Oyster Reef Functions and Services
An Environmental Assessment of Sites Open and Closed to Shellfishing at Hoop Pole Creek

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Fall 2014 Capstone Project, Morehead City Field Site
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The University of North Carolina at Chapel Hill Institute of Marine Sciences
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Background and Policy

Oyster reef habitat has decreased by approximately 85% globally over the past 130 years while landings of Eastern oysters, *Crassostrea virginica*, have been reduced to 1-2% of their peak historic production in the Southeastern and mid-Atlantic United States (Grabowski et al., 2012; Grabowski & Peterson, 2007). This decline is attributable to overfishing and harmful harvesting practices as well as water quality degradation and disease (Grabowski & Peterson, 2007).

Overfishing of oyster reefs has been taking place along the mid-Atlantic and Southeastern coast of the United States for more than a century. By the 1880s, the local commercial oyster fishery was expanding, and overfishing of oysters in Maryland and Virginia pushed dredging operations South to begin exploiting North Carolina’s oyster resources. By 1891, overfishing of North Carolina’s oysters had become so severe that the NC General Assembly passed a law permitting the governor to use military force against dredgers for a period of three months in an attempt to drive out non-native dredge operations (Powell, 2006).

Oyster fisheries on the NC coast continue to be economically and socially important. Oyster harvesting is permitted from October to March for oysters that have a minimum length of three inches. Harvesting is largely accomplished with tongs, rakes or by hand; although dredging occurs in some areas of the Pamlico Sound (NCDENR, 2014). The North Carolina oyster harvest in 2013 produced approximately 61,500 bushels of oysters. This is a substantial decline of 92% from the peak harvest in 1902 of 800,000 bushels (NC Coastal Federation, 2014). The North Carolina Division of Marine Fisheries (NCDMF) currently considers the oyster population in NC to be a species of concern (NCDENR, 2014).

Sharp declines in oyster populations have severe impacts on oyster fisheries as well as local ecology, due to the ecosystem services provided by oyster reefs and their role as ecosystem engineers (Grabowski & Peterson, 2007). For several decades, oyster reef restoration projects have taken place along the NC coast in an attempt to mitigate the negative effects of oyster population decline. The first large-scale restoration project occurred in NC in the late 1930s, in which millions of bushels of oyster seed were planted in shellfishing areas by workers of several New Deal agencies. The NCDMF has periodically deposited marl and other substrate in shellfishing areas since the 1950s for the purpose of facilitating colonization of oyster spat and increasing the area of oyster reefs (NCDENR, 2014; NC Coastal Federation, 2014). Traditionally, oyster reef restoration projects were undertaken for the purpose of supporting oyster fisheries. In recent years, there has been increasing interest by the NCDMF and other organizations such as the North Carolina Coastal Federation and the Nature Conservancy, in building reefs in “no-take” zones to protect oyster populations and the ecosystem services provided by oyster reefs (NC Coastal Federation, 2014).

One major ecosystem service provided by healthy oyster reefs is creating habitat for juvenile fish, crustaceans, invertebrates and other mollusks of commercial and recreational importance. Based on data from oyster reef restoration projects throughout the Southeastern United States, it is estimated that each 10 m² of restored reef produces an additional 2.6 kg of fish and mobile crustaceans per year. The cumulative economic value of the augmented fish production may prove to be more economically valuable than the net profit of the harvested oysters (Grabowski & Peterson, 2007).

Oyster reefs also provide many ecosystem services through water filtration. The Eastern oyster has the ability to filter particles of up to 5 micrometers, which has a marked effect on
turbidity and water clarity. The resulting increased light penetration benefits submerged aquatic vegetation and increases the productivity of the habitat (Ermgassen et al., 2012).

Furthermore, oysters can mediate cycling of nitrogen and carbon in estuaries. Oyster reefs concentrate sediments and increase rates of denitrification, reducing anthropogenic nitrogen in the water column (Grabowski & Peterson, 2007). Reducing nitrogen concentrations could help reduce phytoplankton blooms. Oysters also enhance benthic-pelagic coupling of carbon within the system (Coen et al., 2007).

Oyster reefs attenuate wave energy, which can help to stabilize shorelines and protect critical estuarine habitats such as salt marshes. Oyster reefs may provide an alternative to hard structure shoreline protection methods, such as bulkheads. This service is increasingly important as climate change and sea level rise impact coastal shorelines, and as coastal development and increased boat traffic contribute to higher rates of erosion (Brumbaugh et al., 2010; Coen et al., 2007).

Restoring oyster reefs in areas closed to shellfishing may help to enhance resistance to the protozoan oyster disease (*Perkinsis marinus*), which has impeded many recent restoration projects and decreased oyster success and survival. Sanctuaries enhance genetic diversity by reducing fishing pressure, allowing natural selection to select for resistance to disease over many generations (Powers et al., 2009). In addition, preventing harvest of restored reefs helps ensure long-term reef survival and maximizes ecosystem services. Fishing disturbance on restored reefs can result in habitat degradation and decreased oyster density, reducing the potential for future oyster harvesting and other ecosystem services (Grabowski et al., 2012).

Current research largely supports the idea that it is both economically and ecologically preferable to restore oyster reefs as oyster sanctuaries (Grabowski et al., 2012). Although oyster harvest is not permitted at restoration sites, the use of oyster reef sanctuaries has strong public support. A 2009 public opinion poll conducted by the Nature Conservancy found that 91% of Texas voters and 89% of Louisiana voters support the use of “no-take” oyster reef sanctuaries for the protection of water quality, shoreline, and fish habitat (Brumbaugh et al., 2010). Although there has been some concern that restoration in waters closed for bacterial contamination may create an “attractive nuisance” and encourage illegal harvest, these effects could likely be minimized by increasing public awareness and providing for enforcement of closed sites. The development of oyster reef restoration projects in sites that already prohibit shellfishing has been recognized and supported by both the Nature Conservancy and the Interstate Shellfish Sanitation Conference (Brumbaugh et al., 2010).

In order to examine the differences between open and closed oyster reefs and their potential for restoration projects, we measured the ecosystem services of habitat creation, nutrient cycling, and filtration in two reefs at Hoop Hole Creek in Carteret County, NC and compared the economic value each reef generated from these services. We also tested bacteria levels in each area to test our initial assumptions that the closed reef would have higher levels of bacteria.

Our “open” reef remained open to shellfishing for the majority of our experimentation, although it was temporarily closed from September 9-13 due to heavy rainfall. Our “closed” site has been permanently closed to shellfishing since July of 2008 due to high concentrations of fecal coliforms. The Shellfish Sanitation department within the NC Department of Marine Fisheries follows guidelines for monitoring oyster reefs set by the FDA. All shellfishing sites in NC are monitored six times per year to ensure that shellfish harvesting within an area does not present a human health concern. A site becomes closed if the median or geometric mean of fecal
coliform bacteria exceeds 14 CFUs/100 mL or if the 90th percentile of samples exceeds 43 CFUs/100 mL. Sites are also closed temporarily when rainfall in the area exceeds 1.5 inches over a period of 24 hours (NCDENR, 2014).

The open and closed reefs were in close proximity, with limited environmental differences between them. This allowed us to compare the ecosystem services, and the resultant economic values of these services, between a no-harvest oyster reef and a reef in which harvesting occurs. Although our results are site specific, these findings will give us a better idea of where resources for oyster restoration will provide most economic and ecological benefit.
Chapter 1: Spatial

1. INTRODUCTION

The spatial geometry of an oyster reef can influence its ability to provide ecological services such as habitat structure and water filtration. The features of spatial geometry include reef rugosity, physical dimensions, and density. According to a study done by Peterson and Lenihan (1998), the rugosity, or roughness, and height of the reef affect the fluid dynamics of the surrounding area by altering the water flow and the degree of mixing between water layers. Changes in flow modify the transport of resources to oysters, and consequently affect reef growth and survival. In addition, the height and location of intertidal oyster reefs in particular modify the extent of physical stress (e.g. light, desiccation) endured during low tide. Physical features of oyster reefs also impact their interactions with other biota. Intertidal oysters are only exposed to predation from fish and crabs while underwater during tidal cycles, meaning intertidal oysters at relatively higher elevations experience lower predation pressure. Variations in rugosity, height, and relative location in reference to the water line have the potential to affect oyster survivorship and reef health, as well as habitat quality for associated organisms (Peterson & Lenihan, 1998). We hypothesized that the two reefs studied would show differences in spatial geometry associated with their statuses as open or closed to shellfishing, possibly resulting from crushing and harvesting oysters at the open site.

2. METHODS

To test this hypothesis, we used GPS instruments to determine the locations of each reef and sampling site, took physical reef measurements, and used QGIS, an open-source geographic mapping program, to quantify the data collected. The GPS instruments used were handheld Garmin devices, with maximum error values of 9-12 feet. Measurements were taken at both ends of each reef, and coordinates were later compared to Google Satellite Maps © to determine accuracy. Measurements were taken on each reef along a transect running from the top of the reef down to the waterline. Rugosity was taken by measuring the length of a chain draped over a previously measured distance between two points (chain-link method). Density measurements were taken using quarter-\(m^2\) PVC quadrats that were randomly placed at six points along each reef. All oysters within the quadrat were removed down to the anoxic layer and counted by hand to determine density. The length of the first 30 oysters was measured to predict size distribution at each reef. To measure reef height, one end of the measuring tape was held level to the point where the reef met the marsh, and the other end was extended out in a straight line to the waterside edge of the reef. The vertical distance between these two points was measured using a meter stick. The width of the reef (from marsh-side edge to waterside edge) was measured using a measuring tape. Depth and temperature data were gathered using pressure transducers placed at the submerged edge of each reef. Sampling locations along with height, width, rugosity, and density data were mapped using QGIS.
3. RESULTS

The area studied of open reef (177.54 m²) was significantly less than the area studied of closed reef (370.83 m², p < 0.0001). During an especially low tide, 15.80% of the open reef and 59.74% of the closed reef remained inundated, suggesting that more of the closed reef remains subtidal than at the open. Reef height was significantly different (p < 0.0001), with the open site averaging 25.07 cm, and the closed averaging 86.33 cm (Figure 1). These variations in time inundated likely caused the temperature discontinuities recorded at each site (Figure 2), as the amount of water above the transducers may have affected the temperature recorded at each site. The open reef tended to experience more variation in temperature than the closed.

![Figure 1](image1.png)

**Figure 1.** Physical features of each reef: width and height (cm). Fifteen sampling points were measured on each reef.

![Figure 2](image2.png)

**Figure 2.** Temperatures recorded by pressure transducers. Transducers located at lower reef edge of open and closed sites from Sept 24 to Oct 8, 2014.

Reef rugosities were similarly distinct (p < 0.0001), with the open site averaging 2.89 and the closed site averaging 3.82. Individual oyster sizes did not vary significantly between sites. Oysters averaged 53.67 mm in length at the open site, and 57.63 mm in length at the closed (p =
0.19, Figure 3). The difference in average density per quarter-m² between the two sites was similarly insignificant (p = 0.70, open – 832 oysters/0.25m², closed – 923 oysters/m²).

![Figure 3](image)

**Figure 3.** Oyster length distribution (mm) between open and closed reefs, taken from six quadrats on each reef.

4. DISCUSSION

The physical differences of oyster reefs are important to assess when calculating and comparing ecosystem services and functions. The significantly greater proportion of continuously inundated reef at the closed site, for example, may be reflected in the number and type of biota that it hosts. The significantly higher rugosity of the closed reef is a factor to consider when examining the different biota found at each site. Epifauna (especially crabs), for example, tend to inhabit reefs with high vertical surface complexity, while oyster recruitment and initial survival is higher on lower rugosity reefs (Posey et al, 2004).

The differences in height between the two reefs may not be a reflection of reef health or age, but may instead be due to the location of the closed reef, which is directly adjacent to a deep dredge channel with heavy boat traffic. This boat traffic and deeper channel may increase flow, resulting in greater resource transport to the oysters.

The difference in inundation times may explain the temperature variation experienced at each site. The sites were not far enough apart for local variation to cause discrepancies; thus, the amount of water above each transducer is likely the dominant factor. As the open reef experienced less inundation, we would expect to see a greater influence of the atmospheric temperature. It could have been the case that at the closed site, water – which has a higher specific heat than air – tended to dampen the effects of changing air temperature. This temperature variation is clear in Figure 2, as the open site saw higher highs and lower lows across the entire sampling period.

In considering the variable physical parameters and harvesting policies between the sites, we would expect greater variation in density and size distribution than observed. These results may suggest that the open site, while technically available for year-round harvest, is not heavily shellfished. While specific to one area, the physical evaluation of these reefs may be useful for policy makers as they determine the value of maintaining and/or restoring sites closed to shellfishing.
Chapter 2: Biota

1. INTRODUCTION

Oyster reefs play a crucial role in supporting the biological function of estuarine ecosystems, providing habitat and refuge for a variety of marine invertebrates including mollusks, polychaetes, and crustaceans (Bahr and Lanier, 1981; Grabowski, 2005; Grabowski & Peterson, 2007). Many estuarine fish species benefit either directly or indirectly from oyster reefs, using the reefs as nursing and foraging grounds (Beck et al., 2001; Grabowski, 2005; Grabowski & Peterson, 2007). Oyster reef habitat promotes pelagic fauna by enhancing food availability, increasing shelter, or a combination of these factors (Beck et al., 2001; Scyphers, 2011). This association between oyster reefs and higher organism abundance has been demonstrated with commercially important species, including blue crabs and red drum (Scyphers, 2011). Understanding the role that oyster reefs play in supporting biota is imperative not only to protecting the estuarine ecosystem, but also to sustaining the fishery industry and coastal economy.

We researched the biological ecosystem services that oyster reefs open and closed to shellfishing provide. We examined the range, abundance, and biomass of organisms, particularly macroinvertebrates and fish species, at the open and closed reefs to predict and compare the capacity to which valuable macrofauna utilize these reefs. We hypothesized that the different shellfishing and anthropogenic development pressures at the open and closed sites would cause the biological function of these reefs to differ.

2. METHODS

Six quarter-m² quadrats were randomly placed along the closed reef at daytime low tide on Sept 10, 2014 to assess infaunal and epifaunal reef use. The quadrats were placed on top of each reef. All oysters were collected by hand from within each quadrat area down to the anoxic layer and placed into a 1 mm sieve for oyster density calculations (see Chapter 1). As oysters were counted, all macrofauna were recorded for species and abundance. These methods were repeated at the open reef on Oct 5, 2014. Abundance data were analyzed using two-tailed t-tests of unequal variance to compare catch per unit effort at the open versus closed reef.

To predict larger infaunal reef use, two unbaited crab pots and two unbaited minnow traps were set at each reef at daytime low tide on Oct 8, 2014. One crab pot and one minnow trap were set a meter apart on top of each reef (Figure 1). The remaining crab pots and minnow traps were set a meter apart at the edge of each reef so that they rested upon sediment rather than
reef. All trap and pot pairs were placed at random horizontal distances along the two reefs, and set with their openings perpendicular to the fringing marsh. The pots and traps were collected after 24 hours, and species, abundance, and biomass of all captured fauna were recorded. These methods were repeated twice for a total of three replications. Abundance and biomass data were analyzed using two-tailed t-tests of unequal variance to compare catch per unit effort at the open versus closed reefs.

3. RESULTS

*Ischadium recurvum* (hooked mussel), *Panopeus* spp. (mud crab), *Polychaeta*, *Amphibalanus* spp. (barnacle), *Pyramidellidae* (spiral snail), *Palaemonetes* spp. (shrimp), and *Opsanus tau* (toadfish) were found when sampling fauna by quadrat. There was an overall greater species richness found on the open versus closed reef (Figure 2). There was an overall greater abundance of organisms, however, found on the closed versus open reef.

*Lagodon rhomboides* (pinfish), *Menippe mercenaria* (Florida stone crab), *Opsanus tau* (toadfish), *Palaemonetes* spp. (shrimp), *Orthopristis chrysoptera* (pigfish), *Callinectes sapidus* (blue crab), and *Menticirrhus* spp. (mullet) were found when sampling fauna by crab pot and minnow trap. An overall greater species richness was found on the open versus closed reef (Figure 2).

![Graphs showing quadrat and trap open vs. closed reef biota count](image)

**Figure 2.** Total organisms caught at open and closed reefs via quadrat and trap sampling.

Figure 3 shows total biomass and count averaged by trap on the open versus closed site. Averaged biomass and counts are separated by location—reef top or reef edge. There was an overall greater organism biomass per trap on the open site at both reef top and edge. There was a greater organism catch per trap on the open site at the reef edge, but a greater catch per trap on the closed site at the reef top.

The overall difference in abundance between the open and closed reefs by quadrat sampling was not statistically significant. There were significantly more mussels on the closed versus open reef ($p = 0.0441$), and significantly more barnacles and pyramidellid snails on the open versus closed reef ($p = 0.0153$, $p = 0.0323$). All other differences in organism abundance by quadrat sampling were statistically insignificant. The overall differences in abundance and biomass between the open and closed reefs by trap sampling were not statistically significant.
Differences in abundance and biomass between the two sites by location on the reef were not statistically significant. See Appendix Table 8.1 for a complete results table.

![Figure 3. Biomass (g/trap) and organism count (CPUE) via trap sampling.](image)

### 4. DISCUSSION

Limitations in experimental design and obstacles to implementation introduced potential for error. Time restrictions prevented us from performing rigorous replication. Additionally, two crab pots at the closed site were compromised, possibly due to vandalism. For this reason, two of the replications at the closed site had missing data.

Nevertheless, significant differences were found in the distribution of organisms living directly on or in the reefs. Quadrat sampling indicated a broad range of species inhabiting both sites, but the closed site sampling yielded five times the number of mussels than the open, while the open yielded seven times more barnacles and a greater number of pyramidellids. In contrast, no pyramidellids were found at the closed site. This difference could potentially be explained by the differing topography of the two reefs. As explained in Chapter 1, a greater area of the closed reef was continuously inundated in comparison to the open reef. Consequently, the open site may favor species more tolerant of desiccation, while the closed reef may favor species less tolerant of desiccation. Alternatively, the geography of the sites could be a distinguishing factor. The closed reef’s proximity to a deep, dredged channel with frequent boat activity could cause different flow dynamics between the two sites, thus impacting the types and abundance of organisms that live there.

Sampling with crab pots and minnow traps did not reveal any significant differences in the communities between the two reefs, but did provide a qualitative description of the sites. Most of the main fish species observed consume oysters or the macrofauna associated with oysters, such as those caught via quadrat and trap sampling. Some of the fish collected—primarily pinfish, the species with the greatest observed abundance—are food for species of commercial importance in North Carolina, such as red drum (Jordan et al. 1996).

Four trophic levels were observed through our sampling. It is important to note, however, that the crap pots and minnow traps used would not be able to catch larger consumers. Furthermore, our sampling was only conducted during one season. For these reasons, it is difficult to determine the full range of organisms that use these reefs. Despite this limitation, the structure of the reefs clearly provides habitat for an array of species, and the biomass these reefs support likely propagates upwards to piscivores and other higher-level consumers.
Chapter 3: Water Quality

1. INTRODUCTION

An adult oyster can filter approximately 50 gallons (189.3 L) of water in a 24-hour period (Loosanoff, 1958). Through their spatial placement and spawning locations, oysters physically modify their environment, altering flow rates and facilitating deposition, which decreases turbidity in the water column. Oysters have also been shown to filter suspended solids and chlorophyll $a$, which further decreases turbidity (Haven, 1965).

The Environmental Protection Agency (EPA) defines total suspended solids (TSS) as any dissolved solid that does not pass through a 2-micron filter (5.8 Total Solids, 2012). TSS increases turbidity, which limits light penetration and subsequently hinders photosynthesis in aquatic plants. Heavy sedimentation, in contrast, smothers demersal organisms and essential habitat for many other organisms. Oyster reefs are responsible for biodeposition, the process of sediment filtration, compaction and expulsion as feces and pseudofeces (Haven & Morales-Alamo, 1966). Studies have shown that oysters may increase compact benthic deposition by as much as seven times the rate of normal gravity-driven deposition (Dame, 1999). Such compact deposition decreases turbidity without smothering demersal organisms and degrading benthic habitat.

Chlorophyll $a$ measurements are used as a proxy to determine phytoplankton biomass and relative nutrient abundance (Wang et al., 2012). High levels of chlorophyll $a$ indicate high algal growth. When the algae die and sink to the bottom, they undergo degradation, which depletes oxygen concentrations in the water and causes hypoxia/anoxia (Chlorophyll $a$, 2003). These oxygen deficient environments harm and kill marine organisms trapped in these areas. Field studies have shown that oyster reefs may reduce chlorophyll $a$ concentrations in the water column by more than 75% (Dame et al., 1984).

Our objective was to quantify the drawdown of chlorophyll $a$ and TSS by oysters at both the open and closed reef. We hypothesized that there would be significant difference in drawdown between these sites.

2. METHODS

2.1 Field Sampling

Water samples were collected at a point 3 m upstream of the reef, along the upstream reef edge, at the middle of the reef, along the downstream reef edge, and at a point 3 m and 6 m downstream of each reef. To obtain water samples, a 1 L opaque carboy was attached to a 3 m pole to avoid artificially increasing turbidity. Standing downstream of each sample location, the 1 L carboy was lowered to approximately the middle of the water column. After moving to each sample site, samplers stood still for one minute, allowing any recently suspended particles to settle. The collected water was then transferred into labeled 1 L opaque carboys and stored in a cooler. Samples were collected from the open and closed reefs on two separate days.

2.2 Laboratory Experiment

50 oysters of harvestable length (7.0-7.5 cm) were collected from both the open and closed sites. Twelve outdoor tanks (91 x 91 x 22.7 cm) were cleaned with fresh water and
subsequently filled with water from Bogue Sound. Oysters were placed in two of these tanks 72 hours prior to the trial to acclimate the oysters to water conditions. Water pumps were attached at the same location in the remaining ten tanks to ensure water circulation. Two bricks were placed in the middle of each tank to lift the oysters off the bottom. 1 L of water was taken before oysters were placed in the tanks to determine baseline turbidity. Different oyster densities (1, 2, 4, 8, and 10 oysters per tank) from the open and closed sites were placed randomly in the tanks. After three hours, 1 L water samples were collected from each tank. Two trials (Trial 1, Trial 2) were conducted on the same day.

2.3 Water Sample Analysis

2.3.1 Total Suspended Solids

First 20 mL of distilled water was poured through 52 AP40 millipore glass-fiber filter disks on the filtration tower. This was repeated twice and then the prepared filters were placed in an aluminum weighing tin and dried in an oven at 105°C for two hours. Once dried, filters were weighed on a digital mass balance and masses were recorded. The dried filters were placed back on the filtration tower and 500 mL of each water sample was poured onto the prepared filters after shaking the samples. Each sample filter was then place into an oven and dried at 105°C for two hours. The filters were again weighed and recorded to determine total suspended solids.

2.3.2 Chlorophyll a

For chlorophyll a measurements, 50 mL of each water sample was measured using graduated cylinders after shaking. The 50 mL samples were filtered through Whatman 25.0 mm GF/F filters (pore size 0.7 μm) with the lights off in the laboratory. Filters were then folded and placed in labeled, aluminum foil envelopes. The envelopes were placed in a freezer at -20°C until sonification was performed. To sonicate, the filters were each placed in a BD Falcon 15 mL polystyrene conical centrifuge tube with 7 mL of 90% acetone solution. Test tubes were placed in a VWR Model 150D sonicator for five minutes. Once completed, test tubes were placed back into a freezer at -20°C for no more than 24 hours. Then the samples were run on the Turner Designs Trilogy fluorometer (Turner Designs, Sunnyvale, CA, USA). The fluorometer reading for each sample was used to determine chlorophyll a concentrations.

3. RESULTS

3.1 Total Suspended Solids

The data from the field was analyzed for both sample dates. The comparison of mean TSS concentrations across both reefs, starting at the upstream edge of the reef, did not show a statistically significant difference between the two reefs (p = 0.5091). The open reef showed a 62.1% increase of TSS along the reef, whereas the closed reef showed a 10.4% decrease of TSS along the reef (Figure 1).
Figure 1. Total suspended solid concentrations for open and closed reefs, averaged across two sample dates.

The difference in overall drawdown of TSS was not significant between the closed and open sites (p = 0.1508). The TSS drawdown of open reef oysters displayed a strong, positive, linear correlation with density (R² = 0.9786) (Figure 2). The TSS drawdown of closed reef oysters did not display a linear correlation (R² = 0.3014). When comparing different densities of oysters between open and closed sites, there was no significant difference in drawdown (Open, p = 0.1020; Closed, p = 0.8070).

Figure 2. Percent change in total suspended solids vs. oyster density for open and closed reefs, averaged across two trials.
3.2 Chlorophyll a

Chlorophyll a field samples were analyzed for both dates. The difference in the average chlorophyll a concentration between both reefs, starting at the upstream edge, was marginally significant (p = 0.0906). The open reef showed a 25.5% decrease in chlorophyll a concentrations along the reef, while the closed reef showed a 27.7% decrease along the reef (Figure 3).

![Figure 3. Chlorophyll a concentrations for open and closed reefs, averaged across two sample dates.](image)

The overall difference in drawdown of chlorophyll a was not statistically significant between the closed and open sites (p = 0.8620), or across different oyster densities (Open, p = 0.6960; Closed, p = 0.7866). The open reef displayed a positive linear correlation, as drawdown of chlorophyll a increased with oyster density ($R^2 = 0.7009$), while the closed reef did not exhibit a linear correlation ($R^2 = 0.435$) (Figure 4). See Table 1 for a complete list of p-values.
4. DISCUSSION

4.1 Sampling Error

There were no statistically significant findings, however, this could be due to a number of factors that could have skewed the results. In the lab experiment, Trial 1 took place during midday, whereas Trial 2 was performed from afternoon to dusk. Due to the large variation among chlorophyll $a$ and TSS, triplicate samples should have been applied for greater accuracy.

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**Figure 4.** Percent change in chlorophyll $a$ vs. oyster density for open and closed reefs, averaged across 2 trials.

**Table 1.** Statistical tests and resulting p-values.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSS-Field Open vs Closed</td>
<td>0.5091</td>
</tr>
<tr>
<td>TSS-Lab Open vs Closed</td>
<td>0.1508</td>
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<tr>
<td>TSS-Open Reef Drawdown vs Density</td>
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<tr>
<td>TSS-Closed Reef Drawdown vs Density</td>
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<tr>
<td>Chlorophyll $a$-Field Open vs Closed</td>
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<tr>
<td>Chlorophyll $a$-Lab Open vs Closed</td>
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<tr>
<td>Chlorophyll $a$-Open Reef Drawdown vs Density</td>
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</tr>
<tr>
<td>Chlorophyll $a$-Closed Reef Drawdown vs Density</td>
<td>0.7866</td>
</tr>
</tbody>
</table>
The actual density of chlorophyll $a$ may have fluctuated during the 3 hours between sampling. Collecting samples at $t = 0$ hours and $t = 3$ hours fails to capture any intermediate variations.

The field data was additionally subject to a modicum of error. The retrieval of some samples was difficult due to the current at both sites. Additionally, channels near each reef caused depth differences, which made it challenging to collect samples.

4.2 Total Suspended Solids

While several sources of error were present during sampling, there are still potential conclusions that can be drawn. When performing an analysis of biota, particularly in the field, it is not uncommon for the correlation between variables to be inexact. The presence of slightly significant data, and $R^2$ values above 0.50 in some cases, could indicate there is an underlying trend to the data.

In the laboratory experiment, there was a positive graphical trend between oyster density and drawdown effect. The small number of replicates precludes statistically significant data, however, Figure 2 shows a strong graphical relationship ($R^2 = 0.9786$), and it is apparent that even small quantities of oysters can create significant drawdown. Typical oyster reef densities number in the thousands per m$^2$. A strong decrease in TSS was shown with 10 oysters, and field conditions would likely have greater than ten times that number of oysters per square meter.

4.3 Chlorophyll $a$

Even without statistically significant figures, patterns were evident. There is a clear decrease in the amount of chlorophyll $a$ concentrations across the reef for both treatments, and a slightly significant lower concentration across the closed reef versus the open reef. This shows that chlorophyll $a$ decreased across both reefs, and that some characteristic or characteristics of the closed reef caused it to drawdown chlorophyll $a$ more effectively than the open reef. There was no difference between open and closed reefs when comparing drawdown of chlorophyll $a$ across both trials in the experiment. Potential factors include a difference in the density or lengths of oysters on each reef.
Chapter 4: Nutrient Cycling

1. INTRODUCTION

Excessive nutrient loading is the principal driver of estuarine eutrophication and can degrade the ecosystem through harmful algal blooms, hypoxia/anoxia, and fish kills (Paerl, 1997). Nutrient management and reduction involves a complex interaction between sources and sinks in terrestrial and aquatic ecosystems that are continuously changing (Brush, 2009). Nitrogen is one of the main limiting nutrients in aquatic systems and, despite attempts at reduction through source control, there is still an excess of nitrogen in our North Carolina coastal ecosystem (Lebo et al, 2012). It is therefore important to understand all the components of the nitrogen cycle that alter nitrogen concentrations in the water column.

Support is increasing for combating eutrophication through restoring populations of oyster reefs, which could act as sinks and remove nitrogen from ecosystems (Piehler and Smyth, 2011). Previous studies have shown that oysters can remove nitrogen through assimilation, denitrification and burial (Kellog et al, 2013). Phytoplankton requires biologically available nitrogen to grow and multiply. Oysters remove a portion of phytoplankton biomass from the water column as they filter feed. The nitrogen in the phytoplankton either becomes assimilated into oyster shell and tissue with growth, is dissolved and discharged directly into the water column, or is turned into feces and pseudofeces which settle in the surrounding sediments and are eventually buried (Kellog et al, 2013). Denitrifying bacteria, which reduce nitrite and nitrate and release N₂, further break down nitrogen-rich feces and pseudofeces in the benthic zone.

Oysters also play an important role in the carbon cycle. Studies suggest that they have the potential to sequester carbon in their shell as calcium carbonate. This is countered, however, by the carbon dioxide they produce in the process of shell formation (Dehon, 2008). Oyster reefs can also influence benthic pelagic coupling in the water column through benthic deposition of carbon via bio-deposits of feces and pseudofeces (Grabowski, 2007). A study by Dame et al. (1989) found that an oyster reef in South Carolina took 1200 g C per m² per year from tidal waters.

According to Carmichael et al. (2011), more than 30 studies on the potential for bivalves to remediate water quality have been completed since 1980. Methods and scales, however, varied greatly, and these studies mainly focused on areas open to shellfishing. As a result, it is difficult to compare these previously studied reefs to reefs closed to shellfishing. We investigated and compared the differences in nutrient cycling between areas on- and off-reef and between our open and closed sites. We hypothesized that denitrification rates would be higher in areas with oyster reefs than without, and that the closed oyster reef would have a larger rate of denitrification due to higher oyster densities (see Chapter 1).

2. METHODS

2.1 Nutrient Analysis

Water samples of 50 mL were collected from each reef for nutrient analysis at various points over the semester. Samples were collected from undisturbed water and taken to IMS to be refrigerated until analysis. Additionally, water samples were taken on Oct 8, 2014 near an outflow pipe with runoff from a nearby parking lot, and downstream of the outflow pipe between
the pipe and the open reef. This was done to account for the possibility of the outflow pipe impacting nutrient levels at the open reef site, especially during storm events. All water samples were then filtered using a filter tower and Whatman GF/F filters (25 mm size, 0.7 um nominal pore size). The filtrate was analyzed with a Lachat QuickChem 8000 automated ion analyzer for NO₃, NH₄, and P.

2.2 Denitrification

On the afternoon of Sept 22, 2014, a total of eighteen sediment cores were collected from the open and closed oyster reefs at Hoop Hole Creek. Due to technical difficulties and consideration for oyster reef health, cores were not taken directly through the oyster reefs themselves. Instead, we used two proxies to estimate the sediment cores that would be on the reef. Three cores were taken at the top edge of the reef between the oysters and marsh grass, with each core being approximately three meters apart. After each sediment core was taken, we filled the empty space of the core with on-site water and capped the cores to prevent air bubble formation. We followed the same procedure with the next three cores at the lowest edge of the oyster reef where a solid layer of oysters extended; this area was completely submerged during both high and low tide. Finally, in order to provide information on the background level of denitrification rates in the area and to serve as a control, we took three cores at a sandbar proximal to each site without oysters or marsh-grass, with an elevation that was submerged during high tide and above water at low tide. This resulted in a total of 18 cores, with nine being collected at each site.

To obtain a direct measure of denitrification rates at each site we used continuous-flow core incubations and analyzed concentrations of dissolved gases in the water and sediment cores using the Membrane Inlet Mass Spectrometer (MIMS). The water used in the continuous flow system had been collected at both the open and closed sites earlier in the day of core collection using three 5-gallon carboys at both sites. The carboys were emptied into four separate large holding tanks in the environmental chamber at UNC Institute of Marine Sciences, with two tanks for core incubation and two tanks for flow hoses and pump. After the holding tanks with the pump were filled with appropriate open and closed site water, the pump was turned on to clear the tubes of stray air bubbles and to certify that there were no leaks. The environmental chamber was kept dark and set to 25°C, the temperature of the water at time of collection. Bubblers were inserted in all four tanks to keep constant oxygen concentrations. The cores were put into their corresponding incubation tanks for approximately 18 hours and the top caps were removed after the cores were submerged to allow them to reach equilibrium (Eyre et al. 2002).

The next day, the cores were capped with plexiglass tops equipped with two O-Rings to maintain air and water seals. Cores were capped while submerged in the incubation tanks to avoid air bubbles. If necessary, a brush was used to rid the caps of any stray air bubbles before placement. The capped cores were then switched over to the holding tanks connected to the continuous flow pump, which was set at a rate of 1 mL per minute. Cores were double checked for tight seal and complete lack of air bubbles. Appropriate hoses were attached to the cap ports, which were plumbed with Tygon tubing to divert the flow of water through the cores. 5 mL samples were collected at 18-, 24-, and 36-hour increments (Time 1, Time 2, and Time 3). The 5 mL test tubes were allowed to overflow before capping to avoid excess air. These samples were analyzed using the Membrane Inlet Mass Spectrometer (MIMS), which measures the concentration of dissolved gases in water and the associated denitrification (Kana et al., 1994; Kana et al., 1998).
2.3 Carbon Sequestration

To calculate the carbon sequestration provided by oyster reefs in both closed and open areas, we used the Loss on Ignition method (Byers et al. 1978). We collected the top layer of sediment from each of the 18 cores noted in the denitrification section. This collection followed the completion of denitrification data collection. The top layer of sediment from each core was put in a circular tin tray and frozen until we were prepared to dry them. All trays were covered with tin foil to avoid contamination by any airborne carbon sources. The tin trays were labeled by indent to avoid loss of identification during the combustion process.

To obtain the approximate weight of all sediments and the organic matter (carbon) stored in them, we dried the top layer of the cores in a Fisher Scientific Isotemp muffle furnace at 106°C for approximately 24 hours. Upon removal from the furnace, they were immediately weighed to avoid the absorption of water back into the sediment. We used a Mettler Toledo balance with an accuracy of five decimal places. After weighing the dried sediment, we used a Thermo Scientific Thermolyne combustion furnace to burn off the carbon that had been stored in the sediment. The combustion furnace was set to 525°C for four hours. At the end of four hours, the oven was opened slightly and allowed to cool for half an hour before removing and re-weighing the sediments. Finally, we subtracted the combusted weight from the dry weight to determine the total carbon content that had been present in each set of core surface sediments.

3. RESULTS

Due to a short time frame, cores were collected at only one time point reflecting denitrification rates for a single season and temperature range. The water around both reefs showed NO₃ levels below the detection limit of 0.36g/L for NO₃ throughout almost all time points with the exception of the open reef on Sept 24 with a concentration of 15.4 g/L. NH₄ levels varied from 16.3 to 80.9 g/L between both reefs from Sept 24 and Oct 8, with the peak being on Sept 24 at the open reef. There was no noticeable difference of either NO₃ or NH₄ levels between the two reefs’ associated waters with the exception of peaks on the open reef during Sept 24. The elevated levels of NO₃ and NH₄ could be explained by a storm event with half an inch of precipitation on Sept 24th and the vicinity of the open reef to a parking lot outflow pipe. Although there was a notably higher NH₄ concentration of 107 g/L at the outflow pipe after sampling on Oct 8, samples downstream of the outflow pipe had lower levels of NH₄ of 27.7 g/L, which was more closely associated with the NH₄ levels at the open reef. Oct 8 marked five days since the last storm event. This suggests that, in the absence of rain, the open reef is not close enough to the outflow pipe to be significantly impacted by it. There was little variation in phosphate concentrations between sites, remaining between 5.35 and 22.8 g/L, with the exception of a higher measured level at the outflow pipe (55.2 g/L). Denitrification measurements revealed a higher denitrification rate in every closed reef core versus the corresponding open reef core during all three sampling periods (Table 1).
Table 1. Average denitrification rates by location.

<table>
<thead>
<tr>
<th></th>
<th>Time 1 Average</th>
<th>Time 2 Average</th>
<th>Time 3 Average</th>
<th>Combined Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open Reef Marsh</td>
<td>8.2</td>
<td>20.2</td>
<td>43.6</td>
<td>24.00</td>
</tr>
<tr>
<td>Open Reef Edge</td>
<td>9.7</td>
<td>4.6</td>
<td>18.9</td>
<td>11.07</td>
</tr>
<tr>
<td>Open Off Reef</td>
<td>5.9</td>
<td>18.5</td>
<td>26.1</td>
<td>16.83</td>
</tr>
<tr>
<td>Closed Reef Marsh</td>
<td>16.7</td>
<td>46.6</td>
<td>56.3</td>
<td>39.87</td>
</tr>
<tr>
<td>Closed Reef Edge</td>
<td>12.7</td>
<td>35.8</td>
<td>57.2</td>
<td>35.23</td>
</tr>
<tr>
<td>Closed Off Reef</td>
<td>9.1</td>
<td>42</td>
<td>53.8</td>
<td>34.97</td>
</tr>
</tbody>
</table>

The denitrification rates between reef-marsh, reef-edge, and off-reef at the same site varied greatly. The reef-marsh cores always had a higher denitrification rate than the off-reef cores, but by varying amounts. The reef-edge cores had substantial differences between the open and closed reefs. At Time 1, reef-edge had the highest N$_2$ flux at all zones on the open reef, whereas at Time 2 reef-edge had the lowest in both open and closed areas. Furthermore, the closed reef showed higher N$_2$ flux at every zone on the reef at all times than any zone in the open reef. There was a single exception for one time point in the reef-edge zone between the open and closed reef. This means that even the off-reef area of the closed reef, which should be considered exempt from the impact of the reef, had a higher N$_2$ flux than any open reef area. The denitrification rates between the corresponding areas of the two sites can be compared using Figure 1. The closed reef had higher N$_2$ flux compared to the corresponding area at the open reef through every time point.

![Denitrification Averages Between Reefs](image)

**Figure 1.** N$_2$ flux between open and closed sites at the end of three time periods.

A t-test for statistical significance was used to compare the differences between both the closed and open reefs and the different zones within each reef. Time 2 and Time 3 showed
statistically significant differences between open and closed reef-edge sites and open and closed off-reef sites. See Table 2 for complete statistical results.

The Loss on Ignition method for estimating carbon content revealed the greatest carbon stores in the reef-marsh cores for both the open and closed sites approaching approximately 0.6 g of carbon. The off-reef and reef-edge cores at the closed site contained approximately 0.18 g of carbon while the open site equivalents were approximately 0.1 g of carbon, as seen in Figure 2.

Table 2. T-test and resulting p-values. Significant values are in red.

<table>
<thead>
<tr>
<th>N₂ flux T-test between:</th>
<th>Time 1</th>
<th>Time 2</th>
<th>Time 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reef marsh open and closed</td>
<td>0.1479</td>
<td>0.1497</td>
<td>0.5236</td>
</tr>
<tr>
<td>Reef edge open and closed</td>
<td>0.7357</td>
<td><strong>0.0217</strong>*</td>
<td><strong>0.0354</strong>*</td>
</tr>
<tr>
<td>Off reef open and closed</td>
<td>0.8693</td>
<td><strong>0.0470</strong>*</td>
<td>**0.0050 ***</td>
</tr>
<tr>
<td>Reef marsh and off reef open</td>
<td>0.1479</td>
<td>0.8271</td>
<td>0.1191</td>
</tr>
<tr>
<td>Reef edge and off reef open</td>
<td>0.5073</td>
<td>0.1751</td>
<td>0.5367</td>
</tr>
<tr>
<td>Reef marsh and off reef closed</td>
<td>0.6949</td>
<td>0.7380</td>
<td>0.8924</td>
</tr>
<tr>
<td>Reef edge and off reef closed</td>
<td>0.8574</td>
<td>0.1830</td>
<td>0.6722</td>
</tr>
</tbody>
</table>

Figure 2. Carbon content in each core by reef.

4. DISCUSSION

The closed reef on average had much higher rates of denitrification compared to the open reef. The difference between the two reefs at reef-edge was significant at two of the three time points while the reef-marsh area was not significantly different between sites at any time point. Higher density at the closed site could account for the increased denitrification rates. This higher
density of oysters coupled with a larger average size produces greater amounts of feces capable of being buried or reduced to N₂ by denitrifying bacteria.

Although the overall trends across zones were strong, most zones showed strong variability in the denitrification rates between their individual cores. One possible explanation for the differences in the reef-marsh zone between sites could be the variation in collection of cores. For most of the oyster reef, the area between the upper edge of the oyster reef and marsh was very small if it was present at all. If there was not enough area, cores were taken from gaps in the marsh area nearest to the reef. This could explain some variability in the denitrification rates, as the increased sedimentation due to marsh grass also plays an important role for denitrifying bacteria.

There was a significant difference in the denitrification rates of the cores taken as an off-reef reference (control) at both of the sites. It is possible that the development in the area around the closed reference site caused increased levels of suspended material in the water, which settled out to elevate the surrounding sediment organic matter. Denitrifying bacteria and the denitrification process as a whole is dependent upon a source of oxidizable carbon (Herbert, 1999), indicating that higher organic matter stimulates higher denitrification. This is supported by the regression of carbon content and denitrification at each core evident in Figure 3, showing that higher carbon content is closely associated with higher rates of denitrification. Note the removal of the reef-marsh zone due to the high variability in the reef marsh core location mentioned above.

The increased level of organic sedimentation provides a high background rate of denitrification at the closed site. This explains why the rates on and off the closed oyster reef were more difficult to detect and therefore were not significantly different. This also explains the significant difference between the two control sites. The closed site control core was taken from an area of relatively still water that would allow settling of organic material with low likelihood of resuspension. By contrast, the open site core was taken in an area of higher flow, which could promote the suspension of any settled organic material.

![Figure 3. Regression of carbon and nitrogen content at each core.](image-url)
Although there were relatively definitive trends in carbon and nitrogen cycling between the open and closed reefs during our time scale, there is likely variability across longer temporal scales. Denitrification rates can vary greatly in different environments depending on season, with the greatest denitrification rates being noted in the summer (Piehler and Smyth, 2011). Further replication is necessary to assess denitrification rates during seasons outside of our study and between differing tidal cycles.
Chapter 5: Microbial

1. INTRODUCTION

Microbial water quality and shellfish sanitation has attracted substantial attention and regulation due to the health and economic hindrances that a foodborne or recreational waterborne illness can provide. In order to prevent these instances of illness, indicator bacteria are used to represent possible poor water quality and fecal contamination. These are fecal coliforms (mainly Escheria coli) and Enterococcus for marine waters. E. coli is found on the intestinal lining in most mammals, is very prevalent in sewage, and easily tested for compared to other bacteria so it is used as an indicator bacteria for water quality (Burlage, 2012). Enterococcus are also found in the intestinal lining of mammals and are more persistent than E. coli in brackish waters and so are often used as indicator bacteria for marine environments (Pruss et al., 1998). However, studies show that fecal indicator bacteria do not correlate with levels of viruses and levels of Vibrio, which both are shown to bioaccumulate in shellfish, particularly oysters (Pruss et al. 1998, Jay et al. 2005, Wright et al. 2013).

Oysters are filter feeders and so they can concentrate bacteria and viruses, which is what makes them susceptible to causing foodborne illnesses (Burlage 2012). Two particular Vibrio that are prevalent in North Carolina estuarine systems are Vibrio Vulnificus and Vibrio Parahaemolyticus. They both survive best in 2-4% salinity and 30° to 35°C temperature, but can survive in 1-7% salinity and at temperatures as low as 10°C. Vibrios produce a cytotoxin that causes cells to release ions into the area surrounding cells and creates a hypertonic solution causing cells to desiccate (Jay et al., 2005). In 2010, Vibrio Vulnificus was the cause of 95% of deaths due to bacterial infections from seafood consumption, and Vibrio infections are only increasing. From 1998 to 2010 the number of Vibrio infections went up 116% (Wright et al., 2013). It was hypothesized that bacteria levels in an oyster site that is open to harvesting would be lower than bacteria levels in sites closed to harvesting.

2. METHODS

2.1 Sampling Location

Water and oyster samples were taken at five different sites with variation based on weather conditions. A water sample was taken in an area closed to shellfishing at the site of an outflow pipe carrying water from a local parking lot, which was simply called Pipe (Appendix Figure 8.1). Taking samples from this location depended on whether water was flowing from the pipe or not. The site titled Near Pipe is located directly in front of the outflow pipe. Sampling at this location allowed us to observe the bacteria levels after the water from the outflow pipe mixed with the tidal creek water. A site that was classified as the Additional Closed site is located 0.11 miles downstream from the Near Pipe site and 0.35 miles upstream from the open reef. This allowed us to observe basic fate and transport of bacteria downstream. The final sites, Open Site and Closed Site, were located at the open reef and closed reef. From the Near Pipe through the Closed Site, water and oyster samples were taken during four different events. The majority of sampling events occurred within 48 hours of rainfall, except for Oct 8th, which had minimal rain influence. At each site, the water was tested for temperature, salinity, and turbidity.
Weather conditions were also noted, including air temperature, wind speed, and total rainfall from the last 24 hours, 48 hours and 5 days.

2.2 Water Samples

Water samples were collected from all five sites, transferred in an insulated cooler, and processed within four hours of being taken. Each water sample taken was tested for fecal indicator bacteria by measuring three different forms of bacteria: *Enterococcus* spp., *Escherichia coli*, and total coliforms. Concentrations of fecal indicator bacteria (FIB) to monitor freshwater and saltwater were used since both forms of bacteria could survive in estuarine water (Parker, McIntyre, and Noble, 2010). Colilert-18® media was added to water samples to measure the concentration of total coliforms and *E. coli*, while Enterolert™ media was added to water samples to measure *Enterococcus*. Samples were diluted to 10 mL or 1 mL per 100 mL with deionized water, and dilutions were made in duplicate to increase accuracy of results. After the medias were added, each sample was poured into a Quanti®-Tray/2000 tray, sealed with the IDEXX Quanti®-Tray sealer and incubated overnight. Samples with Colilert-18® were incubated at 35° C and samples with Enterolert™ were incubated at 41° C (IDEXX Laboratories, Westbrook, ME). Nutrient indicators within the medias reacted with the bacteria to either fluoresce or change color to confirm existence of bacteria. Large and small wells on the Quanti®-Tray/2000 trays that fluoresced or changed color were counted and a most probable number (MPN) of FIB per 100 mL was calculated using the number of positive and negative wells on a tray. MPN values from duplicate samples were then averaged using the computer program PRISM, and averaged values were log transformed for graphical representation and comparison.

Water samples were also tested for total *Vibrios*, *V. Vulnificus* and *V. Parahaemolyticus*. Quantities of 1 mL or 5 mL of water were filtered onto filter paper then placed on plates with thiosulfate-citrate-bile-salts sucrose (TCBS) and CHROMagar *Vibrio* (CV) media. TCBS was used to detect total *Vibrios* while CV was used to detect *V. Vulnificus* and *V. Parahaemolyticus*. After the TCBS plates were incubated for 20 hours at 35° to 37°C and the CV plates were incubated for 18 hours at 37°C, the colonies on each plate were counted and recorded based on the amount of water filtered (Hara-Kudo et al., 2001; Harwood, Gandhi, and Wright, 2004).

2.3 Oyster Samples

Oyster samples were collected from all sites but Pipe by hand, transferred in an insulated cooler, and processed within four hours of being taken. Five oysters from each site were scrubbed with deionized water, shucked, and oyster tissue was weighed. The oyster tissue was then blended with 25 mL of phosphate-buffered saline (PBS) until no chunks were present. The blender was sterilized and rinsed between samples. The oyster tissues from each site were then placed into two separate sterile plastic tubes: the first tube contained undiluted oyster tissue while the second tube of oyster tissue was diluted to one-tenth with PBS. 100 μL from each tube was taken and added and spread onto one plate of TCBS and one plate of CV media. After the TCBS plates were incubated for 20 hours at 35° to 37° C, and the CV plates were incubated for 18 hours at 37° C, the colonies of total *Vibrios*, *V. Vulnificus* and *V. Parahaemolyticus*, on each plate were counted (Hara-Kudo et al., 2001; Tamplin and Capers, 1992).
3. RESULTS AND DISCUSSION

3.1 Variations in *E. coli* and *Enterococcus* Between Sites

The Additional Closed site and the Closed site are permanently closed to shellfishing. The closures at both sites are known to be due to high levels of fecal coliform contamination (Ashbolt, 2004). Shellfishing area regulations for microbial contamination focus on concentrations of fecal coliforms and by association *E. coli*, but *Enterococcus* is the standard for recreational waters. Because these sites associate with both functions, it was included in the analysis of the microbial quality of the sites. It was found that both *E. coli* and *Enterococcus* vary significantly between sites, with the Additional Closed Site consistently having higher concentrations of both bacteria (Appendix Table 8.2). However, the *E. coli* concentrations at all three sites exceed the Department of Marine Fisheries suggested 14 CFUs/100 mL at all sites on each (Figure 1). It is important to note here that the North Carolina Department of Marine Fisheries does not use IDEXX laboratories methods of calculating bacterial concentrations. However, this is due to the lower limit of detection colony forming methods allows for. In this study the concentrations were at a high enough limit of detection for IDEXX that this distinction may be inconsequential. The conditionally Open Site was only closed on one of the days of sampling (Sept 10th) due to a rainfall event exceeding 1.5 inches. *Enterococcus* concentrations generally complied with recreational water quality standards, but the concentrations of *Enterococcus* were higher during wet weather (Figure 2).

![E. coli Concentrations in Water](image)

**Figure 1.** Log transformed Most Probable Number (MPN) concentrations of *E. coli* in the water column at each site over all sampling dates. *E. Coli* consistently stayed high during all sampling dates. Concentrations were lowest at the pipe and highest at the Additional Closed site.
Figure 2. Log transformed Most Probable Number (MPN) concentrations of *Enterococcus* in the water column at each site over the sampling dates. Only twice did *Enterococcus* exceed the recreational limit. There were no significant trends and *Enterococcus* levels varied greatly throughout sites and dates.

Figure 3. Log transformed Most Probable Number (MPN) of *E. coli* and *Enterococcus* concentrations against days since last rainfall. Concentrations were significantly higher during active rainfall.

3.2 Temporal Variations of *E. coli* and *Enterococcus*

In addition to the differences between sites, temporal differences in *E. coli* and *Enterococcus* concentrations were also found. From information provided by previous studies, it would be expected that rainfall would increase the concentrations of *E. coli* and *Enterococcus* at all of the sites due to runoff from stormwater transporting these contaminants into the system (Kistemann et al., 2002). Figure 3 shows that this pattern is seen in days since rainfall, but it was not significant when compared to rainfall amount (Appendix Tables 8.4-6). The closer the sampling date was to a rainfall event, the higher the *Enterococcus* and *E. coli* concentrations were. It is difficult with such a small sample size, and only one season represented, to see an explicit positive correlation with contaminant concentration and rainfall amount, but it would be expected to see this pattern with a more comprehensive study as seen in other studies (Ackerman and Weisberg, 2003). For the Additional Closed site, the change between dry and wet weather
was greater than at the Closed site and the Open site. The Closed and Open sites were very similar in that immediately after rainfall there were very high concentrations, but after that initial spike in contaminant levels the concentrations of bacteria did not change significantly. This indicates the possibility that the Additional Closed site is a source or near a source of contamination since the greatest change between wet and dry weather happened at this site. From other studies we know that it is also important to understand the influence of other environmental parameters such as temperature, nutrients, turbidity, and salinity to better understand the driving forces behind temporal differences in microbial populations (Wheeler and Hanks, 1965).

3.3 Spatial Variations of Vibrios

Total Vibrios (Appendix Figure 8.2), *Vibrio Vulnificus* (Figure 4, Appendix Figure 8.3), and *Vibrio Parahaemolyticus* (Figure 4, Appendix Figure 8.3) did not show any statistically significant variations between sites. While there did appear to be a trend on two sampling days with lower concentrations at the Open and Closed sites, they were not significant (Appendix Tables 8.4-6). This demonstrates that the autochthonous *Vibrio* levels in the system are similar in concentrations and contamination throughout, indicating that the risk to public health from *Vibrios* throughout Hoop Pole Creek is likely the same.

![Figure 4. V. Vulnificus and V. Parahaemolyticus concentrations in log transformed colony forming units (CFUs) per mL in oyster tissue at each site across the sampling dates. September 22nd contained high concentrations of both Vibrio species at each site.](image)

3.4 Temporal variations of Vibrios

While differences in *Vibrio* concentrations were not seen between sites, the concentrations did vary temporally for *V. Vulnificus* and *V. Parahaemolyticus*. Total *Vibrios* did not experience significant changes in concentrations (Appendix Figure 8.2, Appendix Tables 8.4-6). Both *V. Vulnificus* and *V. Parahaemolyticus* saw large increases in concentrations on Sept 22nd in both the water and the oyster tissues when compared to both Sept 10th and 24th; this spike occurred at all sites. The increase could not be accounted for by changes in salinity, temperature, or rainfall (Appendix Tables 8.4-6). More information on environmental parameters would be needed to understand what may be driving spikes in the population. From previous studies there is an indication that increased nutrient loading and phytoplankton in the estuarine environment can cause increases in *Vibrio* concentrations in the water. Also, all samples were taken at low tide, so it is possible that the increase in *Vibrio* levels is a function of transport instead of any of these other environmental parameters. Further analysis and research correlating
nutrient levels with *Vibrio* in Hoop Pole and comparing high and low tide microbial levels would give more insight into these speculations (Burns and Galbraith, 2007; Turner et al., 2009).

### 3.5 Risks to Public Health and Improvements in Monitoring Schemes

Understanding the concentrations of possible pathogens in shellfishing waters is necessary to protect public health. While this study examined one open area and two closed areas, bacterial concentrations were continuously above shellfishing regulations at all three sites. Testing currently occurs at a minimal six times throughout the year. Additionally, after 1.5 inches of rainfall, the sites are closed and monitored to reopen after three days (NC Department of Sanitation, 2014). In this study, however, there were rainfall events below the 1.5 inches of rainfall limit that did not cause closures, yet high concentrations of bacteria were found. Studies have previously suggested that the amount of rainfall to cause a closure should be reduced due to input from antecedent rainfall (Coulliette et al., 2007). Our data shows that on Oct 8th, a day where there had been five days since rainfall, the background concentrations of *E. coli* exceeded the limits set forth by Shellfish Sanitation, which further supports Coulliette’s conclusions. The changes in microbial concentrations from temporal variations seen in this study also call for a greater understanding of the ecology of indicator microbes and more inclusive monitoring programs that are able to capture variations in concentrations over time. The forces influencing the concentrations of *V. Vulnificus* and *V. Parahaemolyticus* are also not fully understood, and a large spike in concentrations could not be explained by known environmental parameters. Further research using molecular methods would be able to test pathogenicity of these bacteria and determine true risk to public health.

### 3.6 Conclusions

Overall, the differences, or lack thereof, in bacterial concentrations at the sampled sites at Hoop Pole Creek indicate that the Closed sites may not pose a much greater risk to public health than the Open site. The *Vibrio* and *E. coli* levels did not significantly differ between the Open site and the Closed site. All sites exceeded the 14 CFUs/100 mL limit suggested by Shellfish Sanitation. While this study only provides a small “snapshot” of the microbial concentrations in Hoop Pole Creek and the Shellfish Sanitation limit is quantified spanning over many seasons, the significance in this data rises mostly not from the high levels of contamination in this small sampling period, but in the lack of difference in microbial contamination between the Open and Closed sites which were closed for high levels of Fecal coliforms. This counters the initial assumption that the difference between the Open and Closed sites would be in microbial concentrations. To quantify the actual differences in risk to human health between the sites, a quantitative microbial risk assessment could be used, but this would require more information about actual pathogens and pathogenicity.
Chapter 6: Economics

1. INTRODUCTION

Economic valuation of ecosystem services has gained interest in the past 30 years in the wake of increasing environmental regulations and concerns over reduced ecosystem resilience (Bockstael et al., 2000). McLeod and Leslie (2012) define economics as the study of human well-being. When applying economic values to products of services from nature, it is only possible to prescribe values as these things benefit humans. This can cause philosophical qualms among environmentalists and conservationists, but it is a necessary procedure to promote protection and restoration of natural systems while avoiding bias from personal emotions and opinions towards ecosystem restoration (McLeod and Leslie, 2012; Bockstael et al., 2000).

Valuating market prices of goods taken from the environment (e.g. oysters for consumption) can be fairly straightforward, but valuating non-market values is very difficult. Valuations of ecosystem services are usually underestimates, which can be dangerous in deciding whether a system or area would provide an economic benefit were restoration measures taken (McLeod and Leslie, 2012). If the cost of restoring a system exceeds the net economic value gained from restoring that system, it does not seem cost or time-effective to devote energy and resources into restoration efforts.

In order to better understand how the people of Carteret County potentially gain indirect economic benefit from oyster reefs in areas open and closed to shellfishing, we attempted to provide a monetary representation of just a few of the services provided by our two reefs in the Hoop Pole Creek area. The hopes for this undertaking are that these values will influence how Carteret County citizens view oyster reefs and their benefits beyond consumptive value, and promote restoration efforts in areas where direct-market values are not explicitly reaped.

2. METHODS

Values are presented on a m² scale to give a representation of values of the services studied that more accurately reflects the sizes of these reefs.

2.1 Oyster Market Value

The consumptive market value of potential oyster harvest from the open reef has been included since oysters are a good produced by the system and cannot be ignored from the reef’s potential benefits. An analysis of the market value of the open reef was conducted using oyster density and size data. The estimated average number of harvestable oysters (about 7.6 cm in length) per m² was calculated from the six quadrats taken from the reef. This number was then multiplied by $0.05 per oyster, a value provided by Sammy Corbett of the NC Marine Fisheries Commission (Rich, 2014). This was assumed to be the annual value that could be produced during one oyster harvesting season.

2.2 Habitat Value

The general values of the open and closed reef sites as habitat were derived through an examination of commercially valued fish biomass at each site. It was assumed that all organisms came from within a 10 m² area of oyster reef. Biomass was converted to a per m² value. Using
data from the National Marine Fisheries Service commercial landing statistics database (2013), the value of fish biomass per m$^2$ of oyster reef captured for the three sampling days was calculated and then projected across a year (Grabowski et al., 2012; Grabowski & Peterson, 2007).

### 2.3 Denitrification

Denitrification rates in micromoles per m$^2$ per hour of each reef were calculated from the average of the differences between N$_2$ flux on-reef and off-reef provided by Chapter 4 (Grabowski et al., 2012). These rates were then converted into kilograms of N$_2$ per m$^2$ per year. The economic value of the denitrification services of each reef was calculated using the value of annual nitrogen removal per kg prescribed by the North Carolina Nutrient Offset Credit Program from 2011 – a value of $28.33 adjusted for the 2014 equivalent of $29.87 (North Coast Atlantic Conference Rule no. 15A NCAC 02B .0240 from Grabowski et al., 2012).

### 2.4 Chlorophyll $a$ Removal

Water quality services were valued by converting removal rates of chlorophyll $a$ (an indicator of phytoplankton presence) to removal rates of nitrogen through oyster consumption of phytoplankton using data provided in Chapter 3 (Grabowski et al., 2012). First, concentrations of chlorophyll $a$ from water samples between points 3 m upstream, at the upstream edge, and between the middle of reef and downstream edge at each reef were averaged, and the differences in concentrations between these pairs were calculated (see Chapter 3 Section 2 for more information on sampling points). These differences were inferred to be the average removal rates in micrograms of chlorophyll $a$ per liter of water filtered by oysters. The values for micrograms of chlorophyll $a$ per liter were converted to micromoles of chlorophyll $a$ per liter. Using methods and calculations from Wienke and Cloern (1987), Redfield (1958), and Grabowski et al. (2012), the values for chlorophyll $a$ removal were converted to carbon removal and then to nitrogen removal, producing a quantitative value for micromoles of nitrogen removed per oyster per hour (Table 1). Micromoles of nitrogen were converted to kg and multiplied by the average oyster density for each respective reef per m$^2$ to obtain a value for kg of nitrogen removed per m$^2$ per hour for each, then converted to kg of nitrogen removed per m$^2$ per year. The same value of $29.87 per kg of nitrogen removed set by the NC Nutrient Offset Credit Program was then applied.

**Table 1 - Calculations for value of annual chlorophyll $a$ removal.**

<table>
<thead>
<tr>
<th></th>
<th>Open</th>
<th>Closed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average [Chla] Upstream</td>
<td>*6.62</td>
<td>6.44</td>
</tr>
<tr>
<td>Average [Chla] for On Reef</td>
<td>6.18</td>
<td>4.73</td>
</tr>
<tr>
<td>Difference between Upstream and On Reef [Chla]</td>
<td>0.44</td>
<td>1.71</td>
</tr>
<tr>
<td>Micromoles Chl A/L</td>
<td>4.92E-04</td>
<td>1.91E-03</td>
</tr>
<tr>
<td>Filtration rate L per hour</td>
<td>1.21</td>
<td>1.21</td>
</tr>
<tr>
<td>Micromoles N/oyster/hour</td>
<td>0.004491689</td>
<td>0.017473767</td>
</tr>
<tr>
<td>Kg N/oyster/hour</td>
<td>6.29E-11</td>
<td>2.44E-10</td>
</tr>
<tr>
<td>Oysters/m$^2$</td>
<td>2218.666667</td>
<td>2461.3328</td>
</tr>
</tbody>
</table>
### 3. RESULTS

<table>
<thead>
<tr>
<th></th>
<th>1.22E-03</th>
<th>5.27E-03</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kg N/m²/year</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Value/kg N</td>
<td>$29.87</td>
<td>$29.87</td>
</tr>
<tr>
<td>$ Value/m²/year</td>
<td>$0.04</td>
<td>$0.16</td>
</tr>
</tbody>
</table>

*Outlier value from 3m upstream in Sample 1 removed.

**Figure 1.** Comparison of the value of services presented in this study provided by closed and open reefs per square meter per year. Total value for closed = $22.66. Total value for open = $32.54.

Monetary value of ecosystem services measured in Chapters 2, 3 and 4 is displayed in Figure 1. The value of the open reef as habitat for fish and crustaceans makes up 81% of the reef’s total value per m², whereas this service comprises 15% of the total value per m² of the closed reef. An opposite trend is seen in denitrification value, which comprises 85% of the closed reef’s total value and 15% of that of the open reef. Chlorophyll *a* removal made up a very small fraction of the total value of each reef per m², but the value it contributes to the closed reef is four times greater than that of the open reef. As seen in the pie chart representing the open reef’s value, the predicted market value of harvestable oysters for consumption only makes up 4% of the total value of the open reef per m².

Since the value of ecosystem services is often presented on the scale of hectares per year and is most helpful for managers, the total value of each reef was converted to this scale. The total value of the closed reef per hectare is $226,600 per year, and the total value of the open reef per hectare is $325,400 per year.
4. DISCUSSION

Some clear differences arise between effectiveness of services in the open and closed reefs. For the reefs’ function as a habitat, the open reef seems to boast higher abundances of fish and crustaceans per m$^2$. This initially seems counterintuitive since the open reef would be thought to experience more disturbances from possible fishing pressure. These differences could possibly be attributed to interference in trap sampling on the closed reef or to the relative locations of the reefs. Though the open reef is open to fishing and harvesting, it is not thought to be a popular shellfishing region and sees fairly little human disturbance (although there is no data of actual fishing pressure on this reef available). The closed reef is next to a dredged channel with regular boat traffic, so perhaps disturbance from boat wakes deters mobile organisms from inhabiting this area. More research should be conducted at different points throughout the year to determine what causes the disparity between fish and crustacean abundances on these reefs.

The values of denitrification and chlorophyll $a$ removal display similar magnitudes of disparity between the open and closed reefs. The major differences between these services seen in the field is likely due the differences in density between the two reefs, as is suggested by water filtration laboratory experiments (see Chapter 3, Section 3.2). This characteristic coupled with an average larger oyster size in the closed reef might explain why the closed reef is worth four times more than open reef when it comes to chlorophyll $a$ removal and approximately six times more in terms of denitrification, given no observed or measured differences in oyster quality between the open and closed reefs.

On the open reef, it is clear the consumptive values predicted from estimated harvestable oysters is trivial compared to the indirect, non-consumptive values. It is important to realize that with increasing unsustainable harvesting efforts and degradation to the reef system, the values of ecosystem services provided by the reef could decrease over time. This fact promotes the idea of focusing restoration efforts in closed areas in order to gain the maximum possible economic benefits from services provided by oyster reefs outside of oyster consumption. However, the actual fishing pressures on the open reef are unknown. So with the possibility that the open reef does face pressures from shellfishing, there is much room for optimism for the effectiveness of non-consumptive ecosystem services this reef provides despite disturbance from this disturbance.

It should be noted that these values are rough estimates of potential economic benefits these reefs could provide and the data from which these values were extrapolated were collected during a short amount of time in one season of the year. These values projected across a year represent potential annual value from only a “snapshot” in time. For a more accurate representation of the economic benefits these reefs provide, studies should be conducted periodically throughout the year and on longer timescales. Only a small portion of data collected during this study were able to be used due to restrictions in knowledge of available valuation methods which are still being developed, leading to a rather non-holistic value of the services presented and thus providing an underestimation of their actual value. Additionally, there are a host of other services (such as shoreline erosion protection and decreasing turbidity) provided by oyster reefs not mentioned in this study that should be investigated before prescribing definitive economic values to either of these reefs.
Chapter 7: Synthesis

The physical, biological, and chemical components of oyster reefs interact in many ways to alter the environment. For example, oyster density along reefs potentially affected each groups’ results. Although differences in density between the two reefs were not statistically significant, greater oyster densities were observed on the closed reef. A greater density of oysters on a reef implies greater filtration capacity. Greater drawdown of chlorophyll $a$ and TSS was observed along the closed reef versus the open reef. Higher oyster density at the closed site likely causes increased filtration. Differences in the abundance and distribution of organisms are likely dependent on the topography of the reef. The slightly higher oyster density as well as greater rugosity measured at the closed site could lead to greater species richness. Conversely, greater organism biomass was found at the open reef than the closed reef. This could be attributed to the greater anthropogenic impacts at the closed reef. Our data show that it is plausible that oyster reefs of the same density and size could provide the same magnitude of ecosystem services had they been under similar conditions. Future studies examining a larger variation of densities across different oyster reefs could offer an explanation as to the role density plays in the total value of ecosystem services provided by oyster reefs.

Anthropogenic effects were evident and meaningful for every facet of our project. The rugosity and density of the open reef may have been affected by shellfishing pressures. Despite a lack of documented evidence for shellfishing on the open reef, the data in Chapter 1 suggest that oyster harvest in the open area reduced the density and rugosity of the reef. Furthermore, energy from boat traffic may have caused resuspension of deposited particles, which could then be taken-up by oysters during filtration and deposited in the more compact forms of feces and pseudofeces. Resuspension of particles followed by biodeposition may allow a greater deposit of organic matter, which is necessary for denitrifying bacteria to function. The resuspension of particles also explains the higher content of carbon at the closed reef control compared to the open reef control in Chapter 4. Constant anthropogenic disturbances may cause a reduction in fish abundance through avoidance. This is evident in the finding of greater diversity and abundances of fish biota at the open reef.

Although our premise for comparing the two sites was whether they were open or closed to shellfishing, there were no significant differences between the two reefs regarding shellfishing sanitation standards. There were no significant differences in fecal indicator bacteria and *Vibrio* concentrations between the open and closed sites, indicating that they pose similar levels of risk to human health. Both the open and closed sites possessed microbial concentrations above levels deemed acceptable by the North Carolina Department of Marine Fisheries.

Additionally, the ecosystem services provided by both reefs far outstrip the economic value of harvesting the oysters themselves. With further analysis we could determine where best to place restoration projects to maximize benefits for both fisheries and ecosystem health. While both reefs yielded nearly the same economic value, there were possible errors in sampling, especially regarding the low biomass associated with the closed reef due to vandalism by outside forces.

Overall, there were many similarities between these reefs open and closed to shellfishing. With this knowledge, future studies could investigate different parameters, such as distance from populated areas, size, depth, or climate, to distinguish between reefs, rather than open or closed to shellfishing alone. Such a study could look deeper into broader anthropogenic impacts on oyster reefs.
Acknowledgements

We would like to thank all of the staff and faculty at the University of North Carolina Institute of Marine Sciences for giving us the opportunity to conduct this study, as well as providing us materials and guidance throughout the semester. We would especially like to thank our advisors, Dr. Mike Piehler and Kathleen Onorevole, for their constant support and optimism. A special thanks to Captain Joe Purifoy for always making himself available to transport us to and from the study sites and for gracing us with Disney sing-alongs. Finally, we would like to thank IDEXX Laboratories for providing lab materials, NC Coastal Federation, and NC Division of Marine Fisheries.
Table 8.1 Complete results table for two-tailed T-test of unequal variance between open and closed sites.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mussel Count (Open)</td>
<td>6</td>
<td>10</td>
<td>5.1</td>
<td>0.0441*</td>
</tr>
<tr>
<td>Mussel Count (Closed)</td>
<td>6</td>
<td>53.3</td>
<td>39.8</td>
<td>0.1828</td>
</tr>
<tr>
<td>Mud Crab Count (Open)</td>
<td>6</td>
<td>5.8</td>
<td>2.1</td>
<td>0.3370</td>
</tr>
<tr>
<td>Mud Crab Count (Closed)</td>
<td>6</td>
<td>13.3</td>
<td>11.8</td>
<td></td>
</tr>
<tr>
<td>Polychaete Count (Open)</td>
<td>6</td>
<td>0.5</td>
<td>0.5</td>
<td>0.0153*</td>
</tr>
<tr>
<td>Polychaete Count (Closed)</td>
<td>6</td>
<td>1.7</td>
<td>2.7</td>
<td>0.0323*</td>
</tr>
<tr>
<td>Barnacle Count (Open)</td>
<td>6</td>
<td>7</td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td>Barnacle Count (Closed)</td>
<td>6</td>
<td>1</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>Spiral Snail Count (Open)</td>
<td>6</td>
<td>30.7</td>
<td>25.6</td>
<td></td>
</tr>
<tr>
<td>Spiral Snail Count (Closed)</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Overall Quadrat Biota Count (Open)</td>
<td>6</td>
<td>54.3</td>
<td>28.8</td>
<td>0.0153*</td>
</tr>
<tr>
<td>Overall Quadrat Biota Count (Closed)</td>
<td>6</td>
<td>69.5</td>
<td>53.4</td>
<td>0.8232</td>
</tr>
<tr>
<td>Reef Top Trap Biota Count (Open)</td>
<td>6</td>
<td>1</td>
<td>1.3</td>
<td>0.4850</td>
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<tr>
<td>Reef Top Trap Biota Count (Closed)</td>
<td>4</td>
<td>1.8</td>
<td>1.7</td>
<td>0.5215</td>
</tr>
<tr>
<td>Reef Edge Trap Biota Count (Open)</td>
<td>6</td>
<td>2.3</td>
<td>2.9</td>
<td>0.4779</td>
</tr>
<tr>
<td>Reef Edge Trap Biota Count (Closed)</td>
<td>5</td>
<td>1.2</td>
<td>2.7</td>
<td>0.1013</td>
</tr>
<tr>
<td>Overall Biota Trap Count (Open)</td>
<td>12</td>
<td>1.7</td>
<td>2.3</td>
<td>0.0153*</td>
</tr>
<tr>
<td>Overall Biota Trap Count (Closed)</td>
<td>9</td>
<td>1.4</td>
<td>2.2</td>
<td>0.0323*</td>
</tr>
<tr>
<td>Reef Top Biota Trap Biomass (Open)</td>
<td>6</td>
<td>35.9</td>
<td>52.0</td>
<td>0.1761</td>
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<td>Reef Top Biota Trap Biomass (Closed)</td>
<td>4</td>
<td>18</td>
<td>22.2</td>
<td>0.1013</td>
</tr>
<tr>
<td>Reef Edge Biota Trap Biomass (Open)</td>
<td>6</td>
<td>89.8</td>
<td>130.8</td>
<td>0.0153*</td>
</tr>
<tr>
<td>Reef Edge Biota Trap Biomass (Closed)</td>
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<td>5.6</td>
<td>12.5</td>
<td>0.0323*</td>
</tr>
<tr>
<td>Overall Biota Trap Biomass (Open)</td>
<td>12</td>
<td>62.9</td>
<td>98.9</td>
<td>0.0153*</td>
</tr>
<tr>
<td>Overall Biota Trap Biomass (Closed)</td>
<td>9</td>
<td>11.1</td>
<td>17.5</td>
<td>0.0323*</td>
</tr>
</tbody>
</table>
**Table 8.2** - Water column significant differences (2-way Anova) Differences in concentrations of *E. coli* and *Enterococcus* in the water column were significant at Closed, Open and Additional Closed sites, while differences in concentrations of Total coliforms, *E. coli*, *Enterococcus*, *V. Vulnificus*, and *V. Parahaemolyticus* were significant across the sampling dates.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Site</th>
<th>P-value</th>
<th>Date</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total coliforms</td>
<td>No</td>
<td>0.0525</td>
<td>Yes</td>
<td>0.0070</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>Yes</td>
<td>0.0002</td>
<td>Yes</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><em>Enterococcus</em></td>
<td>Yes</td>
<td>0.0134</td>
<td>Yes</td>
<td>0.0001</td>
</tr>
<tr>
<td>Total Vibs</td>
<td>No</td>
<td>0.0966</td>
<td>No</td>
<td>0.1733</td>
</tr>
<tr>
<td><em>V. vulnificus</em></td>
<td>No</td>
<td>0.8757</td>
<td>Yes</td>
<td>0.0188</td>
</tr>
<tr>
<td><em>V. Parahaemolyticus</em></td>
<td>No</td>
<td>0.2462</td>
<td>Yes</td>
<td>0.0275</td>
</tr>
</tbody>
</table>

**Note:** Excludes data from the Pipe and Near Pipe sites
Table 8.3 - Only the differences in *E. coli* concentrations in oyster tissue were significant across each site, while the concentrations of *V. Vulnificus* and *V. Parahaemolyticus* were significant across the sampling dates. (2-way Anova)

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Site</th>
<th>P-value</th>
<th>Date</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total coliforms</td>
<td>No</td>
<td>0.0552</td>
<td>No</td>
<td>0.7104</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>Yes</td>
<td>0.0002</td>
<td>No</td>
<td>0.3581</td>
</tr>
<tr>
<td>Total Vibrs</td>
<td>No</td>
<td>0.0837</td>
<td>No</td>
<td>0.5160</td>
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<tr>
<td><em>V. vulnificus</em></td>
<td>No</td>
<td>0.8757</td>
<td>Yes</td>
<td>0.0059</td>
</tr>
<tr>
<td><em>V. Parahaemolyticus</em></td>
<td>No</td>
<td>0.1812</td>
<td>Yes</td>
<td>0.0208</td>
</tr>
</tbody>
</table>

Figure 8.2 - Total *Vibrios* concentrations in log transformed colony forming units (CFUs) per mL in oyster tissue and water at each site across the sampling days. Concentrations of total *Vibrio* were consistently high at the Additional Closed, Closed and Open sites.

Tables 8.4-6 - These tables show which relationships revealed significant relationships, the correlation between them, and whether that correlation was positive or negative. Site 3 contained the lowest significant relationships.

<table>
<thead>
<tr>
<th>Site 1</th>
<th>Salinity</th>
<th>P-value</th>
<th>Temperature</th>
<th>P-value</th>
<th>24-hr rainfall</th>
<th>P-value</th>
<th>48-hr rainfall</th>
<th>P-value</th>
<th>5-day rainfall</th>
<th>P-value</th>
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<tbody>
<tr>
<td>TC</td>
<td>no</td>
<td>-</td>
<td>no</td>
<td>-</td>
<td>no</td>
<td>-</td>
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<td>EC</td>
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<td>-</td>
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<td>-</td>
<td>no</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ENT</td>
<td>yes</td>
<td>0.0124</td>
<td>no</td>
<td>-</td>
<td>yes</td>
<td>0.0050</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Vulnificus</em></td>
<td>no</td>
<td>-</td>
<td>yes</td>
<td>0.0483</td>
<td>no</td>
<td>-</td>
<td></td>
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<td>48-hr rainfall</td>
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<td>0.0333</td>
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## Site 2

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<th>Temperature</th>
<th>P-value</th>
<th>24-hr rainfall</th>
<th>P-value</th>
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### 48-hr rainfall

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### 48-hr rainfall

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**Figure 8.3** - *V. Vulnificus* and *V. Parahaemolyticus* concentrations in log transformed colony forming units (CFUs) per 100mL of water at each site across the sampling dates. September 22nd contained high concentrations of both *Vibrio* species at each site. Similar pattern shown in *Vibrio* levels in the oyster tissues as well.
Literature Cited

Ackerman, D. & S. Weisberg. 2003. "Relationship between Rainfall and Beach Bacterial Concentrations on Santa Monica Bay Beaches." *Journal of Water and Health* 1: 85-89.


Paerl, H. 1997. Coastal eutrophication and harmful algal blooms: Importance of


