ABSTRACT

PIERSON, KATHERINE JOAN. Response of Estuarine Fish to Large-scale Oyster Reef Restoration. (Under the direction of David B. Eggleston).

Native oyster species were once vital ecosystem engineers, but their populations have collapsed worldwide because of overfishing and habitat destruction. In response to historic low population sizes of eastern oysters, the North Carolina Division of Marine Fisheries created a network of no-take, oyster broodstock reserves in an effort to enhance the oyster metapopulation in Pamlico Sound. In addition to oyster enhancement, restored oyster reefs increase structural refuge and provide increased feeding opportunities for fish. The overall goal of this study was to quantify estuarine fish use of constructed oyster reefs in Pamlico Sound. Our specific objectives were to (1) compare estuarine fish assemblages on oyster reefs to unstructured bottom, (2) identify dietary habits of bluefish *Pomatomus saltatrix* that cohabitate oyster reefs and unstructured estuarine bottom, (3) identify the short-term change in fish abundance and richness in response to large-scale oyster reef construction using a Before-After-Control Impact (BACI) design, and (4) identify spatiotemporal trends in fish abundance and richness associated with sub-tidal oyster reefs. We quantified transient and reef fish using gill nets and fish traps, respectively.

During this two-year study, 3,031 fish consisting of 51 different species were captured by gill nets and 95 fish of 10 different species were captured by fish traps on oyster reserves. On unstructured bottom, 2,155 fish consisting of 36 species were captured in gill nets and 32 fish consisting of 8 different species were captured in fish traps. There was also strong evidence that oyster reefs harbored more unique species than unstructured bottom, as

might be expected, thereby enhancing the overall diversity of estuarine fish assemblages. Qualitatively, there was evidence that the diet of bluefish, which co-occurred on oyster reefs and unstructured bottom, varied between habitats, with sheepshead *Archosargus probatocephalus* dominating the diet for bluefish captured near reefs, and Atlantic croaker *Micropogonias undulates*, pinfish *Lagodon rhomboids*, silver perch *Bairdiella chrysoura* and spot *Leiostomus xanthurus* occurring only in the diets of bluefish captured from unstructured bottom. Quantitatively, however, there was no difference in the mean relative stomach fullness for fish collected from the two habitats. In general, the relative abundance of transient fish (targeted with gill nets) did not change in experimental reef areas from before to after reef creation ~ 6-8 months later. The creation of oyster reefs affected the mean number of fish species and mean CPUE of fish captured in traps at one restoration site (Clam Shoal) but not a second site (Crab Hole). The mean number of fish species captured in traps at Clam Shoal was higher at control reefs that were already present prior to construction of new reefs (i.e., experimental reefs) than at experimental reefs, whereas after experimental reefs were constructed the mean fish diversity dropped at nearby control reefs, suggesting emigration from control to new reefs. Thus, over a relatively short time frame of 6-8 months post-reef construction, there was little evidence that fish abundance varied from background (control) levels, whereas fish diversity in 1 of 2 sites actually decreased on control reefs. Information on how restored oyster reefs are used by estuarine fish will not only improve our understanding of oyster reefs as Essential Fish Habitat and the trophic role oyster reefs play in estuarine ecosystems, but can augment our growing appreciation of the economic value of oyster reefs beyond simply commercial harvest of oysters.

Response of Estuarine Fish to Large-scale Oyster Reef Restoration

by
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BIOGRAPHY

Katherine Joan Pierson was born in Hartford, Connecticut in October of 1986. She spent much of her youth playing outside, going to the art museum, and convincing her dad to buy her more pets. When she was eight she quit brownies to join 4-H, which became an integral part of her life until she left for University. Another important event happened when she was eight; her parents bought a cottage on a small pond in Eastham, Massachusetts. From then on she spent time swimming, catching turtles, catching bass, sunfish and pickerel and camping out in the backyard. Lucky for her, right down the street was Mass Audubon's Wellfleet Bay Wildlife Sanctuary, and she was enrolled in day camp a couple of weeks every summer from then on. At that early age, due to the Sanctuary's vast property on Cape Cod bay, she decided that she was going to grow up to be a marine biologist.

In the summer before high school she was accepted into a program called "Exploring Diversity through aquaculture," in which she visited four different states to compare and learn about their different aquaculture practices. It was then that she amended her eight year old dream to become a "Marine fisheries biologist." Throughout high school she ran, played the flute and loved her science classes. Before her senior year of high school, she and her best friend traveled up North to Nova Scotia to participate in a five-week French immersion program. In the land of l'Acadie she discovered Acadia University, where she received her BSc in Biology in 2008.

She then moved back "home" to Cape Cod and started working at the Wellfleet Bay Wildlife Sanctuary as an environmental educator and the project manager of the first oyster reef restoration in the state of Massachusetts. She also spent her downtime at the Harwich Shellfish Department, raising quahogs and oysters. With a passion for fish and science, Katie moved to Raleigh to pursue her Master's degree.

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I was back visiting the oyster reef that started it all a couple of weeks ago. Looking out at the reef I was reminded of how many hands built it. It is inevitable that the number of people who have made this thesis possible are greater than just me. I am unable to name everyone but thankfully, I know some inspirational people who will undoubtedly forgive the omission.

At NCSU: I would like to thank my advisor, Dr. Dave Eggleston, for providing financial and logistical support, and helping to push me to be a better scientist. Although the formation of my committee was late in this process, I want to express my sincerest gratitude to them for taking me on. Thank you to Dr. Joe Hightower for always being available and for your clarity. Dr. Jim Rice, thank you for allowing me into your lab space, giving me your time and providing insight when I have been stuck. Thanks to Dr. Geoff Bell. Thank you to my labmates: Ashlee Lillis, Brandon Puckett, Ryan Rindone, Robert Dunn and Gayle Plaia. I would also like to thank Katherine Spears, Hunter Eggleston, Jamie Eggleston, Kate Miller, Matthew Stillwell and Marcos Alaniz Rodrigues for their help pulling nets in and sorting through fish. I would like to acknowledge my funding sources: NOAA, NC Sea Grant and the NC Coastal Federation via the American Reinvestment and Recovery Act/ NOAA.

I have the privilege of knowing some amazing and inspiring people and know that I would not be where I am now without them. I am indebted to the community that I found in the Student Fisheries Society; thank you for recognizing one of your own and never failing to provide support when needed. Specifically, I’d like to thank Zach Feiner for his diet analysis magic, Lindsay Campbell for her help with stomach content identification, Dr. Wayne

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This Master's process has been taxing and I have gotten by with a little help from some friends who have not allowed my life to turn into chaos: Blake Jordan, Nate Naumann and Diane Fielding and of course Will M. I would like to thank my wonderful family without whose support my path would have been much harder.

"No man is an island"- John Donne

Thank you and cheers,

Katie

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Introduction:

Ecosystem destruction and the resulting loss of ecosystem services, such as reduced carbon sequestration, decreased rates of denitrification, and reduced energy transfer between trophic levels (Peterson et al. 2003, Gregalis et al. 2009) has fueled ecosystem restoration projects worldwide. Examples range from restoring the geomorphology of streams, to the construction of riparian buffers to help impede the flow of excess nutrients to receiving waters, to the removal of dams to restore spawning habitat for anadromous fishes (Kauffman et al. 1997, Bednarek 2001, Bash and Ryan 2002). The creation of inter- and sub-tidal habitats in coastal systems, such as salt marshes, seagrass beds and oyster reefs, have also been used to improve water quality, and as a means to enhance essential fish habitat (defined in the next section) (Minton 1999, Coen and Luckenbach 2000, Thom et al. 2005, Beck et al. 2011).

Well-designed monitoring efforts are critical to assessing the efficacy of ecosystem restoration projects, especially given the often relatively long time scale for restored ecosystems to reach functional equivalency with natural ecosystems (Zedler and Callaway 1999). Monitoring is key to evaluating restoration projects in relation to original goals, and in providing an opportunity to adaptively manage the project if certain goals are not met (Thom 2000, Bash and Ryan 2002).

Essential Fish Habitat

The Magnuson-Stevens Fishery Conservation and Management Act of 1996 defined Essential Fish Habitat (henceforth referred to as EFH) as "those waters and substrate necessary to fish for spawning, breeding, feeding and growth to maturity" (US DOC 1997). Within the definition of EFH, "waters" includes all aquatic areas and their physical, chemical, and biological properties, whereas "substrate" includes all communities that make those areas suitable for fish (Rosenberg et al. 2000). Included in this act is a mandate that all U.S. State management plans describe and identify EFH for every managed species (Rosenberg et al. 2000). The EFH mandate prioritizes managing not only single species, but other habitat associated species as well.

Classification and prioritization of EFH , however, remains tenuous due to the mobility of finfish and many crustaceans within a given life history stage, as well as ontogenetic changes in habitat use over time (Eggleston 1995, Coen et al. 1999, Minton 1999, Eggleston et al. 2004). Identification of habitats that are essential at any life stage is vital for the conservation of our fisheries. Ecosystem engineers, such as- oysters and coral that form reefs, provide habitat that serves as refuge for many dependent species and therefore, these habitats have been classified as EFH (Coen et al. 1999, Sarthou 1999, Beck et al. 2011).

The eastern oyster *Crassostrea virginica* provides numerous ecosystem services and functions including structural refuge for mobile and sessile species, biofiltration and subsequent improvement of water quality, carbon sequestration, and shoreline erosion control (Peterson et al. 2003, Gregalis et al. 2009). Oyster reefs have been classified as EFH due to the ecosystem processes they provide (Coen et al. 1999, Coen and Luckenbach 2000, Stunz et al. 2010). Their use by estuarine finfish within the context of a large-scale oyster restoration project within no-take oyster reserves in Pamlico Sound, NC, USA is the focus of this study.

Marine Reserves

Another method of habitat restoration involves creation of no-take marine reserves in which destructive fishing practices (e.g. trawling and dredging) are eliminated, thereby allowing the habitat to re-build. Indirect improvement of seabed complexity and macrobenthic organism abundance often occurs within marine reserves where destructive fishing practices are banned (Watling and Norse 1998, Roberts and Sargant 2002, Rodwell et al. 2003). Coral reefs damaged by fishing gear have exhibited rapid recovery within two years after they were protected as marine reserves (McClanahan and Obura 1995, Edgar and Barrett 1999). The deployment of artificial reefs into marine reserves can result in rapid increases in localized abundance of juvenile target species (Wilson et al. 2002). The combination of ecosystem restoration within a no-take marine protected area (henceforth referred to as MPA) has the potential to mitigate damage done by destructive fishing gear and provide momentum for the restoration of marine species (Wilson et al. 2002).

Oyster Reef Restoration

In the U.S., oyster populations have fallen to historic lows in the last century due to overfishing, habitat loss and disease (Rothschild et al. 1994, Peterson et al. 2003, Beck et al. 2009, Wilberg et al. 2011). Between 1880 and 1910, the U.S. oyster fishery hit its peak and landed 72,574.8 metric tons per year, but by 1995 landings had fallen to only 18,325.1 metric tons per year (Coen and Luckenbach 2000). Oyster landings have not only declined dramatically in the U.S., but from 1995 to 2004 only 10 ecoregions in the world (regional, biogeographic units that have similar ecosystems and species) landed more than 1,000 metric tons per year (Beck et al. 2011). Many factors have influenced this striking drop in oyster fishery landings: overfishing, habitat destruction, shortages of suitable hard substrate for spat (newly settled oysters) to settle on, habitat disturbance, reduced water quality, alteration of natural flow and salinity patterns, natural and introduced predators and competitors, and increased oyster disease (Rotschild et al. 1994, Coen et al. 1999, Beck et al. 2011). Although oyster aquaculture has grown substantially over the past decades, and individual locations and regions have developed seeding programs to supplement wild stocks with hatchery-reared oysters, the majority of the oyster fishery is based upon dwindling wild stocks (Coen et al. 1999, Beck et al. 2011). Motivated by the loss of 85% of the world's oyster reefs, state agencies, academic institutions, nonprofit organizations, and local communities have initiated oyster reef habitat restoration projects (Peterson et al. 2003, Beck et al. 2009).

Oyster reef habitat restoration often includes creating a network of either intertidal or sub-tidal no-take oyster broodstock sanctuaries (Coen et al. 1999, Beck et al. 2011). Other methods of enhancing oyster populations have included the addition of natural or alternative hard substrate, seeding with young and adult broodstock, improvement to habitat quality via clean-up initiatives, and attempts to shift the fishing pressure to hatchery-raised oysters via seeding of oyster beds (Coen et al. 1999, Beck et al. 2011). Restored oyster reefs are created by depositing cultch material on the seafloor, such as recycled and dredged oyster, clam and scallop shells, limestone or granite-based rip-rap material, and crushed concrete, upon which larval oysters settle (Haywood III et al. 1999). In addition to augmenting natural oyster populations, restored reefs in the Southeast United States provide habitat for a variety of recreationally and commercially important species including black sea bass *Centropristis striata*, sheepshead, and stone crab *Menippe mercenaria* (Peterson et al. 2003, Rindone & Eggleston in press, this study). Of these commercially viable species, the southern stock of black sea bass is currently depleted (NCDMF Stock Status Report 2010). Large-scale oyster restoration provides a bio-economically viable solution for rebuilding the black sea bass population in the U.S. Southeastern Atlantic (Millstein and Eggleston in review).

Objectives

The overall goal of this study was to quantify use of constructed oyster reefs by estuarine fish assemblages in Pamlico Sound, North Carolina, USA. Our specific objectives were to (1) compare estuarine fish assemblages on oyster reefs to unstructured bottom, (2) identify dietary habits of bluefish that cohabitate on both oyster reefs and unstructured estuarine bottom, (3) identify the short-term response of fish abundance and richness to large-scale oyster reef construction using a Before-After-Control Impact (henceforth referred to as BACI) design, and (4) identify spatiotemporal trends in fish abundance and richness associated with sub-tidal oyster reefs within Pamlico Sound.

Study system: Oyster broodstock reserves in Pamlico Sound

Pamlico Sound (PS) is the second largest estuary in the United States and is a primarily wind driven system with three major inlets that connect the sound with the Atlantic Ocean (Pietrafesa et al. 1985, Noble et al. 1991, Xie and Eggleston 1999). The mean depth of this shallow estuarine system is 4.9 m, with maximum depths around 7.3 meters (Ross and Epperly 1985, Xie and Eggleston 1999). The mean salinity in PS is 15 ppt, with salinity ranges within the Sound being affected by fresh water inflow that occurs from the west (Tar-Pamlico and Neuse rivers) and the north (Albemarle Sound and tributaries) of PS (Ross and Epperly 1985). Salinities in southern Pamlico Sound are generally the highest, averaging about 25 ppt (Ross and Epperly 1985). PS is a nursery for many fish species, including: spot, bay anchovy *Anchoa mitchilli*, croaker, Atlantic menhaden *Brevoortia tyrannus*, silver perch, and southern flounder *Paralichthys lethostigma* (Ross and Epperly 1985).

The North Carolina Division of Marine Fisheries (henceforth referred to as NC DMF) created a network of no-take oyster broodstock reserves in 1996 to enhance the oyster metapopulation in PS (Figure 1). A secondary goal was to create oyster reefs that would serve as EFH, and support recreational and commercial fisheries (NCDMF Stock Status Report 2010). These oyster restoration efforts were accelerated greatly in 2009-2010 with funding via the American Reinvestment and Recovery Act, which created 17 ha of oyster reefs in nine months (Pelle Holmlund, NC DMF, personal communication), compared to the previous 49 ha that had been created since 1996. As of 2011, 10 broodstock reserves have been established with footprints ranging in size from 1.86 ha to 19.30 ha. Each reserve contains high-relief (2m) limestone marl mounds that are roughly 20 meters apart (Figure 2; NC DMF). Limestone marl was used due to a clear preference by oyster larvae for limestone compared to other geomorphic material (Soniat and Burton 2005). Limestone rip-rap material used to create oyster broodstock reserves was colonized by oysters via natural settlement, and oyster densities have generally increased in these reserves 5 to 15 fold since 2006 (Puckett & Eggleston, in review). Oyster demographic rates such as fecundity, settlement, growth and survivorship, as well as potential larval connectivity among reserves, have been studied extensively on many of the broodstock reserves (Rindone and Eggleston in press, Haase et al. in review, Mroch et al. in review, Puckett and Eggleston in review); however, concurrent information on habitat use by estuarine fishes is absent.

Four oyster reserves were sampled during this study. The longest duration sampling occurred at the Clam Shoal and Crab Hole oyster reserves due to planned and realized large-scale oyster restoration at these locations. Fish at Clam Shoal and Crab Hole were sampled before and after large-scale oyster restoration. To provide an increased perspective of fish assemblages over time and space, two additional sampling locations in Pamlico Sound (Ocracoke & West Bluff) were added in 2010 after reef construction was completed at Crab Hole and Clam Shoal. These sites provided additional range in geographic location, salinity regime and other abiotic factors; Ocracoke and Clam Shoal reserves were located on the eastern part of PS, whereas West Bluff and Crab Hole were located on the western side of the

Sound (Figure 1). Knowledge of fish abundance and richness associated with this large-scale oyster restoration effort will improve our understanding of the role of oyster reefs as EFH, as well as the value of restored oyster reefs within the context of bio-economic costs and benefits (Millstein and Eggleston in review, Beck et al. 2011).

Hypotheses:

To address the hypotheses below, we measured fish abundance (catch per unit effort henceforth referred to as CPUE) and species richness (number of species per unit effort) with two gear types: gill nets that targeted relatively mobile fish, and baited traps that targeted reef-associated species. Each response variable below (e.g., mean CPUE) for a given hypothesis was tested separately for each gear type since these gears have different catch efficiencies and, therefore, cannot be compared statistically (Gunderson 1993).

Objective 1: Fish assemblages on oyster reserves compared to unstructured bottom

Estuarine fish assemblages can vary according to whether or not they are associated with complex structure, such as oyster reefs, which could lead to increased refuge from predators and increased prey for fishes (Harding and Mann 2001a, Grabowski et al. 2005, Gregalis et al. 2009), or reside on unstructured, soft-bottom (Ross and Epperly 1985). We hypothesized that mean (**H1**) CPUE and (**H2**) number of fish species would be significantly higher within oyster broodstock reserves compared to unstructured bottom, irrespective of

Location (Crab Hole, Clam Shoal, Ocracoke, West Bluff) and Month (July, August, September).

<u>Objective 2: Dietary habits of bluefish on oyster reserves compared to unstructured bottom</u>

Bluefish are mobile piscivores that occurred in relatively high abundance within broodstock reserves, as well as unstructured soft- bottom habitats in this study (see Results below). Therefore, we examined stomach contents of bluefish to determine if their dietary habits differed between habitat types. Low sample sizes reduced our ability to test whether or not indices of diet breadth and overlap varied between fish captured on reserves versus unstructured bottom (see Methods below). Thus, we hypothesized that (**H3**) a relative index of stomach fullness would not vary significantly between fish captured within oyster reserves versus unstructured bottom.

<u>Objective 3: Response of estuarine fish to large-scale oyster restoration (2009 through 2011)</u>

We hypothesized that (**H4**) mean CPUE and (**H5**) mean number of fish species per hour would vary significantly according to Year, and be higher upon the creation of the new experimental mounds (2010 and 2011) than before the mounds were created (2009), irrespective of Month (August, September) and Location (Clam Shoal, Crab Hole). We also hypothesized that mean fish abundance and richness on control oyster reserves (created prior to 2009) would vary significantly over time (Month) but not Location (Crab Hole and Clam

Shoal).

Objective 4: Spatiotemporal variation in estuarine fish assemblages

Monthly variation in abundance and species richness of estuarine fish is characteristic of dynamic estuarine environments, and can be driven by changes in abiotic variables such as salinity and tidal cycles, as well as biotic variables such as food or predation risk, the timing of recruitment of young-of-the-year, and spawning stock biomass (Rice et al. 1993, Warlen 1994, Wilson and Sheaves 2001, Searcy et al. 2007a, 2007b, Durham et al. in review). We hypothesized that mean (**H6**) CPUE and (**H7**) number of fish species on established reef mounds would vary significantly among Months (July, August, September, October) irrespective of Location (Crab Hole, Clam Shoal, Ocracoke, West Bluff).

Material and Methods:

Objective 1: Fish assemblages on oyster reefs compared to unstructured bottom

During July through September 2010, we quantified whether or not relative abundance and species richness of estuarine fish varied between oyster reefs and nearby unstructured bottom at the same four oyster reserves described above. At Crab Hole, the unstructured bottom treatment was five points of soft bottom habitat one kilometer away from the reserve. To determine sampling sites in soft bottom areas, the corners of a 0.5 km by 0.5 km square located 1-1.5 km away from the remaining oyster reserves were used to generate random GPS coordinates within the square, with four cells randomly chosen to receive gill nets and four cells randomly chosen to receive traps. Unstructured bottom was confirmed visually by skin divers. During each monthly sampling period, eight randomly selected old established mounds were sampled with gill nets (N=4) and fish traps (N=4). Gill nets measured 18.3 meters long, 3.04 meters deep and were partitioned into four 4.6 meter panels with stretch mesh sizes of 1.3, 2.5, 5.0, and 7.6 centimeters. Use of this type of gill net meant that there was a larger size range of fish sampled, but also that all of our inferences are specific to fish vulnerable to mesh sizes only. Once deployed, all gill nets soaked overnight (15 to 20 hours). Oval fish traps, with door openings of 15.2 centimeters by 13.7 centimeters with square mesh sizes of 0.95 centimeters, were each baited with two Atlantic menhaden fish and soaked overnight (15-20 hours). Sample sites were located via onboard GPS, with verification using a depth finder. Gill nets were then set across each mound by using the depth finder to start deployment on the backside of the mound and moving forward to deploy across each mound. Fish traps were placed directly on each of the sites being sampled.

Upon retrieval of a trap or gill net, all fish were identified to species, counted and measured to the nearest mm Standard length (SL: from anterior portion until the caudle peduncle). Bluefish were retained for stomach content analyses (see Objective 2). Water quality parameters, including temperature (°C), salinity (ppt), and dissolved oxygen (mg/L) were taken at 1 and 3m depths using a YSI-85 probe before deploying nets or traps and after retrieval (Table 1).

Objective 2: Dietary habits of bluefish on oyster reserves compared to unstructured bottom

Initially, we extracted the stomachs of nearly all fish collected during summer 2010 at all four oyster reserves and June 2011 at Crab Hole and Clam Shoal to assess whether or not diets varied according to new versus old oyster mounds, structured versus unstructured bottom, as well as by species. Standard and total lengths (TL: from the anterior portion until the tip of the tail) were measured on every fish sampled. The entire gastrointestinal tract was removed and preserved in 10% seawater-buffered formalin (Harding and Mann 2001a).

As stomach contents were processed in the laboratory, it became apparent that bluefish was the only species that consistently contained stomach contents that were not too digested, and which also occurred in reasonably high numbers within oyster reserves and unstructured bottom. We processed 32 bluefish stomachs from fish captured within oyster reserves in 2010 and 2011, and 36 bluefish stomachs from fish collected on unstructured bottom in 2010.

In the laboratory, the bolus of stomach contents was extracted , blotted with a tissue (i.e., KimWipes) and the wet weight measured to the nearest 0.01g. Once bluefish prey items were identified to the lowest possible taxon, they were placed into eleven prey categories based upon the lowest taxon found in all of the bluefish stomachs (Table 2). Individual prey items were weighed, as well as the empty stomach organ. Initially, empty stomachs were not included in the analysis, leaving 14 bluefish stomachs out of 32 collected on the oyster reserve and 24 out of 36 bluefish stomachs collected off the reserve that contained prey items. Stomach contents were tabulated by a record of presence or absence of prey items found in each fish stomach and analyzed as a percentage of all stomachs (containing food) in which each food category occurred (% frequency of occurrence:%O), as well as by average percent by weight (%W) which denotes the weight of individual prey items as a percentage of the total stomach content weight (Wallace 1981, Eggleston and Bochenek 1990, Murphy and Willis 1996, Harding and Mann 2001a, Plunket 2003). As these stomach samples were processed further, it became apparent that a large majority of the stomachs contained only unidentifiable prey (10 stomachs from reserves and 24 stomachs from unstructured bottom), which left insufficient sample sizes with identifiable prey (4 stomachs from reserves and 7 stomachs from unstructured bottom) to assess indices of diet overlap and breadth. Weights were not available for all fish; therefore stomach contents could not be expressed as a proportion of fish size. However, the weight of the empty stomach was used as an indirect relative body size scalar. Therefore, a derived index of relative stomach fullness was used:

$$X = \frac{weight(g)\ of\ combined\ prey\ items}{weight\ (g)\ of\ empty\ stomach\ organ}$$

I tested if the mean index of stomach fullness varied significantly between stomachs taken from fish captured within reserves versus unstructured bottom with a t-test.

Objective 3: Response of estuarine fish to large-scale oyster restoration

Two different factors were tested separately at each location (Crab Hole and Clam Shoal): (1) type of Oyster Reef (see below), and (2) Year (2009, 2010, 2011). Two different treatment levels of Oyster Reef were sampled within the boundaries of the Clam Shoal and Crab Hole oyster broodstock reserves: (1) “control mounds,” which consisted of older established oyster mounds (e.g., Fig. 2) created before 2006 and sampled in 2009, 2010 and 2011, and (2) "experimental sites before and after mound creation," which consisted of sampling the location where new mounds were going to be constructed, yet prior to their creation in 2009, and then sampling new mounds after their creation in 2010 and 2011. New mounds were created during winter of 2009 and early spring 2010 with the addition of 191 and 144 mounds at Clam Shoal and Crab Hole, respectively (Figure 3; NC DMF 2010).

Within reserves, eight randomly selected mounds or spaces that would (after restoration) be occupied by a mound were sampled with gill nets (N=4) and fish traps (N=4), established mounds were sampled using the same method (Figure 3; experimental sites: 2009 open blue triangles, 2010 and 2011 blue triangles). Gill nets and traps were deployed and fish sampled as described above.

Objective 4: Spatiotemporal variation in estuarine fish assemblages on oyster reserves

To begin to assess spatiotemporal variation in fish assemblages associated with oyster reefs in Pamlico Sound, fish were sampled during 2010 with gill nets and traps on randomly chosen old established oyster mounds at four oyster broodstock reserves (Crab Hole, Clam Shoal, West Bluff, Ocracoke) during four months (July, August, September, October) as described above. Very high relative abundance of clupeids was observed in September (see Results below), therefore hypotheses 6 and 7 were tested (see below) with and without the presence of clupeids so that other patterns might be detected and not swamped by the high abundance of clupeids.

Data analyses

Hypotheses one through six were tested statistically with various multi-way ANOVA models according to procedures described in Underwood (1981), as well as Scheiner and Gurevitch (1993). Data were tested for normality and homogeneity of variance using Shapiro-Wilks test and Levene's test, respectively. Log-transformed (log(x+1)) data were used when necessary to meet assumptions of normality and homogeneity of variance. In some instances, transformed data remained non-normal-- in these circumstances, transformed data was still analyzed with ANOVA due to its robustness and insensitivity to skewness (Glass et al. 1972, Legendre and Borcard 2007). In instances where variances were heavy tailed despite transformation, examination of the residual plots indicated they were close to being normal and therefore were used with transformation for data analysis (D. Dickey, NCSU Statistics Dept., pers. comm.). When higher level interactions were present, they were dissected using lower-level ANOVA models or a two-sample independent t-test (Underwood 1981). Multiple comparisons among treatment levels were tested with Tukey's Honestly Significant Difference (HSD) multiple comparisons test.

Results:

During this two-year study, 3,031 fish consisting of 51 different species were captured by gill nets and 95 fish of 10 different species were captured by fish traps on oyster reserves (Appendix 1). On unstructured bottom, 2,155 fish consisting of 36 species were captured in gill nets and 32 fish consisting of 8 different species were captured in fish traps (Appendix 2).

In general, water temperatures peaked in August (peak range of 27.6 to 30.3 °C) with a notable decrease in October (Table 1). Overall, salinities at West Bluff and Crab Hole located in the western part of Pamlico Sound were lower (mean of 17.3 psu) than at oyster reserves located in the eastern part of Pamlico Sound (mean of 21.1 psu). There was no evidence of hypoxia or anoxia, or any abrupt changes in other abiotic variables during sampling that would likely negatively impact relative abundance and species richness of estuarine fish (Table 1).

.*Objective 1: Fish assemblages on oyster reserves compared to unstructured bottom*

The species composition of estuarine fish captured by gill nets on oyster reserves and the unstructured bottom was different, with oyster reserves having a greater variety of fish species than unstructured bottom (Figure 4). Estuarine fish collected with gill nets within the oyster reserve boundaries were made up of 13% croaker, 12% weakfish *Cynoscion regalis*, and, 6% Atlantic spadefish *Chaetodipterus faber*, whereas fish captured on unstructured bottom were made up of 12% croaker and 9% kingfish (both northern and southern) (Figure 4). Within oyster reserve boundaries, 51 different species were captured with gill nets compared to the 36 different species captured on unstructured bottom (Appendix 1 & 2). A total of 21 species captured with gill nets were unique to oyster reserves and 9 species were unique to unstructured bottom. Fish traps captured 10 different fish species within oyster reserve boundaries and eight different species on the unstructured bottom (Appendices 1 & 2). A total of three species captured with traps were unique to oyster reserves and none of the species captured by fish traps were unique to unstructured bottom.

Clupeid species (i.e., Atlantic menhaden and Atlantic thread herring (31%)) made up a large percentage of the total catch of estuarine fish caught in gill nets in oyster reserves and unstructured bottom (43%; Figure 4). With the inclusion of Clupeids, the relative abundance of transient fish captured with gill nets on unstructured bottom was similar to or higher than the relative abundance within oyster reserves (Figure 5 & 6). Conversely, the relative abundance of reef fish captured with traps within oyster reserves was similar to or higher than the relative abundance on unstructured bottom (Figure 6). The mean CPUE of estuarine fish captured in gill nets varied significantly by Location, as well as Month and Treatment; however, significant interaction effects between Location X Month, as well as Location X Treatment precluded contrasts across the main effects (Table 3). The Location X Month interaction was due to significant increases in mean CPUE at Crab Hole, Clam Shoal and West Bluff in the month of September, whereas there was no significant increase at Ocracoke (Figure 5: HSD multiple comparisons test). Location X Treatment interaction effects were due to Clam Shoal and West Bluff having significantly higher fish CPUE on unstructured bottom than oyster reserve treatments, unlike the Crab Hole and Ocracoke oyster reserves which had no significant differences (Figure 6: Two-sample t-test). The mean CPUE of estuarine fish captured with traps did not vary significantly according to Treatment, Location or Month; there were no significant interaction effects (Table 4, Figure 6).

The mean number of species caught per hour in gill nets varied significantly according to Month and Treatment, but not by Location, although significant interaction effects between Location X Month as well as Location X Treatment precluded contrasts across the main effects (Table 5). Location X Month interaction effects were due to significant increases in species richness in the month of September at all locations except for West Bluff (Figure 7: HSD multiple comparisons test). Location X Treatment interaction effects were due to mean species richness being greater on unstructured bottom than on oyster reserves at Clam Shoal (Figure 8: Two-sample t-test). For fish captured in traps, mean species richness did not follow the same trend as the gill net results and did not vary significantly according to any significant main or interaction effects (Table 6, Figure 8).

Excluding clupeid species, the mean CPUE captured by gill nets varied significantly by Month and Treatment; however a significant two way interaction effect (Location X Month) and a significant three-way interaction effect (Location X Month X Treatment) precluded contrasts across the main and secondary effects (Table 7). The three-way interaction effect was due to greater CPUE on unstructured bottom than in the oyster reserve in July at Crab Hole, in August at West Bluff, but no interaction effects at Ocracoke and Clam Shoal. The mean CPUE at the Ocracoke oyster reserve did not vary significantly by Month or Treatment, and there were no significant interaction effects. At Clam Shoal, mean CPUE varied significantly by Month and Treatment; mean CPUE in September was significantly different than all other months tested and there were significant increases on unstructured bottom compared to oyster reserves (HSD multiple comparison test: Two-sample t-test).

Relative abundance of estuarine fish captured by gill nets increased at both treatments in the month of September compared to all other months sampled. Mean CPUE on unstructured bottom was higher than on oyster reserves at Clam Shoal and West Bluff (Figure 5 & Figure 6). When clupeid species were removed from the data, CPUE in gill nets was highest on unstructured bottom at Clam Shoal, Crab Hole in July and West Bluff in August. Species richness of estuarine fish captured in gill nets, however, displayed increases in September at all but the West Bluff oyster reserve (Figure 7). Clam Shoal exhibited higher species richness on unstructured bottom than within the oyster reserve (Figure 8). Mean CPUE and number of fish species from fish traps did not vary with Location, Month or Treatment (Figure 6). Mean CPUE captured by gill nets on unstructured bottom was similar to mean CPUE on oyster reserves at two locations and higher at two locations, but species richness was similar at all but one site, indicating that generally there is a greater abundance of individuals but not an increase in the number of species on unstructured bottom.

Objective 2: Dietary habits of bluefish on oyster reserves compared to unstructured bottom

Stomach contents of bluefish were grouped into 11 prey categories: shrimp, plant material, unidentified fish, spot, sheepshead, silver perch, pinfish, pigfish *Orthopristis chrysoptera*, croaker, Atlantic menhaden and anchovy (Tables 8 & 9). Stomachs of bluefish from oyster reserves contained seven of the potential 11 prey categories (pigfish, unidentified fish, anchovy, Atlantic menhaden, plant, sheepshead and shrimp), whereas eight prey categories occurred in bluefish caught on unstructured bottom. Only pigfish, Atlantic menhaden and anchovy were unique to stomachs collected on oyster reserves.

The average index of relative stomach fullness on oyster reserves was 0.37±0.19 (mean ±SE) and the mean index of stomach fullness on unstructured bottom 0.47±0.20 (mean ±SE). The mean index of relative stomach fullness did not vary significantly between habitat types (Two-sample t-test: t=-0.34, p=0.74).

Objective 3: Response of estuarine fish to large-scale oyster restoration

The creation of oyster reefs appeared to have no effect on mean relative abundance of estuarine fish. The mean CPUE of estuarine fish captured with gill nets at Clam Shoal did not vary significantly by Treatment ("control" versus "experimental" oyster sites) or Year (2009, 2010 & 2011) (Table 10 A). At the Crab Hole oyster reserve, however, the mean CPUE of estuarine fish captured by gill net varied significantly by Year but not according to Treatment (Table 10 B). The significant Year effect was due to a significant increase in mean CPUE in 2011 compared to 2010 (t=2.633, p=0.0319).

Conversely, at the Clam Shoal oyster reserve mean CPUE captured by fish traps varied significantly by Treatment and Year, as well as a significant interaction effect which precluded contrasts of main effects (Table 11 A). The interaction effect was due to significantly higher mean CPUE captured by fish traps on control compared to experimental sites in 2009, and then no difference in mean CPUE between control and experimental sites after construction in 2010 and 2011 (Figure 9). Mean CPUE of estuarine fish captured in fish traps at Crab Hole did not vary significantly according to any main or interaction effects (Table 11 B).

The mean number of species per hour caught in gill nets at the Clam Shoal oyster reserve varied significantly according to Treatment and Year (Table 12 A). The Treatment effect was due to significantly higher species richness on experimental reefs compared to control reefs (p=0.0168, control: 0.3461±0.1224, experimental: 0.4538±0.1485). The Year effect was due to the significant decrease in mean number of species captured from 2010 to 2011 (p=0.0112). Conversely, the mean number of fish species did not vary according to Treatment or Year at Crab Hole (Table 12 B).

At the Clam Shoal oyster reserve, the mean number of fish species captured by fish traps varied significantly according to Treatment and Year, however, a significant interaction effect precluded contrasts across main effects (Table 13 A). At Clam Shoal there was significantly higher mean number of species in control sites compared to experimental sites in 2009 prior to construction of experimental reefs (p=0.0002), whereas in subsequent years (2010 and 2011) there was no difference in the mean number of fish species found on control versus experimental sites (Figure 10: p=0.7912 and p=0.9789, respectively). Conversely, at the Crab Hole oyster reserve, the mean number of species per hour caught in fish traps did not vary significantly according to any main or interaction effects (Table 13 B, Figure 10).

The creation of the oyster reefs had a profound effect on the estuarine fish captured by fish traps at one of the reserves. At Clam Shoal the relative abundance of estuarine fish and the number of fish species captured by fish traps was much higher on control sites than experimental sites before construction of experimental mounds in 2009, and no difference in mean CPUE of mean number of species (and much lower numbers of species) between control and experimental reefs in 2010 and 2011. The mean CPUE of transient fish captured by gill net at Crab Hole was greater in 2011 compared to 2010. Gill nets at Clam Shoal demonstrated a significant increase in the mean number of species on experimental sites compared to control sites. Gill net catches at Clam Shoal also captured a greater mean number of species in 2010 compared to 2011.

Objective 4: Spatiotemporal variation in estuarine fish assemblages

The mean CPUE of estuarine fish captured with gill nets on control reefs (i.e., old established mounds) varied significantly according to Location and Month; there were no significant interaction effects (Table 14). The significant Month effect was due to higher mean CPUE in September than all other months (Figure 11 A: HSD multiple comparisons test). The significant Location effect was due to a higher mean CPUE of estuarine fish captured in gill nets at West Bluff compared to all other oyster reserves (Figure 11 B: HSD multiple comparisons test). The mean CPUE of estuarine fish captured with traps did not vary significantly according to Location or Month; there were no significant interaction effects (Two-way ANOVA, all $p > 0.44$).

The mean number of fish species captured per hour by gill nets on control reefs varied significantly according to Location and Month, however, a significant interaction effect between Month X Location precluded contrasts across the main effects (Table 15). The interaction effect was due to significant differences in how the mean number of fish species varied by Month within a given Location, with the most consistent trend being highest number of species in September at all Locations (Figure 12: HSD multiple comparisons test). The mean number of estuarine fish species captured with traps did not vary significantly according to Location or Month; there were no significant interaction effect (two-way ANOVA, all $p > 0.26$).

Thus, mean CPUE from gill nets was highest at West Bluff and in the month of September at all locations. Species richness from gill nets was also highest in September. Mean CPUE and number of fish species from fish traps placed on control reefs did not vary with Location or Month.

Discussion:

The overall goal of this study was to determine habitat use by estuarine fish on recently established oyster reefs in Pamlico Sound, NC. In general, we found that the relative abundance of transient (targeted with gill nets) and reef-associated (targeted with traps) fish did not change in experimental reef areas from before to after reef creation. Conversely, the relative species richness did vary on control versus experimental reefs when pooled across year and treatment from before to after reefs were constructed at the Clam Shoal site only. Thus, over a relatively short time frame of 6-8 months post-reef construction, there was little evidence that fish abundance varied from background (control) levels, whereas fish species richness at one of the two sites actually decreased on control reefs. There was also strong evidence that oyster reefs harbored more unique species than unstructured bottom, as might be expected, thereby enhancing the overall diversity of estuarine fish assemblages. Qualitatively, there was evidence that the diet of bluefish, which co-occurred on oyster reefs and unstructured bottom, varied between habitats, with sheepshead dominating the diet for bluefish captured near reefs and croaker, pinfish, silver perch and spot occurring only in the diets of bluefish captured from unstructured bottom. However, there was no difference in mean relative stomach fullness between the two habitats. Information on how restored oyster reefs are used by estuarine fish will not only improve our understanding of oyster reefs as EFH and the trophic role oyster reefs play in estuarine ecosystems, but can augment our growing appreciation of the economic value of oyster reefs beyond simply commercial harvest of oysters (Millstein and Eggleston in review). The role of restored oyster reserves as EFH needs to be continuously monitored due to the dynamic nature of estuarine systems, and future research might more tightly focus on improved methods of sampling reef-associated species.

Gear selectivity

Estuarine fish assemblages are highly variable in space and time due to the dynamic nature of estuaries (Wilson and Sheaves 2001). We tried to account for this variability by sampling across years, months and locations. Fish populations living on oyster reefs are also notoriously difficult to sample because of low visibility for diver observations, complex structure that entangle nets, and difficulties in locating reefs due to inadequate maps (Lenihan et al. 2001, Robillard et al. 2010, Stunz et al. 2010). Initially, we tried four methods to characterize fish assemblages on oyster reefs and unstructured bottom in Pamlico Sound: (i) gill nets, (ii) fish traps, (iii) minnow traps, and (iv) SCUBA diver surveys. We abandoned minnow traps because they never captured any reef-associated fish such as blennies and gobies (whether the traps were baited or un-baited), and abandoned diver surveys due to highly variable visibility that ranged from centimeters to meters. During dives for a related study (Puckett and Eggleston in review) when water visibility was relatively high, we observed numerous large sheepshead swimming around the oyster mounds—these fish were rarely captured by either gill nets or fish traps due to gear avoidance. Despite the fact that both gill nets and fish traps suffer from reduced gear efficiency for certain species such as sheepshead, we assumed that the efficiency of a given gear type did not vary across habitat types (oyster reefs versus unstructured bottom) (Gunderson 1993), and therefore provided a relative comparison of fish abundance and species richness across habitat types for those species that were vulnerable to them.

Fish assemblages on oyster reefs compared to unstructured bottom

In general, the relative abundance of transient fish captured with gill nets on unstructured bottom was similar to or higher than the relative abundance within oyster reserves. Conversely, the relative abundance of reef fish captured with traps within oyster reserves was similar to or higher than the relative abundance on unstructured bottom. Our findings for transient fish are contrary to many studies that indicate oyster reefs harbor greater relative abundance and species richness of transient fish than unstructured bottom

(Lenihan et al. 2001, Plunket 2003), although Gregalis et al. (2009) found that CPUE of transient fish on their control (unstructured bottom) was greater than both their low and high relief oyster reefs. Possible explanations for increased transient fish CPUE on unstructured bottom include the broad habitat associations of these fish combined with their ability to travel across the estuary (Gregalis et al. 2009) and their use of open water for foraging grounds (Robillard et al. 2010), all of which may pre-dispose transient fish to a weak or insignificant response to the large-scale creation of oyster reefs.

For reef fish captured by traps, although the data were highly variable, there was a trend of higher abundance on oyster reefs compared to unstructured bottom. Oyster reefs provide abundant refuge from predators, spawning substrate, and food resources (Coen et al. 1999, Tolley and Volety 2005, Deaton et al. 2010). For example, Tolley and Volety (2005) found that abundances of gulf toadfish *Opsanus beta* captured by lift nets were an order of magnitude higher on oysters (dead or alive) compared to sand bottom. During this study, oyster toadfish *Opsanus tau* captured by fish traps were more abundant on oyster reserves than on unstructured bottom (Appendices 1 & 2). Toadfish have been documented feeding on xanthid crabs that are found on oyster reefs (Tolley and Volety 2005). Toadfish also lay eggs on the underside of consolidated oyster shells (Breitburg 1999).

In this study, restored oyster reefs in Pamlico Sound harbored 18 different species that have important recreational and commercial value in NC: Atlantic croaker, Atlantic menhaden, black sea bass, bluefish, gag grouper *Mycteroperca microlepis*, red drum *Sciaenops ocellatus*, southern flounder, southern kingfish *Menticirrhus americanus*, spanish mackerel *Scomberomorus maculates*, spot, spotted seatrout *Cynoscion nebulosus*, striped bass *Morone saxatilis*, summer flounder *Paralichthys dentatus*, weakfish, and white perch *Morone americana*, as well as shark species such as Atlantic sharpnose *Rhizoprionodon terraenovae*, smooth dogfish *Mustelus canis* and spiny dogfish *Squalus acanthias*. Of the 18 important fishery species found utilizing oyster reefs in this study, six have been documented as previously inhabiting similar oyster habitat (Lenihan et al. 2001). Unlike past studies, Atlantic menhaden, bluefish and cownose rays *Rhinoptera bonasus* were found on both restored oyster reefs and unstructured bottom (Lenihan et al. 2001). Unique to oyster reefs, in this study, were commercially and recreationally important fishery species such as black sea bass, gag grouper, red drum, spotted sea trout, striped bass, and white perch, as well as shark species such as smooth and spiny dogfish. There were some distinct differences in the fish species caught between oyster reefs and unstructured bottom. For example there was a shift from species that were common in unstructured habitats, such as clupeids, kingfish, croaker and hogchoker *Trinectes maculates*, to an assemblage where those were still present, but with a greater percentage of weakfish, spadefish and pigfish. Atlantic spadefish is classified as a reef residential species (Coen et al. 1999), and was the only reef resident that we captured in relatively high numbers. In this study, highly sought-after recreational species, such as croaker, weakfish, bluefish and spot were found on both oyster reefs and unstructured bottom, but constituted a greater percentage of the catch on oyster reefs.

The estuarine fish captured by gill nets on the oyster reserves in this study made up 51 different species, the gill net catch was greater than the 14 transient fish species captured in Chesapeake Bay, MD using gill nets (Harding and Mann 2001b), the 23 fish species captured in Barataria Bay, LA with gill nets and substrate trays (Plunket 2003) and 31 fish species captured in the Yangtze River estuary, China using gill nets and fish traps (Quan et al. 2009). To our knowledge, there are no European studies of the response of fish to oyster restoration. Oyster reefs in Pamlico Sound and Chesapeake Bay are targeted by recreational anglers via private boats, as well as charter and party boats, and constitute a growing sector of estuarine-based recreational fisheries (Efland and Eggleston, unpubl. data, Hicks et al. 2004).

Dietary habits of bluefish on oyster reserve and off reserve

The majority of the bluefish diets were comprised of unidentifiable fish, due to either the high level of decomposition or the propensity of bluefish to bite off chunks of their prey. This large, unidentifiable component of the fish diets masked possible quantitative differences in diet composition between the two habitats. A greater number of stomachs contained prey items on the unstructured bottom compared to the stomachs collected within the oyster reserve.

Due to the small sample size of stomachs that contained identifiable prey items, a more in-depth study of the comparison between oyster reserve versus off reserve should be conducted to better assess diet-overlap and niche breadth indices (e.g. Schoener's (1970) or Levin's (1968), respectively) in stomach contents between habitats. Collection of stomachs directly after the main crepuscular feeding period for bluefish (Buckel and Conover 1997) would help ensure that there would be food in a majority of the stomachs sampled and more identifiable items. Moreover, future diet studies should target larger samples sizes within a more narrow range of gill net soak times to reduce the level of digestion of prey items.

<u>Response of estuarine fish to large-scale oyster restoration</u>

The creation of oyster reefs in this study appeared to have no short-term (scale of months) effect on mean relative abundance of transient estuarine fish. For example, the relative abundance of fish captured by gill nets on experimental reef sites before reefs were built did not differ on these same sites after reefs were built, nor did they differ from control ("old") reefs. Transient estuarine fish such as weakfish, bluefish, menhaden, and croaker generally have broad habitat associations and can travel across the estuary (Gregalis et al. 2009). Both attributes may pre-dispose transient fish to a weak or no response to the large-scale creation of oyster reefs. Moreover, gill net catches in this study were relatively low and generally ranged from 1.0-2.0 fish/hour, which could decrease the likelihood of detecting a reef effect on transient fish in this study. In related studies, an average CPUE of 6.4 fish/ hour were captured in experimental gill net panels of 25.4, 38.1, 50.8 and 63.5 mm in Barataria Bay, LA (Plunket 2003), and a mean CPUE of 1.9 fish/hour were captured in gill net mesh of 5 and 10 cm in Mobile Bay, AL (Gregalis et al. 2009). In contrast, for relatively constricted tidal creeks in Alabama, creation of oyster reefs resulted in increased abundance of demersal southern flounder and decreased abundance of silver perch, whereas there was no difference in whole fish community relative abundance in creeks with and without restored oyster reefs (Geraldi et al. 2009).

The creation of oyster reefs in this study did have a pronounced effect on the number of fish species at the Clam Shoal site, but in a manner that was somewhat unexpected. The pattern was striking for trap data- the number of fish species as well as mean CPUE was 7-fold higher on control reefs compared to experimental reef sites before construction of mounds (which demonstrated a short term response to mound creation). However, after experimental reefs were built, the mean number of species and relative abundance of estuarine fish nearly doubled on experimental reefs, whereas the mean on control reefs dropped nearly 6-fold (Figures 9 & 10). In a related study, fish traps that sampled natural oyster reefs as well as restored reefs captured a total of 4-12 fish in each 12 hour soak time (Lenihan et al. 2001), which was an order of magnitude higher than the values reported in this study. Lenihan et al. (2001) used box-shaped fish traps that were un-baited which could account for discrepancies between their catch and values reported in this study. The size frequency of fish captured on experimental versus control reefs was similar (data not shown) suggests that colonization of experimental reefs within 6-8 months post reef construction was probably via immigration from adjacent reefs rather than via new recruits. The idea of a "drawdown" of resident fish from control to experimental reefs is consistent with the "attraction mechanism" associated with artificial reefs (Pickering and Whitmarsh 1997), however, a definitive test requires some type of mark-recapture or biotelemetry experiment before versus after reef creation (e.g., Briones-Fourzan et al. 2007).

Spatiotemporal variation in estuarine fish assemblages

The relative abundance of estuarine fish on "old" oyster mounds was about twice as high in September compared to other months, and about twice as high at West Bluff compared to other sites. The mean number of fish species followed this same general pattern in time. Possible explanations for the increase in relative abundance and number of fish species in September could include recruitment of some size classes into our gear due to growth throughout the summer months (Attrill and Power 2004), and immigration of certain species (e.g., black sea bass and Atlantic menhaden) as they move across the estuary in preparation to emigrate to the continental shelf in fall for subsequent winter spawning (Ross and Epperly 1985, Warlen 1994). Moreover, there is evidence of a positive correlation between number of fish species in Pamlico Sound and increasing water temperature (Ross and Epperly 1985), this was also found in a study of estuarine fish in the Thames estuary in which fish abundance increased with temperature (Attrill and Power 2004). Temperature affects fish physiology, with indirect effects on growth and survival adaptations that correspond to changes in fish abundance (Attrill and Power 2004). Water temperatures in this study were the highest in August, which corresponds to a slight delay in species richness and abundance when water temperature increases. Unlike this study, Ross and Epperly (1985) found that species richness peaked in April and July and remained high until September and October throughout all stations in PS. Similar studies of seasonal variability in fish abundance on oyster reefs in Alabama (Gregalis et al. 2009) and in the lower Noosa estuary in Australia (Miller and Skilleter 2006) found that fish CPUE was lowest in winter, increased in spring and fall, and was highest in summer months. This study only encompassed the summer and early fall months.

The high relative abundance of fish at West Bluff in this study may have been due to seafloor bathymetry that splits PS into two separate basins of differing depth and sediment composition, causing distinct fish assemblages (Ross and Epperly 1985). Different environmental factors, such as salinity regimes, proximity to inlets, and current flow create a complex estuarine ecosystem that could account for spatial variation within PS (Wilson and Sheaves 2001). Although this study did not target larval fish, spatiotemporal variation in larval supply might also impact spatiotemporal patterns in abundance of estuarine- dependent species in PS (e.g., Etherington and Eggleston 2003, Reyns et al. 2006). Differences in fish abundance between oyster reserves in PS could also be due to the surrounding habitat; notable differences in fish abundance have occurred when oyster reefs have been placed near seagrass beds or marshes (nursery habitat for juvenile fishes) (Heck 2003).

Future studies

A key component of addressing fish habitat utilization is fish movement and site fidelity, which can be addressed in tagging studies (Topping et al. 2006). In addition to the previous suggestions regarding ways to increase the sample size and quality of stomach contents for diet analyses, future studies of estuarine fish use of oyster reefs would do well to have a movement component. Tagging studies can provide information on fishing mortality, natural mortality, immigration, emigration and site fidelity (Pine et al. 2003). Moreover, tag recapture studies, as well as those that examine condition indices such as biomass, growth and survival can help inform how oyster reefs may or may not enhance growth, physiological condition, and survival compared to unstructured bottom. For example, Bacheler et al. (2009) used conventional tags paired with ultrasonic transmitters to determine monthly fishing mortality values (which could be partitioned into recreational and commercial components) as well as natural mortality values for red drum in coastal North Carolina. In a telemetry study, Ng et al. (2007) found that tagged striped bass had annual returns into the New Jersey estuary as well as small home ranges which indicated high habitat quality. Similar information would demonstrate specific species site fidelity and inform about habitat quality of oyster reserves, which would serve to further refine the EFH definition for oyster reefs. A study using tag recapture on spiny lobsters found that the deployment of artificial reefs (casitas) resulted in significantly higher biomass, apparent survival and local persistence of juveniles than areas with no casitas (Briones-Fourzán et al. 2007). Determining mortality values for fish that are utilizing oyster reefs would provide information on fish survival on oyster reserves compared to unstructured bottom, and information about returning fish can refine the definition of EFH for transient fish using oyster reserves.

Oyster reefs are inherently structurally complex, which often limits the use of traditional gear. Many recent studies have tried to find alternatives to present conventional gear methods, such as the use of epibenthic sleds in deep subtidal habitats (Robillard et al. 2010, Stunz et al. 2010). An alternative non-invasive technique to quantify relative abundance of fish is hydroacoustic sampling. Hydroacoustics have been widely used in sampling fishery resources in rivers, lakes and other deep water, with recent studies concentrating on the horizontal beam technology application in shallow water (Taylor et al. 2006, Boswell et al. 2007). Hydroacoustic surveys of an artificial reef in the Gulf of Mexico found that the relative abundance of fish decreased with distance from the artificial reef complex and that the horizontal area of influence was within 20 m of the reef structure (Boswell et al. 2010). The major benefit of this technique is the ability to cover large surface areas in relatively short periods of time, however, ground-truthing methods (i.e. gill nets, fish traps, divers) are necessary to partition the sonar signatures into specific species (Boswell et al. 2007).

Conclusion

Management strategies for oyster restoration include creating no-take broodstock reserves, enhancing the fishery via planting cultch and seed oysters in no-take areas that are periodically opened for harvest, utilizing oyster reefs for erosion control in nearshore zones, improving water quality via biofiltration, and as a by-product enhancing fishing opportunities for recreational fishers targeting reefs for finfish (Eggleston 1999, Beck et al. 2011). Because of the numerous ecosystem functions and services provided by oyster reefs, restoration has been used to simultaneously attain multiple management goals. Our gill-net catches indicated that the relative abundance and number of species of transient fish was similar between oyster reserves and unstructured bottom. Conversely, our fish-trap catches indicated that the relative abundance and number of reef fish species was, in some instances, higher on oyster reefs, thereby enhancing the overall diversity of estuarine fish collected.

Determining potential differences in bluefish stomach contents indicated that although certain prey items were exclusively found in fish stomachs taken from oyster reserves versus unstructured bottom, relative stomach fullness was similar for fish captured on oyster reserves versus unstructured bottom. Using a BACI design, we found that fish colonization of newly created reefs was relatively rapid (6-8 months). Monthly sampling indicated that September had the highest relative abundance of all months sampled. Continued identification of spatiotemporal trends in estuarine fish use of oyster reefs in Pamlico Sound as well as future monitoring of new experimental mounds such as in this study will help define the role of oyster reefs as EFH.

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Table 1: Water quality data during 2010 for all four oyster reserves in Pamlico Sound, NC. Readings were taken at 1 and 3 meters water depth (measurements at 3 m are in parentheses). Blanks denote times when data could not be taken due to weather events.

	Before setting gill nets and traps			**After pulling gill nets and traps**		
Oyster Reserve	***Salinity (ppt)***	***Temperature (^oC)***	***Dissolved O_2 (mg/L)***	***Salinity (ppt)***	***Temp. (^oC)***	***D.O. (mg/L)***
West Bluff						
July	14.2 (14.6)	28.7 (27.7)	7.77 (7.39)	14.5 (14.5)	28.3 (28.2)	7.36 (7.4)
August	17.7 (17.7)	30.3 (30.2)	8.05 (8.08)			
September	18.6 (18.7)	27.2 (27.0)	7.94 (7.57)	18.9 (18.9)	26.1 (26.2)	7.31 (7.36)
October	19.8 (20.1)	21 (20.3)	9.65 (8.5)	18.7 (19.0)	20.4 (20.4)	8.91 (8.92)
Crab Hole						
July	12.6 (12.7)	27.7 (27.8)	7.31 (7.31)	14.3 (12.7)	28.3 (27.8)	7.64 (7.31)
August	18.8 (18.8)	27.6 (27.6)	6.55 (6.44)	18.5 (18.7)	28.4 (28.2)	6.85 (6.56
September	17.9 (17.9)	26 (26.4)	8.55 (8.57)	18.7 (19)	26.5 (25.8)	7.64 (7.03)
October	17.7 (18.7)	21.5 (21.2)	10.2 (5.68)	17(18.7)	20.2 (20.9)	10.43 (5.41)
Ocracoke						
July	19.1 (19.2)	23.8 (23)	7.66 (7.8)	19.2 (19.2)	28.4 (27.6)	7.55 (7.37)
August	21.9 (22)	29.9 (29.5)	7.24 (7.13)	21.9 (22.4)	28.9 (29.1)	6.76 (6.8)
September	21.8 (21.8)	27.2 (27.2)	7.62 (7.82)	22 (21.9)	26.4 (26.4)	6.47 (6.39)
October	20 (20.8)	22.3 (22.2)	8.52 (8.44)	20.6 (20.6)	21.4 (21.4)	7.89 (8.2)
Clam Shoal						
July	21.3 (21.9)	29.7 (28.5)	7.65 (7.32)	21.5 (21.6)	28.3 (28.1)	6.2 (5.85)
August	23.3 (23.4)	29.8 (29.7)	6.72 (6.61)	23.4 (23.5)	28.9 (28.9)	5.85 (5.75)
September	20 (20)	26.6 (26.4)	7.02 (6.85)	20 (20.1)	26.7 (26.5)	7.18 (6.8)
October	19.4 (19.6)	22.9 (22.4)	9.15 (9.24)	20.1 (20.2)	21.6 (21.4)	7.47 (7.62)

Table 2: Names, categories and descriptions of bluefish prey categories used in diet analysis.

Category	Description
Anchovy	Identified Anchovy part via jaw, skull and pectoral fin structure
Atlantic menhaden	Identified Atlantic menhaden via structure and scale analysis
Croaker	Identified Croaker part
Pigfish	Identified Pigfish part via scale analysis
Pinfish	Identified Pinfish part via scale analysis
Plant material	All plant material
Sheepshead	Identified Sheepshead part via pharyngeal tooth structure
Shrimp	All shrimp parts
Silver perch	Identified Silver perch part
Spot	Identified Spot via scale analysis
Unidentified fish	Fish parts that were unable to be identified

Table 3: Results of ANOVA for the effects of Location (Crab Hole, Clam Shoal, Ocracoke, and West Bluff), Month (July through September 2010) and Treatment (Oyster reserve, and unstructured bottom) on mean estuarine fish CPUE that were captured in gill nets. * Indicates significant effect.

Factors	**Sum Sq**	**Df**	**F value**	**Pr (>F)**
Location	3.8716	3	20.2981	1.43e-09 *
Month	4.0100	2	31.5355	1.72e-10 *
Treatment	1.4230	1	22.3816	1.13e-05 *
Location X Month	3.0985	6	8.1224	1.13e-06 *
Location X Treatment	0.8128	3	4.2612	0.0080 *
Month X Treatment	0.2142	2	1.6844	0.1930
Location X Month X Treatment	0.4082	6	1.0700	0.3888
Residuals	4.4506	70		

Table 4: Results of ANOVA for the effects of Location (Crab Hole, Clam Shoal, Ocracoke, and West Bluff), Month (July through September 2010) and Treatment (Oyster reserve, and unstructured bottom) on estuarine fish CPUE that were captured in fish traps. * Indicates significant effect.

Factors	**Sum Sq**	**Df**	**F value**	**Pr(>F)**
Location	0.0273	3	1.5314	0.2140
Month	0.0098	2	0.8256	0.4422
Treatment	0.0123	1	2.0756	0.1541
Location X Month	0.0359	6	1.0058	0.4287
Location X Treatment	0.0006	3	0.0316	0.9924
Month X Treatment	0.0119	2	1.0014	0.3726
Location X Month X Treatment	0.0228	6	0.6406	0.6974
Residuals	0.4159	70		

Table 5: Results of ANOVA for the effects of Location (Crab Hole, Clam Shoal, Ocracoke, and West Bluff), Month (July through September 2010) and Treatment (Oyster reserve, and unstructured bottom) on mean estuarine fish species caught per hour with gill nets.
* Indicates a significant effect.

Factors	**Sum Sq**	**Df**	**F value**	**Pr(>F)**
Location	0.0269	3	1.0294	0.3850
Month	0.9543	2	54.8306	4.71e-15 *
Treatment	0.2134	1	24.5188	4.90e-06 *
Location X Month	0.2265	6	4.3371	0.0009 *
Location X Treatment	0.1232	3	4.7186	0.0047 *
Month X Treatment	0.0269	2	1.5479	0.2199
Location X Month X Treatment	0.0492	6	0.9424	0.4706
Residuals	0.6092	70		

Table 6: Results of ANOVA for the effects of Location (Crab Hole, Clam Shoal, Ocracoke, and West Bluff), Month (July through September 2010) and Treatment (Oyster reserve and unstructured bottom) on mean number of fish species per hour captured in fish traps. * Indicates a significant effect.

Factors	**Sum Sq**	**Df**	**F value**	**Pr(>F)**
Location	0.0147	3	2.7077	0.0518
Month	0.0019	2	0.5327	0.5894
Treatment	0.0025	1	1.3760	0.2448
Location X Month	0.0136	6	1.2548	0.2898
Location X Treatment	0.0034	3	0.6204	0.6042
Month X Treatment	0.0021	2	0.5845	0.5601
Location X Month X Treatment	0.0086	6	0.7933	0.5783
Residuals	0.1246	69		

Table 7: Results of ANOVA for the effects of Location (Crab Hole, Clam Shoal, Ocracoke, and West Bluff), Month (July through September 2010) and Treatment (Oyster reserve and unstructured bottom) on mean estuarine fish CPUE excluding Atlantic menhaden and Atlantic thread herring that were captured in gill nets. * Indicates significant effect.

Factors	**Sum Sq**	**Df**	**F value**	**Pr(>F)**
Location	0.8016	3	1.0791	0.3637
Month	2.4681	2	4.9839	0.0095 *
Treatment	3.0674	1	12.3880	0.0008 *
Location X Month	10.3139	6	6.9423	8.14e-06 *
Location X Treatment	0.9475	3	1.2755	0.2895
Month X Treatment	0.0710	2	0.1434	0.8667
Location X Month X Treatment	4.9493	6	3.3314	0.0061 *
Residuals	17.3326	70		

Table 8: Percent composition by weight (%W) of 11 different prey categories for buefish stomach contents found on oyster reserves and unstructured bottom.

Prey Category	Oyster Reserve %W	Unstructured Bottom %W
Anchovy	4.0237	0
Atlantic menhaden	7.6923	0
Croaker	0	4.5455
Pigfish	7.6923	0
Pinfish	0	3.3093
Plant	0.2442	2.5674
Sheepshead	15.3846	4.5455
Shrimp	7.6923	10.3164
Silver Perch	0	4.5455
Spot	0	4.5455
Unidentified Fish	57.2706	65.6250
	n=14	n=24

Table 9: Frequency of occurrence (%O) of 11 prey categories of bluefish diets collected on oyster reserves and unstructured bottom.

Prey Category	Oyster Reserve % O	Unstructured Bottom %O
Anchovy	7.1429	0
Atlantic menhaden	7.1429	0
Croaker	0	4.1667
Pigfish	7.1429	0
Pinfish	0	4.1667
Plant	14.2857	8.3333
Sheepshead	14.2857	4.1667
Shrimp	7.1429	12.5000
Silver perch	0	4.1667
Spot	0	4.1667
Unidentified fish	64.2857	70.8333
	n=14	n=24

Table 10: Results of two-way ANOVA for the effects of Treatment (Control vs. Experimental reefs) and Year (2009, 2010 & 2011) on estuarine fish CPUE captured by a gill net separated by Location (A. Clam Shoal, B. Crab Hole). * Indicates effects that are statistically significant.

A. Clam Shoal

Factors	**Sum Sq**	**Df**	**F value**	**Pr (>F)**
Treatment	0.1230	1	0.7198	0.4021
Year	0.7184	2	2.1029	0.1377
Treatment X Year	0.0102	2	0.0298	0.9706
Residuals	5.8077	34		

B. Crab Hole

Factors	**Sum Sq**	**Df**	**F value**	**Pr (>F)**
Treatment	0.1336	1	0.7474	0.3934
Year	1.4475	2	4.0489	0.0265*
Treatment X Year	0.9240	2	2.5847	0.0902
Residuals	6.0775	34		

Table 11: Results of two-way ANOVA for the effects of Treatment (Control & Experimental reefs) and Year (2009, 2010 & 2011) on mean estuarine fish CPUE captured by fish traps separated by Location (A. Clam Shoal, B. Crab Hole). * Indicates effects that are statistically significant.

A. Clam Shoal

Factors	**Sum Sq**	**Df**	**F value**	**Pr(>F)**
Treatment	0.1917	1	11.8338	0.0016*
Year	0.2142	2	6.6113	0.0038*
Treatment X Year	0.3018	2	9.3128	0.0006*
Residuals	0.5346	33		

B. Crab Hole

Factors	**Sum Sq**	**Df**	**F value**	**Pr (>F)**
Treatment	0.0004	1	0.1664	0.6859
Year	0.0016	2	0.3659	0.6863
Treatment X Year	0.0003	2	0.0684	0.9340
Residuals	0.0755	34		

Table 12: Results of two-way ANOVA for the effects of Treatment (Control vs. Experimental reefs) and Year (2009, 2010 & 2011) on the mean number of estuarine fish species captured per hour with gill nets separated by Location (A. Clam Shoal, B. Crab Hole). * Indicates effects that are statistically significant.

A. Clam Shoal

Factors	**Sum Sq**	**Df**	**F value**	**Pr (>F)**
Treatment	0.1158	1	8.0461	0.0076*
Year	0.1779	2	6.1776	0.0051*
Treatment X Year	0.0367	2	1.2761	0.2921
Residuals	0.4895	34		

B. Crab Hole

Factors	**Sum Sq**	**Df**	**F value**	**Pr (>F)**
Treatment	0.0236	1	1.3374	0.2556
Year	0.0538	2	1.5235	0.2325
Treatment X Year	0.0136	2	0.3848	0.6835
Residuals	0.5999	34		

Table 13: Results of two-way ANOVA for the effects of Treatment (Control vs. Experimental reefs) and Year (2009, 2010 & 2011) on the mean number of estuarine fish species captured per hour with fish traps separated by Location (A. Clam Shoal, B. Crab Hole). * Indicates effects that are statistically significant.

A. Clam Shoal

Factors	**Sum Sq**	**Df**	**F value**	**Pr(>F)**
Treatment	0.0220	1	13.6869	0.0008*
Year	0.0223	2	6.9405	0.0030*
Treatment X Year	0.0353	2	10.9630	0.0002*
Residuals	0.0531	33		

B. Crab Hole

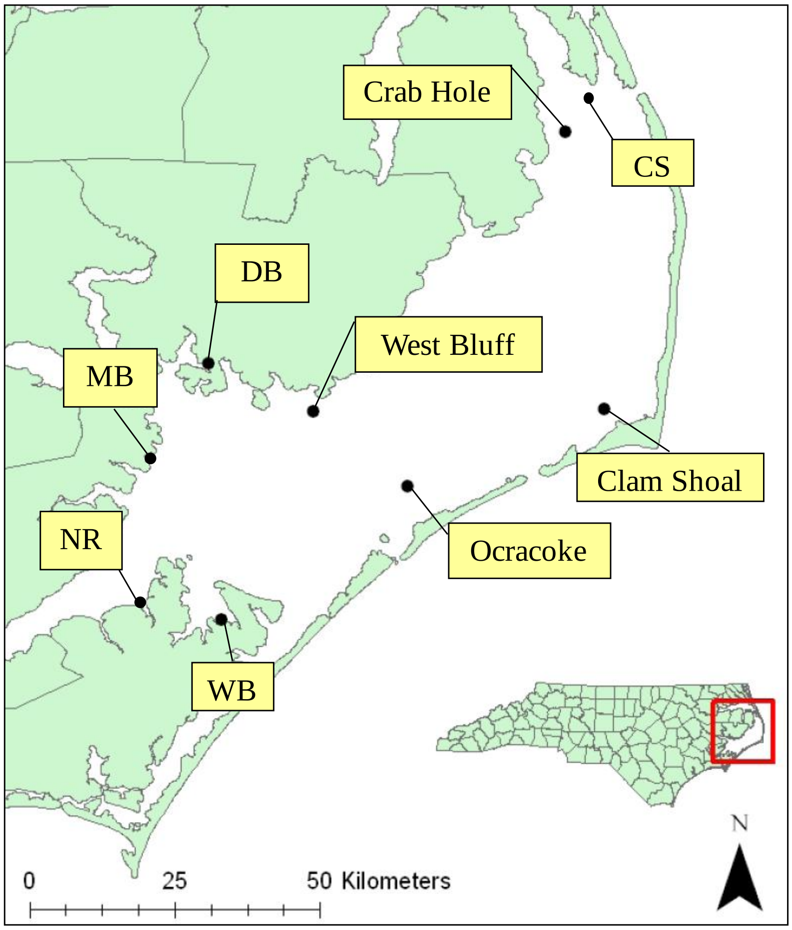
Factors	**Sum Sq**	**Df**	**F value**	**Pr (>F)**
Treatment	0.0004	1	0.2344	0.6314
Year	0.0007	2	0.2076	0.8136
Treatment X Year	0.0003	2	0.0949	0.9097
Residuals	0.0544	34		

Table 14: Results of ANOVA for the effects of Month (July through October 2010) and Location (Clam Shoal, Crab Hole, Ocracoke and West Bluff) in Pamlico Sound, NC on mean CPUE of estuarine fish captured by gill net. * Indicates effects that are statistically significant.

Factors	**SUM of Sq**	**Df**	**F**	**Pr(>F)**
Location	8.4719	3	4.3976	0.0083*
Month	11.2948	3	5.8610	0.0017*
Location X Month	11.3961	9	1.9712	0.0644
Residuals	30.1915	47		

Table 15: Results of ANOVA for the effects of Month (July through October 2010) and Location (Clam Shoal, Crab Hole, Ocracoke and West Bluff) on mean number of fish species captured per hour with gill nets. * Indicates effects that are statistically significant.

Factors	**Sum Sq**	**Df**	**F value**	**Pr(>F)**
Location	0.1561	3	6.7085	0.0007*
Month	0.6288	3	27.0167	2.64e-10*
Location X Month	0.2614	9	3.7444	0.0013*
Residuals	0.3646	47		



CS: Croatan Sound
DB: Deep Bay
MB: Middle Bay
NR: Neuse River
WB: West Bay

Figure 1: Oyster broodstock reserves in Pamlico Sound, NC, USA. Broodstock reserves sampled in this study are Crab Hole, Clam Shoal, Ocracoke and West Bluff.

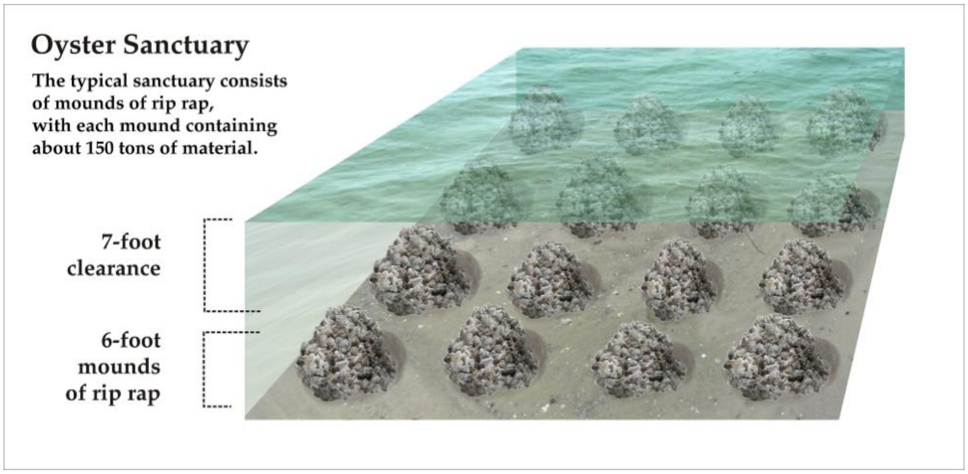


Figure 2: Schematic of restored oyster broodstock reserves constructed in Pamlico Sound, NC by the NC Division of Marine Fisheries. Mounds were constructed of piles of limestone rip-rap boulders of approximately 50cm diameter, with overall height of the mound ~2 m. Figure from NC DMF website at: http://www.ncdmf.net/shellfish/sanctuary1.htm .

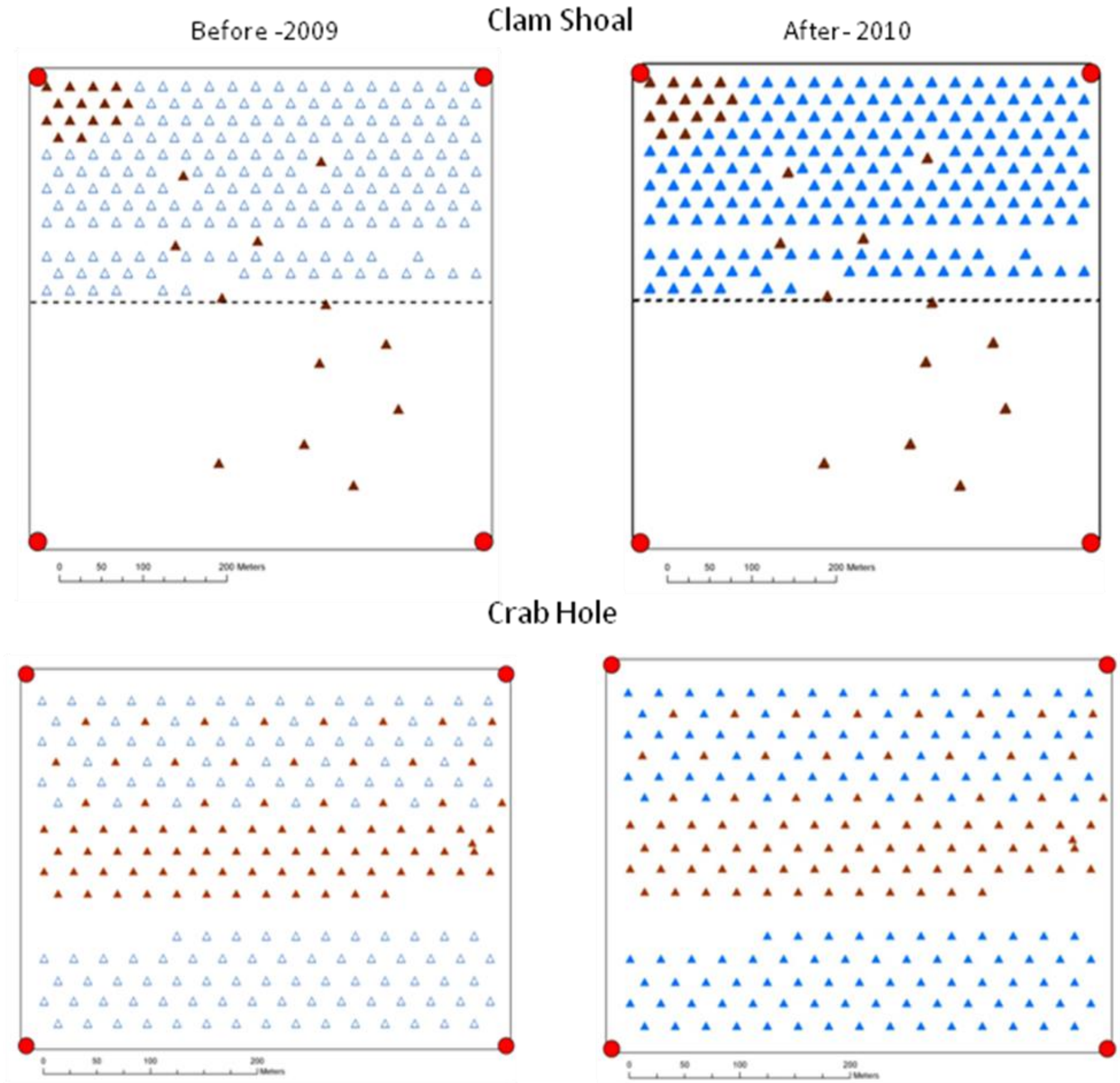


Figure 3: Schematic of Clam Shoal and Crab Hole oyster reserves before (2009) and after large-scale construction of mounds of rip-rap during winter 2009 and early spring 2010. The location of the old, “control reefs” is shown by red triangles in each schematic, whereas the location of “experimental reefs” prior to their construction in 2009 is shown by open blue triangles and the location of “experimental reefs” after they were constructed in 2010 is shown by closed triangles. Figure by Gayle Plaia.

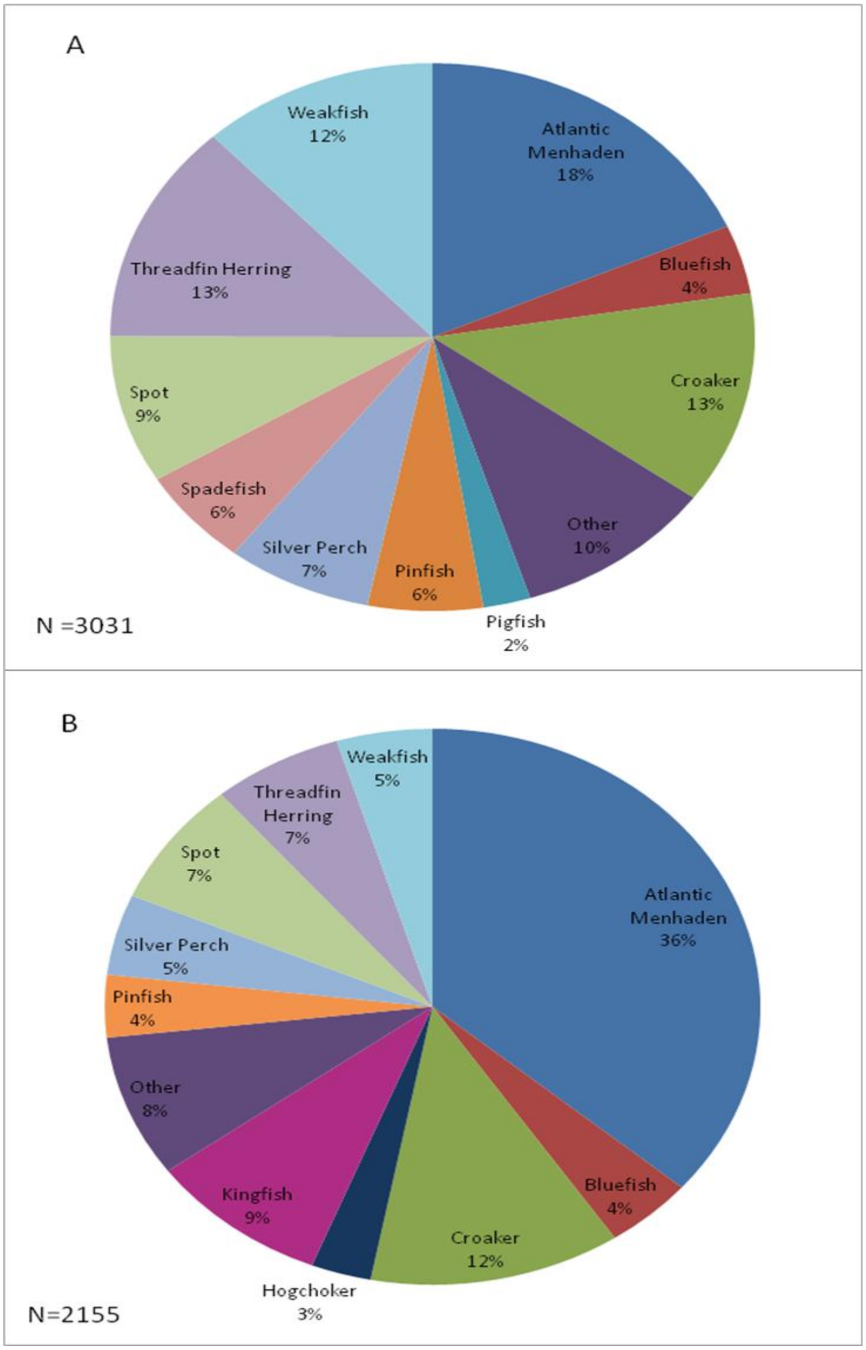


Figure 4: Percentages of total gill net catch by fish species (top ten and other category) for oyster reserves (A) and off reserve unstructured bottom (B). The other category on oyster reserve (A) was made up of 41 different species and the other category off reserve unstructured bottom (B) is made up of 26 other species.

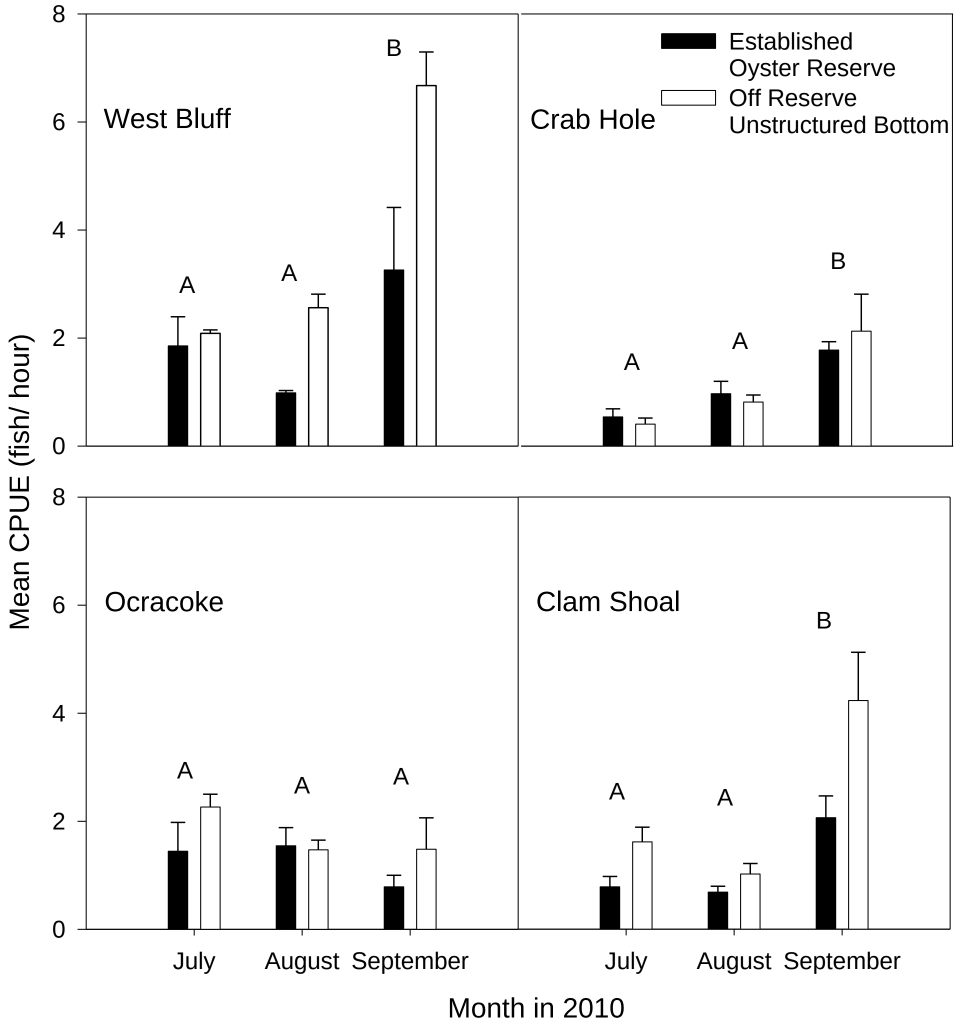


Figure 5: Mean estuarine fish CPUE (+SE) captured by gill net over the months of July through September at each oyster reserve (Crab Hole, West Bluff, Clam Shoal, Ocracoke). Months with the same letters were statistically similar as revealed with a Tukey's Honestly significant different multiple comparisons test on pooled treatment effects.

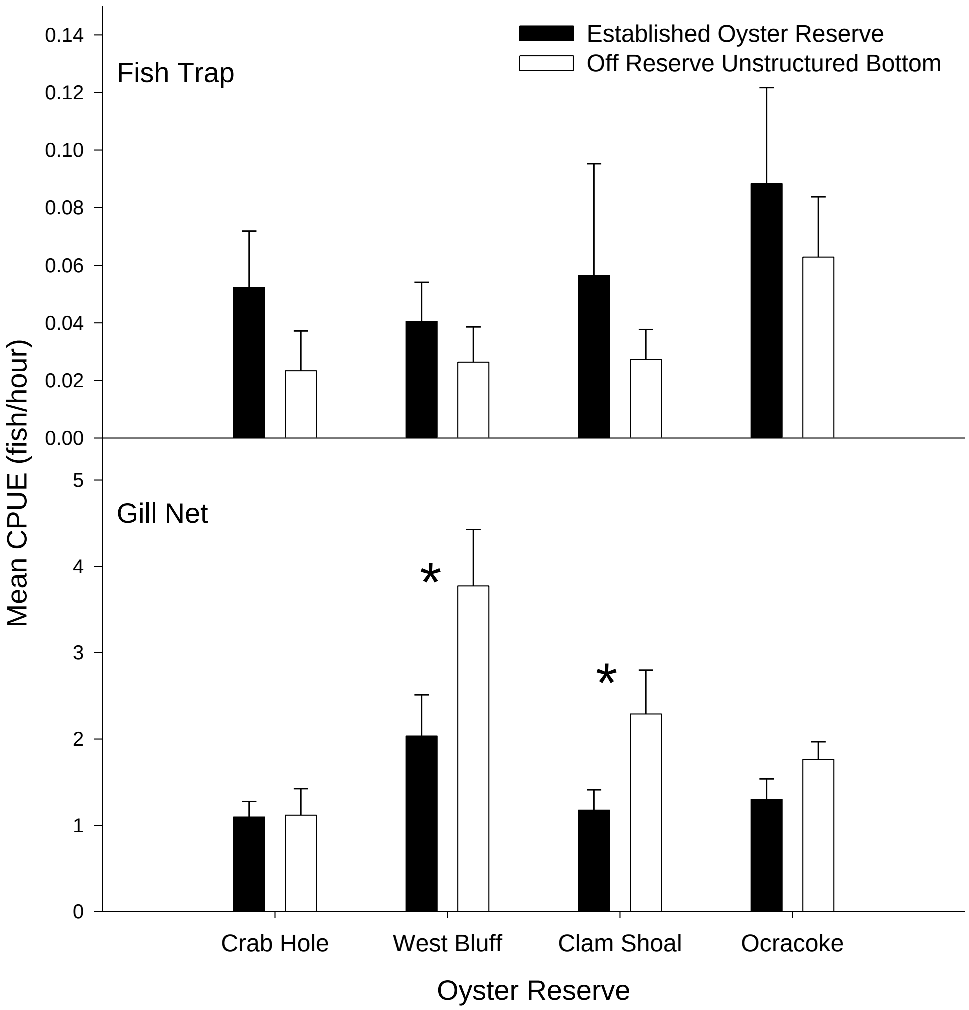


Figure 6: Spatial variation in mean CPUE of gill net and fish trap catches at Crab Hole, West Bluff, Clam Shoal and Ocracoke pooled across July through September 2010. Error bars are ±1SE . * denotes where the difference between established oyster reserves and unstructured bottom was statistically significant.

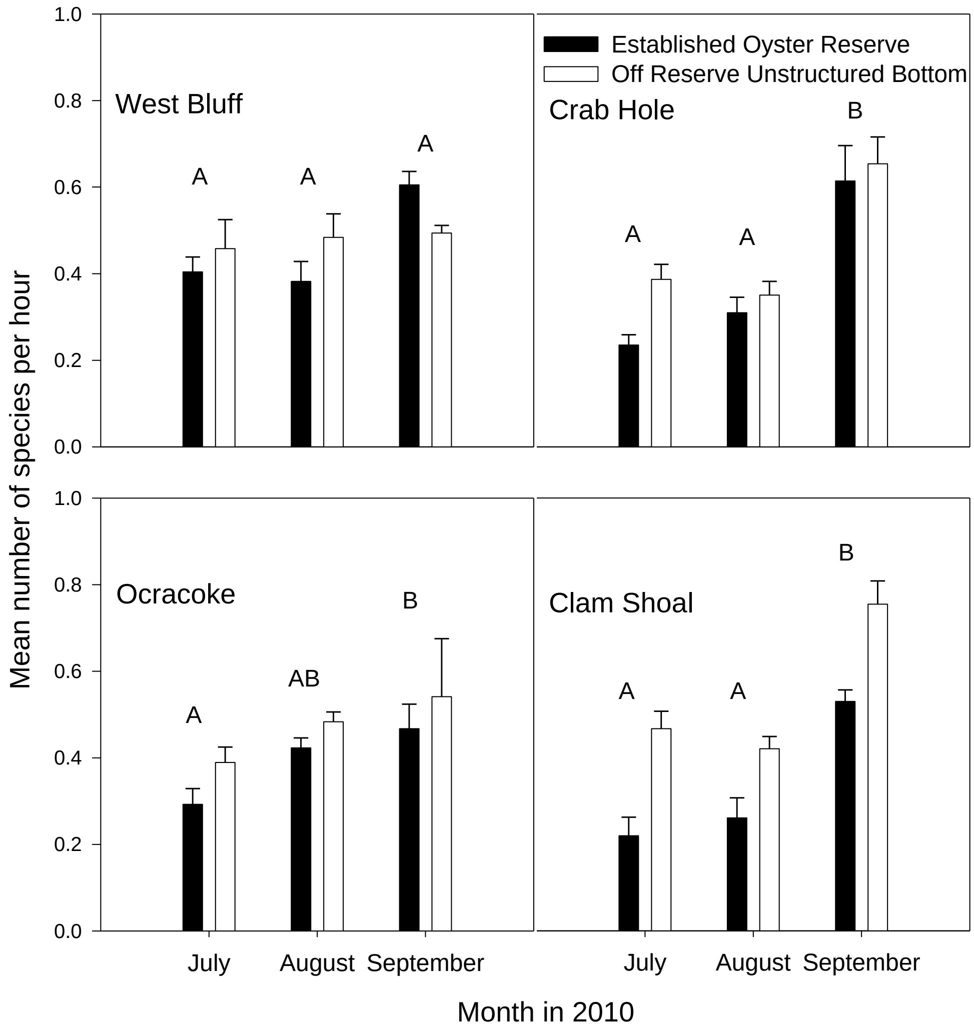


Figure 7: Mean gill net number of species per hour (+SE) over the months of July through September at each of the four oyster reserve (Crab Hole, West Bluff, Clam Shoal, Ocracoke). Months with the same letters were not significantly different as revealed with a Tukey's Honestly significant different multiple comparisons test on pooled treatment effects.

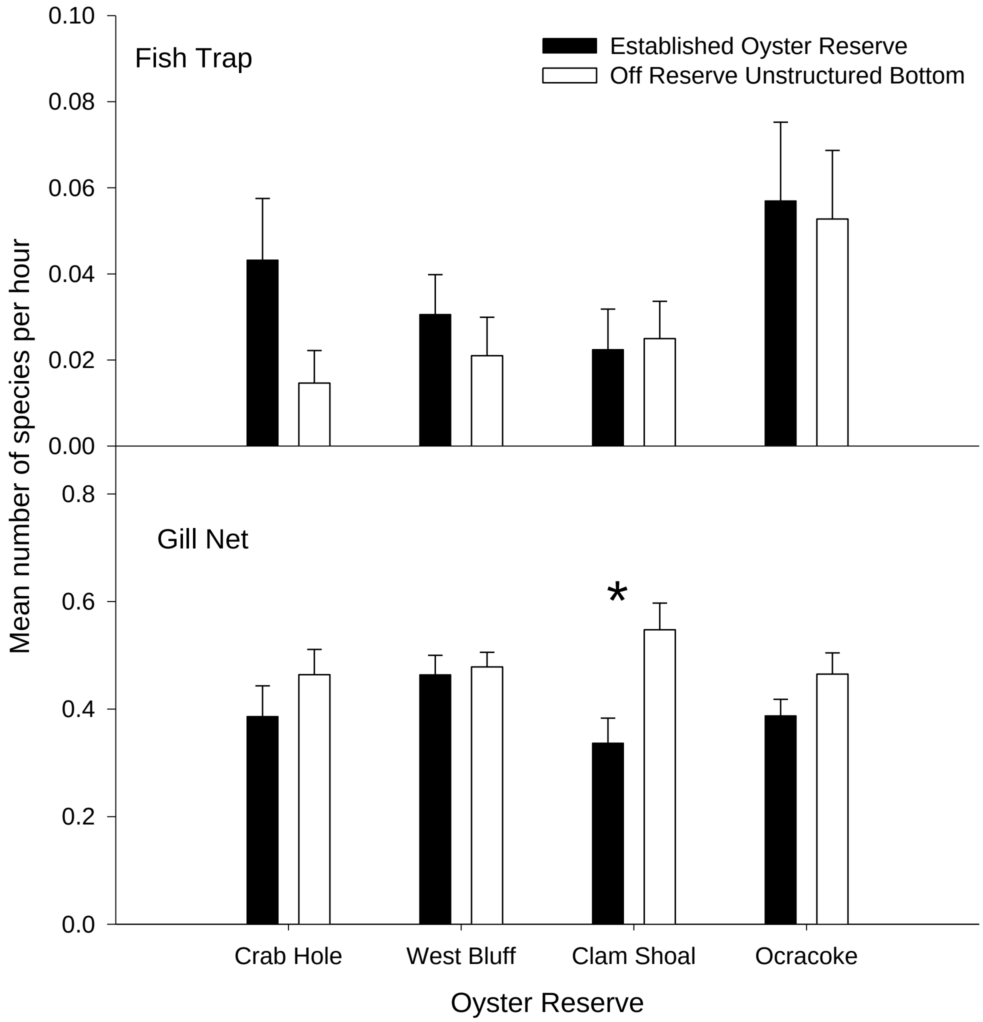


Figure 8: Spatial variation in mean number of species per hour (+SE) of gill net and fish trap catches at Crab Hole, West Bluff, Clam Shoal and Ocracoke pooled across July through September 2010. * denotes where the difference between established oyster reserves and unstructured bottom was statistically significant.

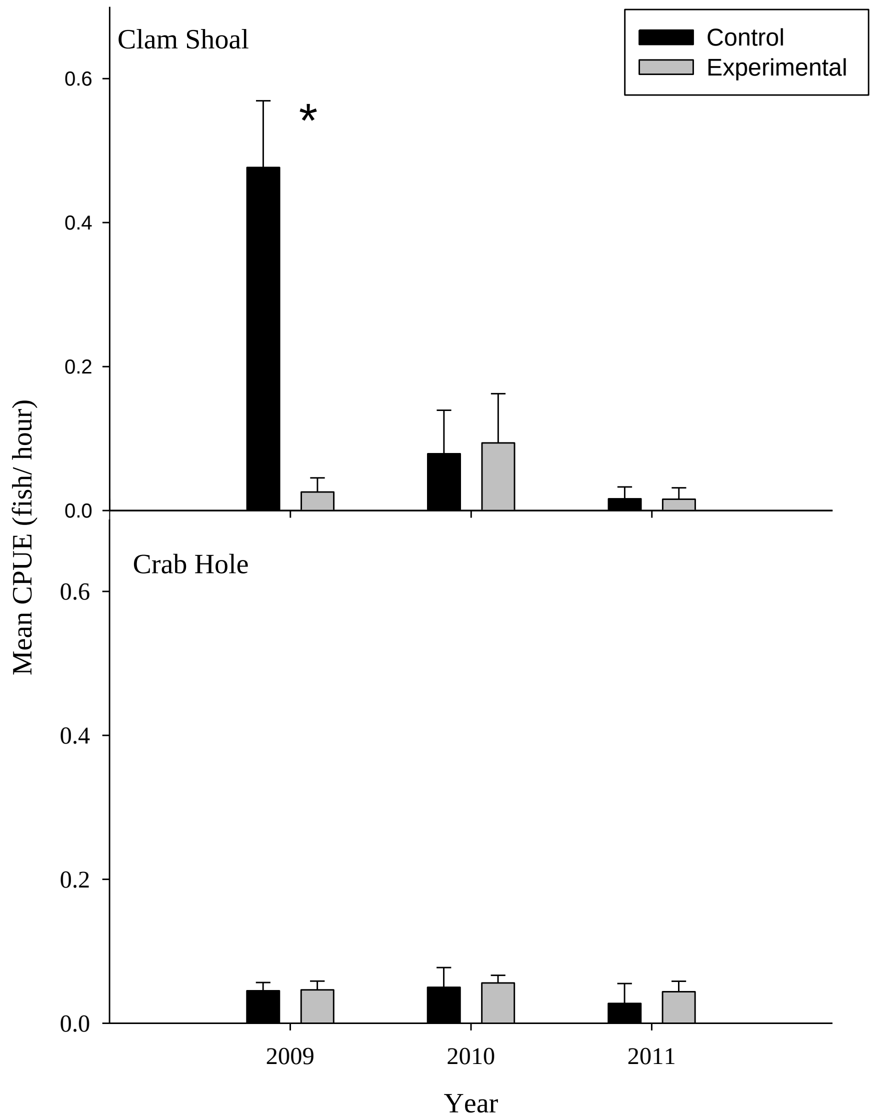


Figure 9: Mean CPUE (+SE) captured by fish traps as a function of Year at Clam Shoal and Crab Hole. The creation of new experimental mounds took place between 2009 and 2010. * denotes where difference between control sites and experimental sites was statistically significant.

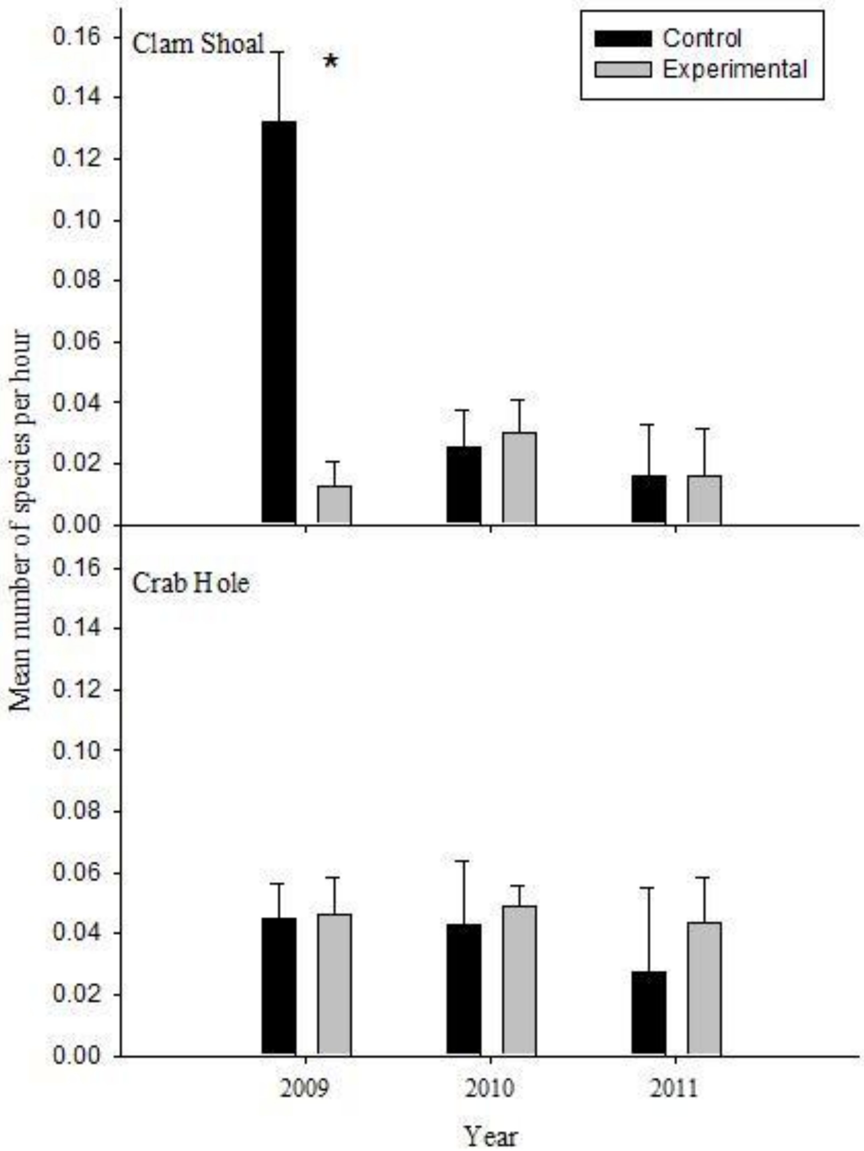


Figure 10: Mean number (+SE) of estuarine fish species captured by fish traps as a function of Year at Clam Shoal and Crab Hole. The creation of new experimental mounds took place between 2009 and 2010. * denotes where difference between control sites and experimental sites was statistically significant.

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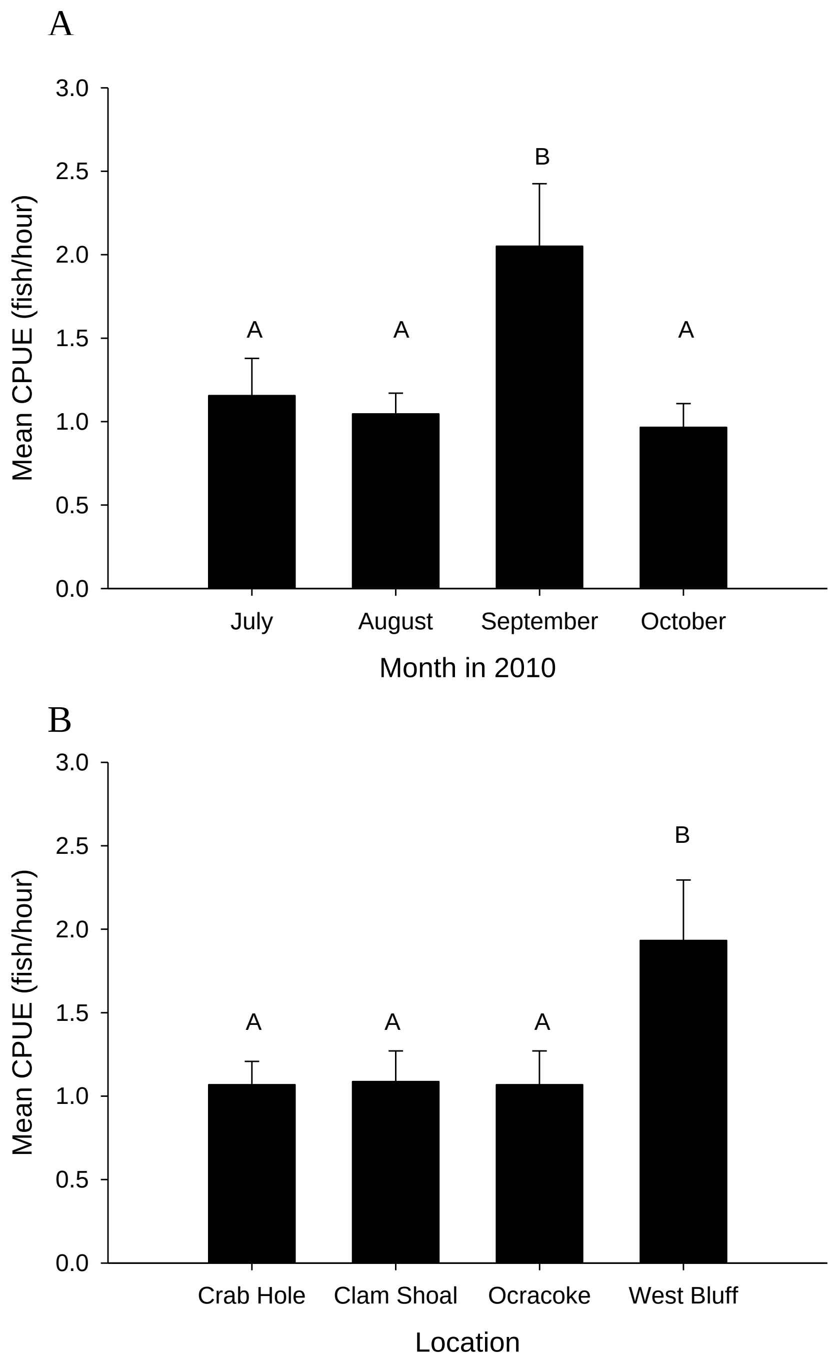

Figure 11: Effects of Month (i) and Location (ii) on mean estuarine fish CPUE (+SE) from gill net sampling of "old" oyster mounds. Months with the same letters were not statistically different as revealed with Tukey's HSD multiple comparisons test.

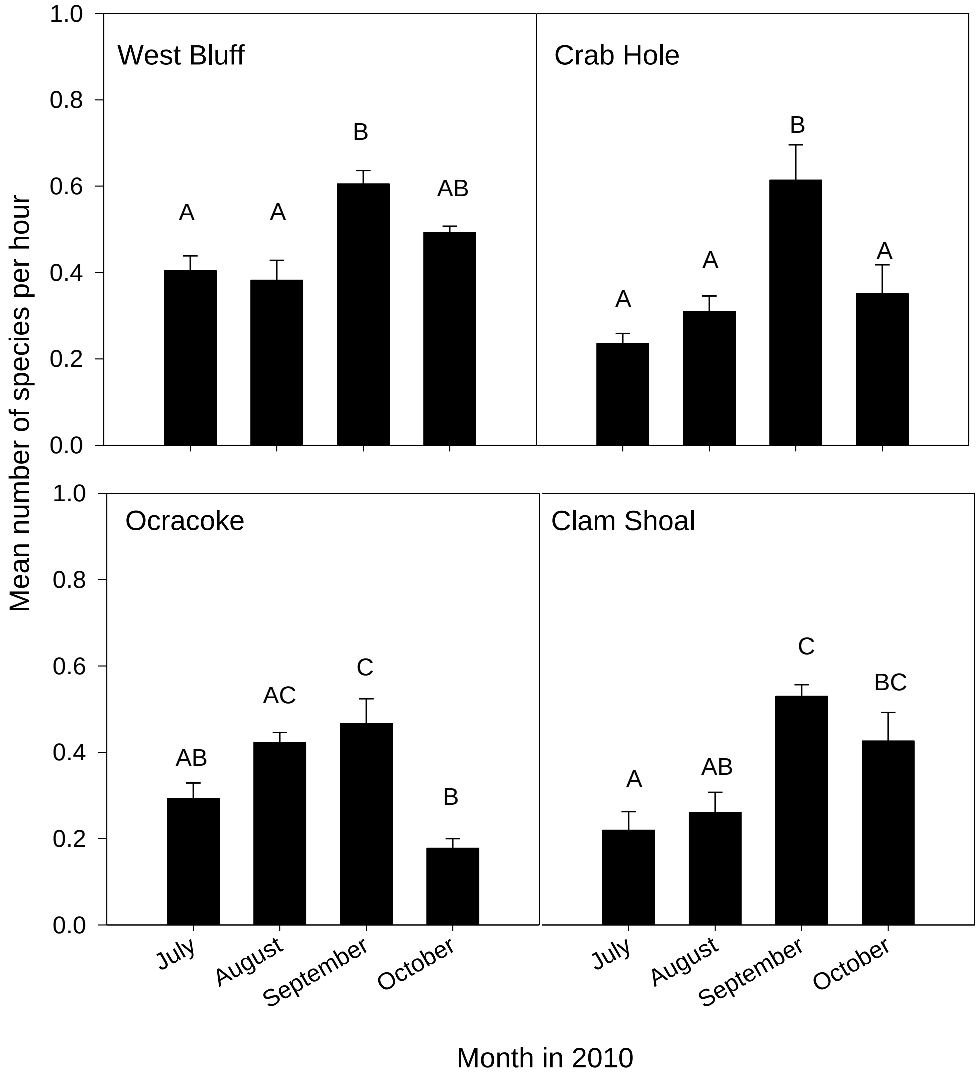

Figure 12: Month X Location interaction effect on mean number of estuarine fish species captured per hour (+SE) with gill nets in 2010 of "old" oyster mounds. Months with the same letters were not significantly different as revealed with Tukey's HSD multiple comparisons test.

APPENDIX

Appendix 1: Finfish species collected on four restored oyster reserves in Pamlico Sound, North Carolina from 2009 through 2010 as part of a study of oyster reef restoration.
* Indicates an important coastal fishery in North Carolina

Species	Collection method	Number collected
Actinopterygii		
Anglefin whiff *Citharichthys gymnorhinus*	Gill net	4
Atlantic bumper *Chloroscombrus chrysurus*	Gill net	4
Atlantic croaker *Micropogonias undulates* *	Gill net; Fish trap	380; 9
Atlantic menhaden *Brevoortia tyrannus* *	Gill net	557
Atlantic spadefish *Chaetodipterus faber*	Gill net; Fish trap	174; 5
Atlantic thread herring *Opisthonema oglinum*	Gill net	395
Banded drum *Larimus fasciatus*	Gill net	4
Big eye anchovy *Anchoa lamprotaenia*	Gill net	1
Blackcheek tonguefish *Symphurus plagiusa*	Gill net	11
Black sea bass *Centropristis striata* *	Gill net; Fish trap	13; 4
Blueback herring *Alosa aestivalis*	Gill net	2
Bluefish *Pomatomus saltatrix* *	Gill net	123
Bluntnose jack *Hemicaranx amblyrhynchus*	Gill net	1
Butterfish *Peprilus triacanthus*	Gill net	13
Conger eel *Conger oceanicus*	Fish trap	1
Gag grouper *Mycteroperca microlepis* *	Gill net	1
Guaguanche *Sphyraena spp.*	Gill net	3
Harvestfish *Peprilus paru*	Gill net	12
Hogchoker *Trinectes maculates*	Gill net	35
Inshore lizardfish *Synodus foetens*	Gill net	1
Ladyfish *Elops saurus*	Gill net	3
Needlefish *Strongylura marina*	Gill net	2
Oyster toadfish *Opsanus tau*	Gill net; Fish trap	12; 26
Pigfish *Orthopristis chrysoptera*	Gill net; Fish trap	72; 5
Pinfish *Lagodon rhomboids*	Gill net; Fish trap	175; 42
Red drum *Sciaenops ocellatus* *	Fish trap	1

Sea bream *Archosargus rhomboidalis*	Gill net	6
Sheepshead *Archosargus probatocephalus*	Gill net	13
Silver perch *Bairdiella chrysoura*	Gill net	222
Silver porgy *Diplodus argenteus*	Gill net	4
Southern flounder *Paralichthys lethostigma* *	Gill net	19
Southern kingfish *Menticirrhus americanus* *	Gill net; Fish trap	39; 1
Spanish mackerel *Scomberomorus maculates* *	Gill net	9
Spot *Leiostomus xanthurus* *	Gill net	265
Spotted seatrout *Cynoscion nebulosus* *	Gill net	1
Striped bass *Morone saxatilis* *	Gill net	1
Striped anchovy *Anchoa hepsetus*	Gill net	16
Striped searobin *Prionotus evolans*	Gill net	1
Summer flounder *Paralichthys dentatus* *	Gill net	13
Tautog *Tautoga onitis*	Gill net	1
Unidentified filefish Family Balistidae	Fish trap	1
Unidentified fish	Gill net	3
Unidentified flounder Order Pleuronectiformes	Gill net	1
Unidentified lizardfish Family Synodontidae	Gill net	3
Unidentified kingfish *Menticirrhus spp.* *	Gill net	3
Unidentified sea robin Family Triglidae	Gill net	1
Unidentified sennet *Shyraena spp.*	Gill net	10
Weakfish *Cynoscion regalis* *	Gill net	361
White perch *Morone americana* *	Gill net	15
Whiting *Merluccius spp.*	Gill net	5
Elasmobranchii		
Atlantic sharpnose *Rhizoprionodon terraenovae* *	Gill net	5
Cownose ray *Rhinoptera bonasus*	Gill net	5
Little skate *Raja erinacea*	Gill net	1
Smooth butterfly ray *Gymnura micrura*	Gill net	1
Smooth dogfish *Mustelus canis* *	Gill net	3

Spiny dogfish *Squalus acanthias* *	Gill net	1
Unidentified butterfly ray *Gymnura spp.*	Gill net	4
Unidentified skate *Raja spp.*	Gill net	1
Grand total number of species 51; 10	**Gill net; Fish trap**	**3031; 95**

Appendix 2: Finfish species collected in Pamlico Sound, North Carolina from 2009 through 2010 on unstructured bottom as part of a study of oyster reef restoration.
* Indicates an important coastal fishery in North Carolina

Species	Collection method	Number collected
Actinopterygii		
Atlantic bumper *Chloroscombrus chrysurus*	Gill net	1
Atlantic croaker *Micropogonias undulates* *	Gill net; Fish trap	268; 2
Atlantic cutlassfish *Trichiurus lepturus*	Gill net	1
Atlantic menhaden *Brevoortia tyrannus* *	Gill net	781
Atlantic spadefish *Chaetodipterus faber*	Gill net; Fish trap	19;7
Atlantic thread herring *Opisthonema oglinum*	Gill net	139
Big eye anchovy *Anchoa lamprotaenia*	Gill net	1
Black cheeked tonguefish *Symphurus plagiusa*	Gill net	8
Bluefish *Pomatomus saltatrix* *	Gill net	94
Cobia *Rachycentron canadum*	Gill net	4
Guaguanche *Sphyraena spp.*	Gill net	4
Harvestfish *Peprilus paru*	Gill net	1
Hogchoker *Trinectes maculates*	Gill net; Fish trap	64; 1
Inshore lizardfish *Synodus foetens*	Gill net	4
Leopard searobin *Prionotus scitulus*	Gill net	1
Northern kingfish *Menticirrhus saxatilis*	Gill net	16
Northern searobin *Prionotus carolinus**	Gill net	3
Oyster toadfish *Opsanus tau*	Gill net; Fish trap	1; 2
Pigfish *Orthopristis chrysoptera*	Gill net; Fish trap	27; 5
Pinfish *Lagodon rhomboids*	Gill net; Fish trap	78; 10
Porgy Family Sparidae	Gill net	1
Remora *Remora remora*	Gill net	1
Ribbonfish Family Trachipteridae	Gill net	1
Silver perch *Bairdiella chrysoura*	Gill net	101
Southern flounder *Paralichthys lethostigma* *	Gill net; Fish trap	6; 1

Southern kingfish *Menticirrhus americanus* *	Gill net; Fish trap	168; 2
Spanish mackerel *Scomberomorus maculates* *	Gill net	11
Spot *Leiostomus xanthurus* *	Gill net	157
Striped anchovy *Anchoa hepsetus*	Gill net	9
Striped searobin *Prionotus evolans*	Gill net	1
Summer flounder *Paralichthys dentatus* *	Gill net	8
Unidentified fish	Gill net; Fish trap	6; 2
Unidentified kingfish *Menticirrhus spp.* *	Gill net	9
Unidentified searobin Family Triglidae	Gill net	2
Weakfish *Cynoscion regalis* *	Gill net	102
Elasmobranchii		
Atlantic sharpnose *Rhizoprionodon terraenovae* *	Gill net	46
Cownose ray *Rhinoptera bonasus*	Gill net	1
Southern stingray *Dasyatis americana*	Gill net	2
Unidentified butterfly ray *Gymnura spp.*	Gill net	8
Grand total number of species 36; 8	**Gill net; Fish trap**	**2155; 32**