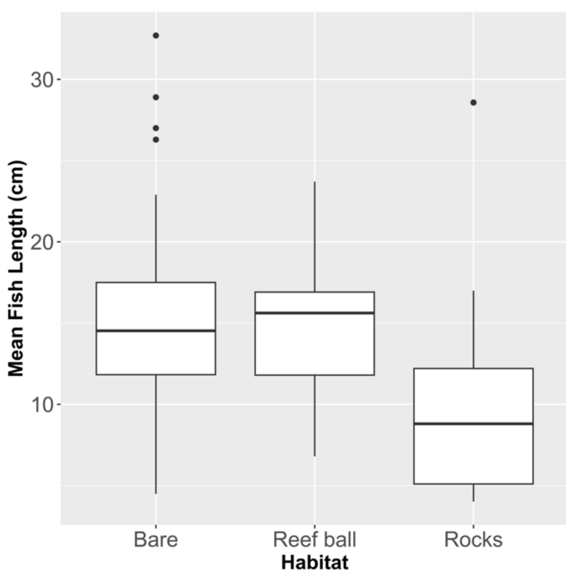

Figure 7: Mean fish length vs. habitat type within oyster sanctuary. *P*-value of 0.0106

The average number of fish across sanctuaries varied between sanctuaries by up to a factor of 29.16 (Figure 8 Table 1). The average fish value in Table 1 (i.e., Avg. Fish) includes fish abundance values for the bare control area outside the reef, where less fish were typically observed, but this metric provides an overview of fish abundance in the region on the sampling date. The surface and bottom temperatures remained relatively similar, with little difference being observed between surface and bottom values at most of the oyster reef sanctuaries sampled (Table 1). There was slightly more stratification seen in the dissolved oxygen and turbidity values (Table 2). For example, Little Creek had the lowest dissolved oxygen stratification and Long Shoal showed the greatest difference in surface and bottom dissolved oxygen. Indeed, sea conditions when sampling at Long Shoal were relatively calm, which would have reduced the ability of more oxygenated surface waters to mix deeper into the water column. Pea Island showed the highest level of stratification in turbidity, with a lower bottom than surface turbidity (Table 2).

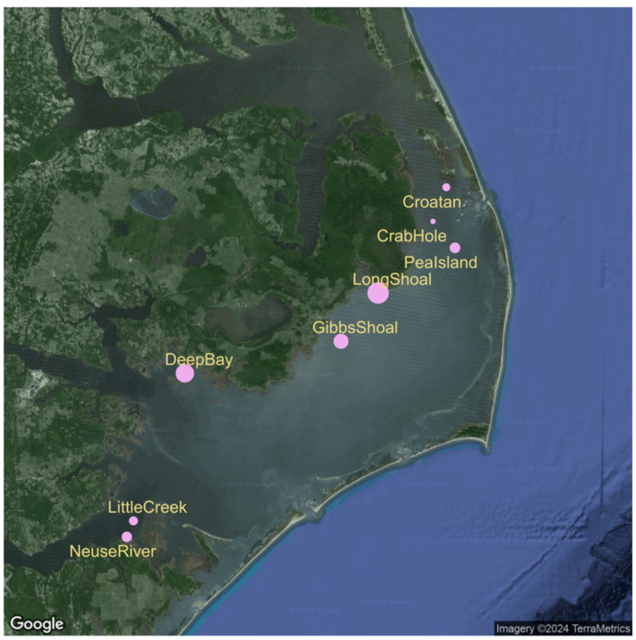


Figure 8: Map of fish abundance at each sanctuary sampled. Size of dot represents relative fish abundance.

Table 1: Water quality data averages for oyster reef sanctuaries

| Sanctuary (# of water quality samples) | **Date Sampled** | **Avg. Surf. Temp. (ºC)** | **Avg. Bot. Temp (ºC)** | **Avg. Surf DO (%)** | **Avg. Bot DO (%)** | **Avg. Surf DO (mg)** | **Avg. Bot DO (mg)** | **Avg. Surf Sal.** | **Avg. Bot Sal.** | **Avg. Surf Turb** | **Avg. Bot Turb** | **Avg. Turb (Secchi)** | **Avg. Fish** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Croatan (n=3)** | 6/15/22 | 27 | 27 | N/A | N/A | 7.5 | 7.0 | 12.9 | 13.4 | N/A | N/A | N/A | 0.43 |
| **Deep Bay (n=4)** | 7/11/22 | 27 | 27 | N/A | N/A | 6.9 | 6.8 | 19.7 | 19.7 | N/A | N/A | 0.8 | 4.95 |
| **Long Shoal (n=26)** | 5/22/23 | 21 | 21 | 94 | 74 | 7.8 | 5.9 | N/A | N/A | 5 | 10 | 1.2 m | 7.29 |
| **Crab Hole (n=14)** | 5/30/23 | 20 | 18 | 114 | 107 | 9.4 | 8.4 | 23.9 | 28.5 | 4.7 | 3.1 | 1.1 m | 0.25 |
| **Pea Island (n=11)** | 5/30/23 | 20 | 18 | 106 | 87 | 8.4 | 6.9 | 24.0 | 29.5 | 3.9 | 1.8 | 1.3 m | 1.03 |
| **Gibbs Shoal (n=26)** | 6/9/23 | 22 | 22 | 93 | 85 | 7.2 | 6.5 | 23.9 | 24.1 | 6.3 | 10.4 | 1.0 m | 2.83 |
| **Neuse River (n=14)** | 10/25/23 | 18 | 18 | 103 | 97 | 8.5 | 8.0 | 22.4 | 23.0 | 2.15 | 3.4 | 1.5 m | 0.96 |
| **Little Creek (n=14)** | 10/25/23 | 19 | 18 | 102 | 99 | 8.2 | 8.1 | 22.4 | 23.7 | 1.9 | 3.8 | 1.5 m | 0.61 |

Table 2: Stratification Index for DO (mg) and Turbidity (YSI value)

| | **Stratification Index for DO (mg)** | **Stratification Index for Turbidity** |
|---|---|---|
| **Croatan (n=3)** | 0.933 | N/A |
| **Deep Bay (n=4)** | 0.986 | N/A |
| **Long Shoal (n=26)** | 0.756 | 2 |
| **Crab Hole (n=14)** | 0.894 | 0.660 |
| **Pea Island (n=11)** | 0.821 | 0.462 |
| **Gibbs Shoal (n=26)** | 0.903 | 1.651 |
| **Neuse River (n=14)** | 0.941 | 1.581 |
| **Little Creek (n=14)** | 0.988 | 2 |

**Discussion**

The oyster reef sanctuaries sampled in Pamlico Sound do affect the fish communities in the area in which they are established. This effect is seen primarily in shifts in size structure and abundance of the fish populations. Larger numbers of fish are observed within these sanctuaries and the mean size of the fish in these sanctuaries is larger within the sanctuaries as compared to the control area. It is possible that the larger sized fish observed within the sanctuaries indicates that there are different species assemblages present inside as compared to outside the sanctuaries. As older fish tend to be larger, the larger fish observed within the sanctuaries could also be older individuals that prefer the oyster reef structure. When analyzing the random frame subsamples from the ARIS, I noticed that much larger fish with more distinct body types that might suggest larger reef dwelling and predatorial fish species, such as sheepshead and possibly even cobia. In multiple frame subsamples, large fish with elongated bodies and short dorsal fin spines were observed. These fish were also generally observed alone, indicating that they were likely not schooling fish species. It does seem though, especially considering the scarcity of other highly structured habitat like these sanctuaries in Pamlico Sound, that the restoration oyster reef sanctuaries are providing and enhancing habitat for fish in this area.

It is usually not possible to determine the specific species of fish based on the ARIS imagery, but there is evidence that larger fish such as sheepshead (*Archosargus probatocephalus*) were present in the oyster reef sanctuaries, which likely was a major driver of the pattern we observed in mean fish length across the sanctuary boundaries. Recreational anglers were observed catching sheepshead on multiple of these sanctuaries while sampling was occurring. Indeed, many of the larger fish observed around reef ball substrate appeared to have the sheepshead body form. Interesting patterns in small-schooling fish were also apparent within sanctuaries. The observation of smaller fish over rocky habitat is likely due to smaller prey or schooling fish aggregating in these areas. It is possible that the aggregation of these smaller fish might attract larger predators to these oyster reef sanctuaries and further contributed to the larger fish sizes seen inside the sanctuaries as compared to on the bare control area outside. These smaller fish are likely utilizing the sanctuaries as habitat and possibly feeding ground, as oyster sanctuaries can house small invertebrates and shellfish that fish, especially those species often found on reefs, might commonly feed on.

Fish abundance varied among the eight sanctuaries sampled, which was to be expected (Figure 4; Table 1). Differences in fish abundance between the different oyster reef sanctuaries sampled is likely due to a number of factors, including seasonal effects and sanctuary distance from shore. For example, the two sanctuaries with the highest overall fish abundance, Long Shoal and Deep Bay, were the two oyster reef sanctuaries closest to shore. The eight sanctuaries were also sampled at times with variable seasonal temperatures and effects between the dates of June 2022 and October 2023. The seasonal effects influencing the sanctuaries may have impacted the fish abundance on the reefs at the time of sampling. Little Creek and Neuse River sanctuaries were both sampled later in the year, when temperatures were colder, and lower than average fish abundances were observed at both. Repeated sampling of these sanctuaries over the course of one or more seasons would be required to truly understand how the time of year and seasonal climate affects fish abundance on these reefs, but I hypothesize that warmer water temperatures are positively related to fish abundance.

In addition to temperature, dissolved oxygen levels, especially bottom values, can play a role in the number of fish present in an area. If the bottom dissolved oxygen levels are too low, it can drive fish higher up in the water column or out of the area altogether. However, Deep Bay and Long Shoal both showed lower dissolved oxygen values, in milligrams, than many of the other sanctuaries, so this may not have been a significant contributing factor to the variation in fish abundance observed between these eight oyster reef sanctuaries. Despite the reduced levels of DO on those sampling dates, and higher stratification at Deep Bay on that day, a threshold of oxygen limit.

## Conclusion

The foremost and most significant conclusion that can be drawn from this data is that both fish abundance and length are higher inside oyster reef sanctuaries as compared to control bare area outside the reef structures. This, in conjunction with the finding that there are high numbers of small schooling fish aggregating over rocky habitat within the oyster reef sanctuaries, supports the assumption that oyster reef sanctuaries can and do function as artificial reef habitat for fish. This also helps justify the funding devoted to establishing restoration oyster reefs in Pamlico Sound by the state of North Carolina. In addition to supporting shellfish restoration and improving water quality, these oyster reef sanctuaries serve as additional habitat for fish. It is possible that the funding of these sanctuaries could also be justified by their support of recreational fishing and because they attract fish while having other positive ecological impacts on the environment. An economic benefit analysis on recreational fishing use of the oyster sanctuaries would be useful for the state of North Carolina. Further questions for study could include more detailed analysis of how these oyster reef sanctuaries affect and possibly benefit the broader populations within the estuary (e.g., fish spawning aggregations).

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