

COASTAL RECREATIONAL FISHING LICENSE

FINAL REPORT

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Abstract

Oyster reefs are one of the most depleted and degraded marine habitats worldwide. To reverse the current trend of oyster reef declines, North Carolina has established subtidal oyster sanctuaries in the Pamlico Sound, initiated by creating many large mounds of marl boulders. North Carolina has seeded sanctuary mounds and harvest areas with hatchery-raised juvenile oysters set on recycled adult shell to enhance development of oyster reefs. These costly restoration efforts, which are widely used for the eastern oyster, are carried out despite limited information on whether seed oysters accelerate reef development and, if so, how oyster size and time of deployment maximize oyster survival. Three sanctuaries differing abiotically and biotically were seeded during summer 2010. We experimentally manipulated mounds at each sanctuary and varied recycled shell and seed presence, seed size, and deployment date of shell and seed. Although oyster settlement varied spatially, natural recruitment swamped any measurable effect of seeding. Our findings, in combination with information from 3 additional sanctuaries seeded in 2006 and 2008, indicate that seeding does not enhance oyster reef restoration efforts in Pamlico Sound. Financial resources used for oyster seed would be better used to increase the amount of substrate for oyster settlement. Although our results may apply to areas with less natural oyster recruitment, this study highlights the need to quantify basic ecological processes on appropriate spatiotemporal scales to optimize restoration actions.

Introduction

Over the past two years, the State of North Carolina has made the decision to invest substantial resources in purchasing and planting seed oysters to enhance the recovery of the oyster populations on oyster reef sanctuaries as part of a large-scale oyster reef habitat program in the Pamlico Sound region. Seed oysters set on shell have been provided to the State by contract from a private hatchery. In the 2008 legislative short session, funding was appropriated for the first of perhaps three state-owned oyster hatcheries. This first hatchery is being designed for research but with production capacity to mass produce seed oysters to allow scaling up of the process of seeding the newly constructed artificially built oyster reef sanctuaries. In the future, as many as two production hatcheries may be constructed. Unfortunately, the scientific and technical information necessary to optimize habitat benefits from oyster seed plantings does not exist. Quite simply, we need to know how to organize the seed planting to get the most habitat benefits for each buck spent on seed.

The current practice of creating artificial oyster reef sanctuaries is intended to expand oyster reef habitat for its ecosystem services (Lenihan and Peterson 1998). The most important of these services include providing habitat to enhance production of fishes, crabs, and shrimps (Lenihan et al. 2001, Peterson et al. 2003), enhancing water quality by filtration and denitrification (Grabowski and Peterson 2007, Coen et al. 2007), and growing oyster spawning stock biomass to enhance larval production and recruitment on oyster production grounds, natural reefs and cultch planting areas (Rothschild et al. 1994). The NC sanctuary program typically uses large boulder-sized marl to construct artificial oyster reefs rising multiple meters off the estuarine bottom at locations spread across the Pamlico Sound. Many of these artificial reefs are then seeded with small hatchery-raised oysters set on oyster shell by planting the seed on the tops of the reefs. The sizes of seed planted and the dates of planting are now based upon when the nursery at South River is filled with enough seed oysters to plant. Seeding alone, without knowing how seed survival varies with size and date of planting, does not insure optimal growth and survival of the seed oysters, which is a necessary prerequisite for maximizing the ecosystem services that are the goal of the program.

This project was a collaboration with the DMF oyster reef sanctuary program (Craig Hardy and Pelle Holmlund) to implement tests of how those variables to which seed shellfish survival is most sensitive affect the success of oyster reef habitat development in the Pamlico Sound region of North Carolina. Shellfish seed survival typically varies strongly with seed size at planting and season (date) of planting (Blankenship and Leber 1995, Peterson et al. 1996). The quantitative relationship between survival and design variables of seed size and planting date is not itself constant but varies with ecosystem condition, largely driven directly and indirectly by those environmental parameters like salinity that change with location. For example, the community of oyster seed and juvenile predators and

competitors for space changes from high-salinity areas like the sanctuary near Clam Shoal to intermediate-salinity areas like Gibbs Shoal to low-salinity areas like Crab Hole off the mainland Dare County shore. Furthermore, the size- and season-dependent variation in risks of predation and competitive mortality can vary greatly among places with differing environmental conditions. Our project will uncover these relationships and thus optimize seed planting benefits.

Objectives

The overall objective was to gather and analyze field and laboratory data to allow the design for seed oyster planting on oyster reef sanctuaries to maximize the survival of the seed oysters and establishment of oyster reef habitat on the artificial oyster reefs.

Overall objective with four interconnected components:

- (1) In collaboration with the DMF oyster reef habitat sanctuary program, we conducted field trials in which we varied oyster seed size (2 size classes) and planting date (two dates from June and Aug.) in test plantings on constructed oyster reefs at three sanctuary sites in the Pamlico Sound differing in geographic region and salinity. Monitoring over two years after planting will reveal how size-dependent and season-dependent survivorship of seed and establishment of reef habitat structure varies with sanctuary location and environment.
- (2) We determined if natural oyster settlement varies across artificial oyster reefs as a function of seeding. Seeding may enhance development of oyster cover and habitat structure through promoting settlement of oyster larvae attracted by biochemical settlement clues. Three different settlement collectors, composed of unaggregated adult shell or PVC spates, will be tested and used to measure natural recruitment of oyster spat on the marl reefs.
- (3) On three dates over the warm season of early seed growth and survival (June through Oct), we conducted field sampling programs to characterize the abundances and size frequency distributions of all potential predators on seed and juvenile oysters at each of the three sanctuary locations and 3 additional sanctuaries. Predators of most concern include mud crabs, stone crabs, blue crabs, black drum, and sheepshead.
- (4) To test the hypothesis that predation is the major cause of mortality of seed oysters, we installed seed oysters set on shell inside and outside of predator exclusion cages planted out on each sanctuary reef on two dates distributed across the warm season (June through Oct). Cages had three designs, one intended to exclude small predators the size of mud crabs, one to exclude large crabs and a third to exclude large fishes.
- (5) Using the predators captured during sampling of the predator fields at each sanctuary site, we conducted laboratory experiments in outdoor mesocosms at the UNC IMS laboratory in Morehead City in which we tested for size preferences and size limitations of each predator feeding on seed and juvenile oysters over a range of size classes. These trials were run using multiple size classes of the predators, as present in the sanctuary sites.

Methods

Experimental trials testing how seed size and planting date influence success of oyster reef creation (Objective 1) and experimental tests of how much the presence of seed oysters enhances natural oyster settlement on created reefs (Objective 2).

As a result of DMF losing funding to seed the reefs during the first year, we survey multiple sanctuaries during the summer of 2009 for fauna and physical characteristics because we were not sure which reefs we would seed in 2010 (Figure 1). Salinity and temperature were recorded using continuous loggers, which recorded measurements every 30 seconds. Current was recorded at each sanctuary using an S4 current meter which recorded measurements every 30 seconds and was deployed for a minimum of 2 weeks at each site.

Reef creation and seeding procedure

To test how three combinations of seed size and planting date, plus substrate type (recycled shell and marl), influence the success of oyster reef development as a function of location, this experiment was repeated at three NCDMF oyster sanctuaries in Pamlico Sound, North Carolina (Clam Shoal, Crab Hole and Gibbs Shoal; Fig. 1). These sites were chosen because they span the wide range of salinities that exist in the Pamlico Sound and because they contained newly created mounds (constructed after Dec. 2009). Each oyster sanctuary consisted of 50 to 300 mounds of rip-rap marl rock. Each mound contained approximately 15 tons of marl elevated 3m high with a footprint diameter of 4m. Mounds were created in a uniform grid with mounds separated by approximately 25 m in the diagonal rows. Mean water depths at Clam Shoal, Crab Hole and Gibbs Shoal were approximately 3.4, 4.0, and 4.3 m, respectively.

Oyster larvae, spawned from 15 oysters collected from the West Bluff oyster sanctuary in Pamlico Sound, were purchased from Middle Peninsula Aquaculture in Foster, VA. Substrate for seeding consisted of harvested oysters shells > 7.2 cm shell height (SH) from the NCDMF recycling program that were thoroughly cleaned by repeatedly dunking them in seawater and then moved to completely fill 2-bushel plastic crates (2.5-cm² openings separated by 1-cm thick plastic on sides and bottom with open tops). Eighteen crates were placed into large tanks (4.9 x 0.9 x 0.8 m) located on the NCDMF dock in Morehead City, NC filled with unfiltered seawater from Bogue Sound. Approximately 2.5 million eyed larvae were added to each tank and fed plankton provided by the Middle Peninsula Aquaculture twice a day. The larvae were given 3 days to settle, after which unfiltered seawater was pumped (4.4 l s⁻¹ for each tank) directly from Bogue Sound until the seeded shells were deployed on reefs. Salinity was measured weekly at the NCDMF dock using a Sontec YSI and ranged from 21 to 31 psu. One to three days before deployment, crates were divided into 9 sections (3x3 grid when viewing the broad side of the crate) and 1-3 shells were haphazardly chosen from each section to ensure the nine shells were sampled evenly throughout the crate, seed oyster abundance and size were determined by

counting the number of juvenile oysters per shell and measuring the height of 5 haphazardly chosen spat on each shell.

Larvae were set on shell in two independent additions of eyed larvae on May 25th and July 5th 2010. Large seed oysters were produced on half the seeded shells from the May larva, and these shells were kept in separate tanks until the second deployment. Treatments consisted of: 1) recycled shell deployed in late June 2010, 2) small seed oysters set on recycled shell (approximately 5 mm SH) deployed in late June 2010, 3) small seed oysters set on recycled shell (approximately 2 mm SH) deployed in mid August 2010, 4) large seed oysters set on recycled shell (approximately 10 mm SH) deployed in mid August 2010, and 5) no shell addition (marl). These treatments will be referred to as early shell, early small seed, late small seed, late large seed, and marl only, respectively. Ten mounds at each sanctuary were haphazardly assigned one of five treatments for a total of two mounds per treatment (two replicates per treatment per sanctuary). Mounds with shell treatments received 16 bushels of shells (seeded or unseeded depending on the treatment), which were deployed on the top of the mounds. The early deployment was achieved by transferring the oyster-filled tanks into a dump truck and transporting them from Morehead City to boat launches near the sanctuaries, where they were then delivered to the mounds by boat. Transport in the tanks took no longer than 5 hours. Seed and shell were deployed at Crab Hole, Gibbs Shoal, and Clam Shoal on June 21, 22 and 23, respectively. For the second deployment, oyster tanks were transported from their original location on the NCDMF dock to the sanctuaries by barge. Oysters remained in tanks on the barge deck with a continuous supply of unfiltered seawater for approximately 10, 20 and, 24 hours as shells were deployed sequentially in the three sanctuaries. Seeded shell was deployed in Clam Shoal on Aug. 10th and in Gibbs Shoal and Crab Hole on Aug. 11th. Prior to depositing shell on a mound, divers marked the center of each mound with a surface buoy attached to a weight. Immediately after deployment, divers inspected the mounds to ensure shells were on top of the mound and spread the shell out so that the shell layer was no greater than 5 cm. At Clam Shoal and Crab Hole, two additional mounds at each sanctuary, created in 2005 and 2006, were monitored to serve as a baseline for established reefs. Gibbs Shoal was first established as a sanctuary in 2009 and had no previously constructed mounds. A temperature-salinity data logger was deployed on the top of one mound at each study sanctuary to measure environmental

conditions. Temperature-salinity data were recorded every 30 minutes from June 2010 to September 2011, except when loggers malfunctioned (Fig 2).

Reef monitoring

To quantify the success of oyster reef development on the reef mounds, we collected two sets of measurements: abundance and size of oysters on deployed shell, and oyster density on the surface of the marl boulders. Abundance of seed oysters and their size frequency were measured on two occasions in fall of 2010 (10/7-10/15) and 2011 (9/8-9/13). Divers searched the mound top for deployed shell and retrieved 50 deployed shells or as many shells as could be located. Deployed shells could be distinguished from naturally recruited shells because deployed shells were larger and thicker. Retrieved shells were returned to the lab. We recorded the number of oysters on each shell and measured the shell height of 5 haphazardly chosen oysters on each of the retrieved shells to obtain a size frequency for each mound.

We quantified the density and size of oysters on the marl mounds in each sanctuary during three samplings in the fall of 2010 and 2011 (same dates as shell sampling) and spring of 2011 (5/25-6/3). Divers haphazardly removed 2 marl pieces from both the top and bottom (<50cm from the base) of the mound and immediately placed the marl in separate plastic sacks. Care was taken to ensure that oysters remained attached to the marl or that any oysters that did fall remained in the sack for quantification. Marl pieces were labeled with location on the mound (top or bottom) and mound type (early small seed, late small seed, large seed, shell, marl only or old mound) and brought back to the lab for processing. The surface area of the marl that was exposed on the mound and available for organisms to occupy was measured by orienting the marl as it was on the mound (oysters oriented vertically and side of marl with little or no epifauna on the bottom) and the “bird’s eye view” surface area was estimated by using a 5 cm grid quadrat held directly over the marl. Oyster size frequency was determined by measuring 50 haphazardly chosen oysters attached to the marl from both top and bottom samples of each mound. Oysters that recruit on the shells of existing oysters and small oysters can be difficult to find, especially on the highly complex 3d structure of oysters on the marl. To ensure accurate counts, three different people counted the number of oysters on each marl piece. The 3-observer average abundance for each piece of marl was combined with the area of exposed marl to determine oyster density (m^{-2}). This procedure was used instead of harvesting all oysters within a

quadrat on the mound because of the difficulty in removing all oysters from pieces of marl in a defined area.

NC DMF data

To account for temporal variability in oyster recruitment in our study, we analyzed data from the NCDMF sanctuary program. We only analyzed data from sanctuaries that had mounds seeded and unseeded within 1 year of the mounds being built. This criterion was met 4 times. In 2006 South River had 14 mounds built in June and July and 9 of the mounds were seeded in Aug. Sound River had 8 mounds built in Mar. 2008 and 7 of the mounds were seeded in June in 2008. In 2008 West Bluff had 5 mounds built in June and July and 3 of them were seeded in Aug. Finally, Ocracoke had 14 mounds built in Sept. 2006 and 6 of these mounds were seeded in Aug. 2007. In these instances seed production and deployment were similar to methods described above, except approximately 20 bushels (instead of the 16) were added to each mound after seed reached approximately 1 cm SH. These sanctuaries were sampled throughout the year, once a year starting in 2007, with sampling within a sanctuary being completed in less than one week. NCDMF sampling was similar to our methods, except 3 instead of 2 pieces of marl were collected from the top, middle (half way between the crest and bottom), and bottom of the mound, for a total of 9 pieces of marl per mound. NCDMF's procedure for estimating oyster density (m^{-2}) differed from the method used in our study, and consisted of estimating surface area of the marl by measuring the length, width, and height of the marl and used 50% of the calculated surface area to determine the oysters m^{-2} . The abundance of oysters on each piece of marl was estimated by taking the sum of the total number of oysters counted within 10 cm increments of SH.

Statistical analysis

Differences in salinity among sites were analyzed using a non-parametric Kruskal-Wallis test with site as the independent variable. The mean salinity per day (from measurements taken every 30 min) was used as a replicate and only days that had data from all sites were used. To determine whether large seed oyster were larger than small seed oyster before deployment, we ran a non-parametric Kruskal-Wallis test with oyster seed size as the dependent variable, and treatment (early small, late small, and late large seed) as the independent variable. Non-parametric tests were necessary because data were non-normal and had heterogeneous variances. The numbers of oysters on shell or marl were not normally distributed and heavily skewed

towards 0 and a mixed effects-generalized linear mixed models (GLMM) were used to determine significant effects (R software, GLMM ADMB package using Laplace approximation). Independent fixed factors were shell/seed treatment (early shell, early small seed, late small seed, and late large seed), site, and sampling date. Shell/seed treatment included marl only as a level (mounds with no shell addition) when running analyses on oyster density on marl. Sampling date was a fixed factor and not a random factor because including temporal variation in recruitment was ecologically relevant. Mound was included as a random factor in all models. Model family (Poisson or Negative binomial) and inclusion of factors and interactions were chosen based on lowest AIC scores. Model creation started with treatment factor only and then additional models were created by adding site and sampling date with and without interactions. The model with the lowest AIC was chosen. If this model had interactions that were not significant, the highest order interaction was removed to determine if the model could be improved (lower AIC). This was repeated until the best model was found. Model selection was performed separately with the following dependent variables: number of oysters per shell, oyster density (m^{-2}) on marl, and oyster density (m^{-2}) on marl from DMF seeded sites. Depth was included as an additional factor in model selection for number of oysters m^{-2} on experimental mounds. Model selection for NCDMF data included an additional fixed factor, year created, and sampling date was referred to as age of mound. Size of oysters on shells was analyzed using a general linear model (GLM; R software, glme package with AIC) because it was a continuous variable with homogeneous variance (Bartlett's test; $p > 0.05$). Procedures for model selection were identical to those previously described.

To answer the primary question of the study, which was to determine if seeding increased oyster abundance, as well as the best model for number of oysters per shell and oyster density on marl were complex and included 3 independent factors with 3 significant interactions, separate tests were run for each sanctuary with shell/seed treatment as the independent variable using only data from 1.5 years after shells were deployed (fall 2012 sampling). The simplified models were run with the same GLMM procedure as previously described. The significant levels for these additional tests were adjusted to reduce type I error when running multiple tests ($p < 0.012$; Bonferroni's correction).

Comparison on recruitment collectors (Objective 2)

We deployed three different settlement collectors to test consistency of patterns of settlement among collectors, ease of data collection, and level of variability among replicate collectors and thus strength of signal. The first collector comprised of a set of individual empty and cleaned scallop shells strung on a rope attached to a cement block at the bottom and a styrofoam float (Figure 2A). A version of this oyster settlement collector has been used for 60 years along the east coast from Delaware Bay (Hal Haskins) through North Carolina sounds (Al Chestnut) to measure oyster settlement. Consequently, using this collector design will allow comparison of present settlement at sanctuary sites to historical data for many other sites in- and outside North Carolina. The second settlement collector was modeled after that developed by Lenihan and Peterson (1998), consisting of 10 adjacent adult oyster shells evenly spaced and attached to the top surface (0.06 m^2) of a cement block using eye bolts drilled into the block (Figure 2B). The final settlement collector was a 0.01 m^2 piece of 0.5-cm thick PVC, anchored by attachment to a cement block (Figure 2C).

The square cement board collector was chosen to estimate recruitment at 6 sanctuaries. Oyster spat collectors were deployed in two positions on reef mounds, on the table top crest and half-way up the side. Three replicate collectors at each position on two reef mounds at each sanctuary site were exposed to potential oyster settlers for about 6 week periods. Deployment periods were late-May to mid-July, and mid-July to Sept. Collectors were retrieved from the field, replaced where necessary to begin the next collections, and returned to the lab where they were frozen until counting of settlers can occur. Settled oysters were counted using Wild M5 dissecting microscopes. Significant differences between sites and collectors were tested by a 2-way ANOVA with number of recruits as the independent variable and site and collector type as the dependent variable.

Sampling reefs for predators of small oysters (Objective 3). At each oyster reef sanctuary site, we conducted field sampling of the presence and relative abundance of those animals that prey on spat, juvenile, and adult-sized oysters. The site-specific assessment of predator fields was done on three dates each year, spread out between early July and late October so as to test whether field information on site-specific and date-dependent predation risk can explain observed variation in survival of seed, juvenile, and older oysters from our deployment trials. The predators of most likely significance include crustaceans (mud crabs, spider crabs, blue crabs) and demersal fishes (black drum, sheepshead, and cownose rays). Small, less mobile predators, such as oyster drills and mud crabs, were sampled by deploying 2 replicate plastic trays (30 x 18 x 10 cm) filled with oyster shells and marl on each experimental mound which were sampled twice during each summer (approximately 6 week soak). While still in place on the bottom, scuba divers covered the tray with screen mesh before transport to the boat to retain all animals that have entered the trays. The trays were returned to the lab and all predators

identified, measured, weighed and kept alive for subsequent laboratory feeding experiments. To estimate relative density of blue crabs, crab pots were augmented with fine mesh covers (0.5 cm) to retain smaller crabs and then deployed. Six crab pots were baited with fish and were set on top of separate mounds at each sanctuary site for 4 hours. Larger predatory fishes were sampled using 30-m, multi-sized mesh gillnets. The nets had panels of each of four mesh sizes (2.5, 5, 7.5, 10 cm square measure, each 7.5 meters long). The nets were set for 4 hours through the center of the mounds at each sanctuary. All fishes were brought back to the lab, in aerated tanks for feeding experiments if alive or on ice if dead. At the lab lengths were measured and fish wet-weighed.

Experimental tests to measure the impact of predation on seed oysters, (Objective 4). At each sanctuary site, we conducted predator exclusion experiments on using seed oysters identical to the ones deployed on the experimental mounds test whether predation is a major source of seed oyster mortality and to quantify how risk of predation varies with seed size and planting date. The oysters within the cages were sampled at the same time as the mounds/shell (See Objective 1). Five different sorts of predator exclusion cages were used, open, control, roof, large mesh and small mesh. The small mesh (1.3 cm) was chosen to exclude small predators such as mud crabs or oyster drills and the larger mesh (4 cm) to exclude fishes such as sheepshead and black drum. Cage control had mesh on only two sides, thereby allowing predator access but including artifacts associated with presence of mesh (Summerson and Peterson 1984). The roof cage had only the 4cm mesh on top, but did not have sides. This design allowed crabs to enter through the side but excluded large fishes (sheepshead and black drum). The mesh was made out of vexar (polypropylene) and surrounded cement blocks of the same size as those blocks anchoring settlement collectors. The mesh was wrapped around the block and secured with zip ties to form a dome 30 cm high (small and large mesh only). All cement blocks were rapped with 1 cm mesh aqua-netting, which enabled attachment of shells. Prior to adding seed oysters on shell, each shell had a 4-mm hole drilled near the umbo. Nine seeded shells were zip-tied to the cement blocks via the aqua-netting. This design allowed predators to manipulate and move the adult oyster shell just as they would a normal shell, yet still ensure that the shells remain in the cages. One cage of each mesh type was deployed on the table top of each experimental reef mound by divers.

Mortality within exclusion cages and partial cages were assessed in July 2009 (Control, shell only, and small early seed mounds), Sept 2010, May 2011, and Sept 2001. Mounds were thoroughly search to recover all cages, which were returned to the boat. Blocks were removed from the cages, five out of nine shells were haphazardly chosen to sample. Sampling consisted of count all oysters and measuring five for size frequency, and the cages were redeployed. The mortality of seed oysters inside exclusion cages and on cage controls were compared. The impact of predators on seeded oyster was

analyzed using an ANOVA with cage type and seed size/ deployed as the (fixed) independent variables and the difference from the initial deployment averaged over all sampling periods as the dependent variable.

Experiments on prey preferences and size limitations of oyster predators (Objective 5). Field experiments are valuable because they are conducted in the natural environment, but experimenting in outdoor laboratory mesocosms like those at IMS allowed more carefully controlled experiments and more opportunity for observing behavior and mechanistic processes. Consequently, to gain more insight into the mechanisms of size- and species-specific predation on seed oysters and to extend our understanding of predation risk to juvenile and adult-sized oysters, we conducted a series of predation experiments in the outdoor ponds at IMS. Each experiment offered an individual predator oysters of three different sizes and mussels of two different sizes. Predators used included black drum, sheepshead, stone crabs,, blue crabs, and mud crabs of at least two different size classes. The sizes of the mesocosms varied, scaled upwards as predator size and mobility increases. Prey were placed in natural life positions relative to the bottom substrate, which will be a shell reef with surrounding sediments. Prey trials were ended when 50% of the preferred size class was consumed to avoid biases from modifying prey frequencies (Murdoch 1969). Thus, the duration of each experiment was determined after initial test runs. Fish experiments were run in large cement mesocosms (3 x 4 m, .5 m depth) for 3 to 6 days). Blue and stone crab feeding preference were tested in metal mesocosms (2 x 1.5 m, .5 m depth) and run for 3-5 days. Mud crab trials were run in small cages (10 x 10 x 10 cm) in tater tables with a water depth of 112 cm. Please refer to the attached manuscripts (Macreadie et al. 2010, Macreadie et al. in press, and Geraldi and Macreadie submitted) for additional experiments on predator-prey interactions on oyster reefs.

Results

Overall data from multiple sanctuaries

The temperature/salinity loggers collected data from 10/09-5/12. Temperature was consistent throughout Pamlico Sound (Figure 2), while salinity was highly variable and ranged from 0 to 31 psu (Figure 3 and 4). Loggers did malfunction, which left gaps in data collection. Snap shots of current at 7 sanctuaries was also collected and ranged from 0 to 30 cm/sec (Figure 5). Initial observations indicate that recruitment is highly correlated with current.

Experimental trials testing how seed size and planting date influence success of oyster reef creation (Objective 1) and experimental tests of how much the presence of seed oysters enhances natural oyster settlement on created reefs (Objective 2).

Salinity was recorded for an average of 194 days at each sanctuary where we conducted our experiments (Fig. 2). All three sites only had 60 days of contemporaneous data. Crab Hole, Gibbs Shoal and Clam Shoal, experienced salinities (mean \pm 1 SE) of 14.8 ± 0.51 , 19.7 ± 0.41 , and 21.2 ± 0.64 , which were statistically different (Kruskal-Wallis chi-squared = 57.52, df = 2, p-value < 0.001). The salinity at the three sanctuaries ranged from 0 to 32 psu, which spans the documented salinity of the Pamlico Sound (Williams et al. 1973).

The 16 bushels (approximately 560 l) of shell deployed on each mound contained an average of 32,000 seed oysters. We deployed approximately 588,000 seed oysters to the Pamlico Sound. On average, late large seed had the highest number of seed oysters per shell (6.0 ± 0.3 , mean \pm SE), followed by early small seed (4.4 ± 0.6) and late small seed (2.9 ± 0.2) before deployment (Fig. 6A). The size of large and small seed oysters on recycled adult shell were significantly different immediately before deployment (Kruskal-Wallis chi-squared = 662.78, df = 2, p-value < 0.001; Fig. 7A).

The number of oysters per seeded shell was best described by a negative binomial model with shell/seed treatment, site, and date sampled as factors (see Supplemental Material for all Appendices, Appendix A for all models). As a result of shells being overgrown by oysters and/or moved by wave action, only one shell originally deployed was found on the late large seed mounds in Gibbs Shoal during the second sampling, which negated producing a model with all interactions. The difficulty in finding shells after two summers of growth is evident in the total number found per two mounds as shown in Fig. 6C. The shells deployed in June without seed or with small seed had more oysters than the shells deployed in August with small or large seed (Fig. 6, Appendix B). Posthoc comparisons were based on the models standard errors around the mean not overlapping for the variables being compared. Crab Hole had more oysters on shells than the other two sites and these differences were consistent across sampling dates. Most two-way interactions were significant (Appendix B) and additional models were run for each sanctuary separately with data from the fall 2011 sampling. In the fall of 2011 the shell/seed treatment was not significant ($p > 0.012$) when analyses were run for each site separately (Fig. 6C, Appendix C).

The size-frequency of oysters on deployed shell was analyzed using a parametric model with shell/seed treatment and site as factors (Appendix D). The model would not run with year as a factor because of the lack of data for late large-seed mounds at Gibbs Shoal during the second

sampling. Shells deployed in June 2010 had larger oysters than shells deployed later, regardless of seed presence or deployment size of seed (Fig. 7C, Appendix E). Shells deployed at Gibbs Shoal had larger oysters than shells at Clam Shoal or Crab Hole. There were no significant interactions, but the inclusion of the interactions improved the model.

The density of oysters on marl was best described by a negative binomial model with site, depth, and sampling date as factors (Appendix F). The three-way interaction was not included because it did not significantly change the model and parsimonious models are preferred (Crawley 2007). Treatment was not included in the model because it did not explain a significant amount of the variation (Fig. 8, Appendix G). Significant interactions resulted from: more oysters on the top than on the bottom of the mound at Clam Shoal, the reverse of the pattern at the other sites; oyster density at Gibbs Shoal increased through time, which was the opposite trend of the other 2 sites (Fig. 8); and bottom marl had more oysters in the first two samplings, but mean oyster density was similar on the top and bottom of mounds at the final sampling (Fig. 8). Although the interactions prevent conclusions about the main effects the following trends among the main effects were evident; the mean density of oysters on marl was 4 times greater at Clam Shoal than at the other two sites in the fall after deployment (Fig. 8, Appendix G); oyster density at the bottom of the mound was greater than on the top of the mound; and the third sampling in fall of 2011 had fewer oysters than the samplings in fall of 2010 and spring of 2011. Because of the complexity of the overall model, separate models were run for each sanctuary at the fall 2011 sampling with shell/seed treatment as a fixed factor and mound as random factor. There was no difference in shell/seed treatments in any of the sanctuaries (Fig. 8C, Appendix H).

Our analysis of NCDMF data at three additional sanctuaries where mounds were both seeded and unseeded within 1 year of being created was limited because all four fixed factors (seeded or not, mound age, site, and year created) could not be included in one model due to inconstant sampling of mounds each year. The best model included whether the mound was seeded, mound age, and year of creation as factors (Appendix I). Seeded mounds had a lower density of oysters than unseeded mounts (Appendix J, Fig. 9). Mounds created in 2008 had a higher density of oysters than mounds created in 2006 and the density of oysters increased with mound age.

Comparison on recruitment collectors (Objective 2)

The number of spat recruiting to settlement collectors varied by both ($F_{2,164}=17.67$, $p<0.001$) site and collector type ($F_{2,164}=42.85$, $p<0.001$; Figure 10). The interaction between site and type was also significant ($F_{4,164}=5.87$, $p<0.001$). Oyster shells had the greatest number of recruits. Squares mimicked the pattern of recruitment on oyster shell, highest at IMS and Ocracoke, but lowest at South River. Scallop shells were rather consistent among sites, unlike the other two collectors. As a result of the ease in quantifying oyster recruits, the square cement board collectors were deployed at 7 sites (Figure 11). Recruitment was highly variable in 2009 and 2010. Clam Shoal had the highest recruitment followed by West Bluff and Ocracoke.

Predator surveys (Objective 3)

Crab traps and gillnets were set May, July, and Sept. of 2009 and 2010, then again in May and Sept of 2011. Trap catch consisted primarily of black seabass, pigfish, pinfish, blue crab and toadfish (Figure 12). Black seabass, an economically important fish was caught at West Bluff, Carb Hole and Clam Shoal. Blue crab, an oyster predator, was not the prevalent at any sanctuary except Gibbs Shoal. The most common (non-pelagic) fish caught were bluefish, croaker and spot, which are all economically important species (Figure 13). Other economically important species caught included Black drum, sheepshead, southern flounder, speckled trout, and tautog. Sheepshead was the most prevalent oyster predator followed by black drum and cownose rays. Sheepshead may be underestimated in gillnet catch because they remain directly over the reef and less likely to get caught in gillnets than less sedentary species. Sheepshead ranges in length between 176 to 495 mm (Figure 14). Two species of mud crabs were caught in bins.

Eurypanopeus depressus was much more abundant than *Panopeus herbstii* (Figure 15), although *P. herbstii* was larger. Crab carapace width varied from 3 to 58 mm (Figure 16) and crabs were most abundant at 5-10 mm, which was almost exclusively *E. depressus*.

Exclusion cages (Objective 4)

Initial analysis indicated that predation did not affect oyster abundance on deployed shells (2-way ANOVA, $F_{4,143}=0.22$, $p=0.93$) and sanctuary was marginally significant ($F_{2,143}=2.36$, $p=0.09$; Figure 17). Further, more complex analysis is necessary to ensure that this findings is accurate and not a result of over simplifying data (pooling all shells within cage and among sampling dates).

Feeding experiments (Objective 5)

Consumption of bivalves by sheephead was affected by size of sheephead and bivalve (Figure 18). All sheephead preferred small mussels. Although only two small sheephead were run in the experiment, they only eat small oyster and small mussels. The medium sheephead followed the same pattern, but consumed at least one of the other sized bivalves. Although large sheephead preferred smaller bivalves, they readily consumed all sizes and species. Black drum showed a similar feeding preference and preferred small mussels (Figure 19). Although the large black drum was not replicated, the findings indicate that large black drum consumed more oysters than the smaller sizes.

Small and medium mud crabs consumed a greater amount of smaller oysters than large oysters, while large mud crabs did not show a preference for a specific size of oyster (Figure 20). When offered multiple sizes of attached and unattached oysters (small < 2.5 cm, medium >2.5 and < 5 cm, large > 5 cm shell height) and mussels (2-3 cm shell height), mud crabs preferred mussels and unattached small oysters (Figure 21). Blue crabs were similar to mud crabs and preferred mussels and unattached small and medium oysters. However, large blue crabs consumed large attached and unattached oysters. Stone crabs preferred large oysters, attached and unattached as well as mussels.

Discussion

The sanctuaries in this study extended over the entire area of Pamlico Sound and the temporal scale of results included 3 different years of reef creation. In the fall following experimental seeding, shells deployed without seed in June had as many oysters of equal or greater size than any of the shells deployed with seed. Our results indicate that seeding recently created artificial reefs is neither necessary nor enhances oyster reef development in the Pamlico Sound.

Seeding artificial reefs could have been expected to increase natural recruitment because oyster larvae are thought to be gregarious settlers (Kennedy et al. 1996). Laboratory experiments have found that the presence of seed oyster on shell (Hidu and Haskin 1971, Keck et al. 1971) and presence of chemical cues from adult conspecifics (Hidu et al. 1978, Turner et al. 1994, Tamburri et al. 2008) increase settlement of larvae. Although we did not directly measure settlement, natural oyster recruitment overwhelmed any benefit of seeding. Our results are not consistent with laboratory findings because the presence of seed oysters did not increase recruitment on shell or on the mound substrate (marl). Discrepancies between this study and past laboratory experiments could result from seed oysters not producing a strong enough chemical cue to attract larvae, or the larvae could have been equally attracted to cues coming from biofilms on the marl, shell, and shell with seed (Tamburri et al. 1992, 2008).

Oyster recruitment and abundance varied within and among sites. Abundance of oysters on shell was highest at the low-salinity site, but oyster density was highest on marl at the high-salinity site. Greater recruitment in higher salinities has been found in the Pamlico Sound (Ortega and Sutherland 1992), Maryland (Beaven 1954), and the Gulf of Mexico (Butler 1954). The low recruitment to shell at the high-salinity site compared to the low-salinity site could have resulted from an earlier settlement pulse at the high salinity site, with deployment of shell occurring after this pulse. Within sites, recruitment of oysters was higher at the bottom of the mounds than on the top at the first sampling. Lenihan (1999) monitored high and low relief oyster reefs and also found higher oyster recruitment at deeper depths. But 1 year after reef deployment, the density of oysters was similar between the top and bottom of the reef. In addition, oyster density decreased over time at the low- and high-salinity sites, but increased at the mid-salinity site. This could indicate that moderate salinities within Pamlico Sound may be the best areas for oyster restoration with the goal of maximizing oyster densities. However, the average density of oysters remained above 400 oysters m⁻² at all experimental sites, which is greater than the highest densities found on oyster reef sanctuaries throughout North Carolina and

is 40 times higher than the 10 oysters m^{-2} that has been used as a indicator for a functional reef (Powers et al. 2009).

Data from NCDMF support our experimental findings that seeding is neither necessary for nor beneficial to oyster restoration efforts in Pamlico Sound. Results from these data are not as clear as our experimental study results because of inconsistent sampling, but conclusions can still be made. At NCDMF-monitored sites, density of oysters on the marl varied between the 2 years that mounds were created. Seeded mounds had significantly fewer oysters than unseeded mounds. Addition of seeded shell could reduce oyster abundance on mounds because added shell is easily dislodged and redistributed during storm events, and this shell movement can destroy oysters attached to marl or remove deployed shell from the mound. Shell overgrowth and removal was evident at the experimental sites by the decreasing number of shells found on the mounds through time.

In principle, addition of seed oysters could be advantageous for restoration efforts where oyster recruitment is limiting or mortality is high for recently settled oysters. These situations would exist if: populations are reduced low enough that gametes released by adults are not fertilized; habitat is highly degraded (i.e. anoxia) and the existing oyster population has very low reproductive output; or predators or disease cause high mortality of recently settled oysters. Although oyster recruitment did vary, recruitment was not limiting because natural recruitment swamped any effect of seeded shell. Oyster predators (mud crabs, sheepshead fish, black drum and oyster drills) were prevalent throughout Pamlico Sound, but recruitment seemed to exceed the effect of predators because the density of oysters remained above $400m^{-2}$ regardless of shell/seed treatment and there was not significant effect of cage or cage type on oyster survival. This is surprising because of the high density of mud crabs and their consumption of oysters in feeding preference trails. Although mud crabs have been hypothesized to have an impact on oyster populations based on natural density of feeding (Rindone and Eggleston 2011), we did not find increase oyster survival when mud crabs were excluded. This probably results from high recruitment and the abundance of alternative prey (mussels). Our results indicate a drastic decrease in oyster density in high-salinity areas by the last sampling at Clam Shoal and approximately 3 years after reef creation at Ocracoke. Powers et al. (2009) surveyed a protected reef near (within 5 km) Clam Shoal in 2002-2003 and found no live oysters. They attributed the oyster absence to recruitment limitation, but our results and findings of Ortega and Sutherland

(1992) indicate that post-recruitment mortality is probably the reason for low oyster abundance on the east side of Pamlico Sound. The cause of the high mortality is presently unknown, but this region had the highest recruitment to marl (Clam Shoal, approximately 8000 oysters m⁻²). High recruitment and subsequent high mortality indicates that this region could be used for transplantation in oyster reef restoration schemes. Transplantation consists of deploying substrate in high-recruitment areas and then moving the substrate to an area with lower mortality after recruitment occurs. Such oyster transplanting is common in many areas and is used to increase oyster harvest and restore oyster reefs (Powell et al. 1997, Southworth and Mann 1998, Brumbaugh and Coen 2009, Kennedy et al. 2011).

Beck et al. (2011) estimated that oysters are only 5-10 percent of historic abundances in North Carolina. However, our findings indicate that extant oyster populations in the areas surrounding the studied oyster sanctuaries have larval production sufficient to develop oyster reefs on deployed substrate, which confirms historical observations that oyster recruitment is not limiting south of the Chesapeake Bay (Wallace 1952, Andrews 1954). Ortega and Sutherland (1992) found that recruitment along the western side of Pamlico Sound seemed to be decreasing from 1988 to 1990, which they attributed to decreasing oyster populations. Our study two decades later was different from their finding. Moreover, no-harvest oyster sanctuaries throughout the Pamlico Sound have remained viable for longer than 10 years (Powers et al. 2009), which would indicate that recruitment is neither limiting nor decreasing. Determining which factors contribute to the high recruitment in Pamlico Sound and why recruitment is low in other areas, such as the Chesapeake Bay (Mann and Powell 2007), is an important step to facilitate widespread oyster restoration.

To our knowledge, few experimental studies have tested the benefit of seeding restored oyster reefs, which is unexpected given the widespread use of seed oysters to restore and maintain oyster populations. One study on whether seed oysters augmented artificial reefs found 100% mortality of seed oysters from oyster drill predation in Mobile Bay, AL (Wallace et al. 2002). Although the benefit of seeding for oyster restoration will vary depending on where and when seeding is used, experiments are needed to determine if seeding is beneficial to oyster restoration.

Restoring habitats, whether because of widespread degradation or extirpation, is one of the great challenges of our century (Hobbs and Harris 2001). Management restoration efforts are

usually limited by management schemes that led to the degradation, the amount of money allocated for restoration and the complexity of ecological processes. Managers and stakeholders should invest in experiments that test whether recruitment is limited before artificially augmenting natural recruitment, a strategy commonly used to restore other biogenic habitats such as seagrass meadows (Bell et al. 2008, Orth et al. 2012) and coral reefs (Clark and Edwards 1995, Edwards and Clark 1998). As habitat restoration efforts increase, restoration techniques need to be firmly grounded in experimental ecology so that invested resources are maximized based on the spatial and temporal dynamics of recruitment and the overall restoration goals.

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Figures

Figure 1. Map of seeded and monitored oyster sanctuaries in the Pamlico sound.

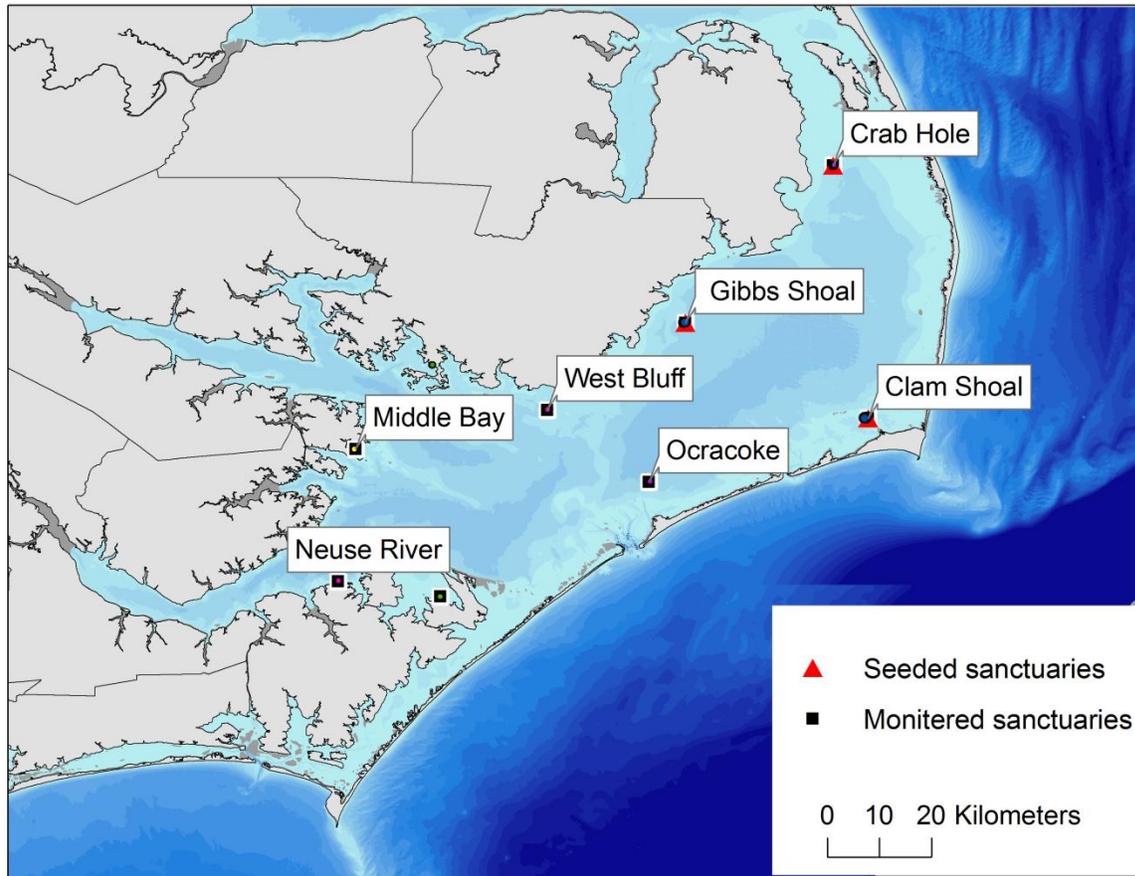


Figure 2. Temperature over time at oyster sanctuaries in the Pamlico Sound.

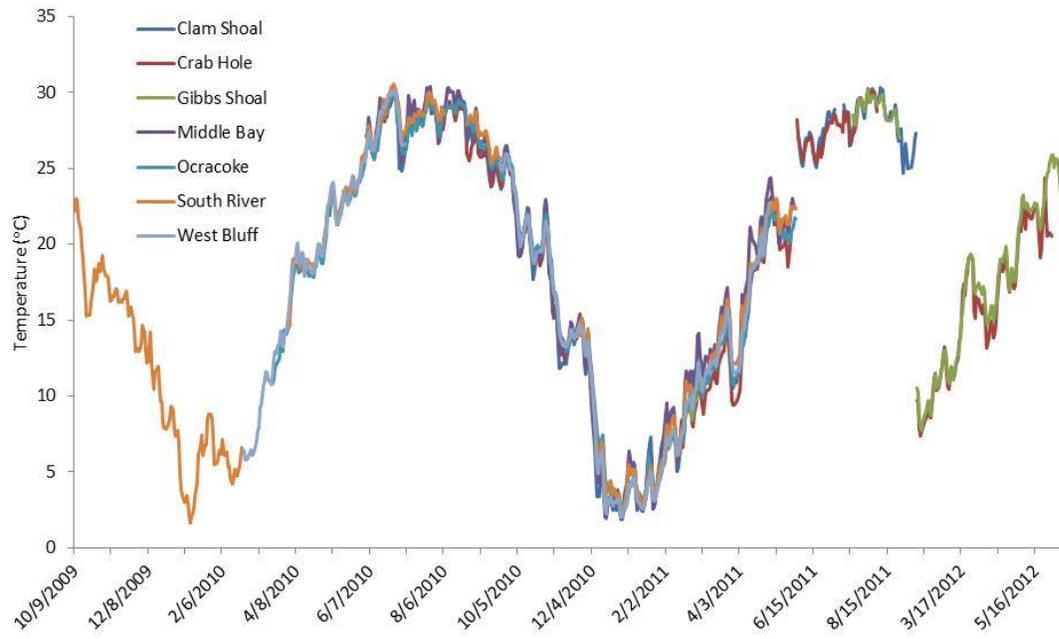


Figure 3. Salinity over time at oyster sanctuaries in the Pamlico Sound.

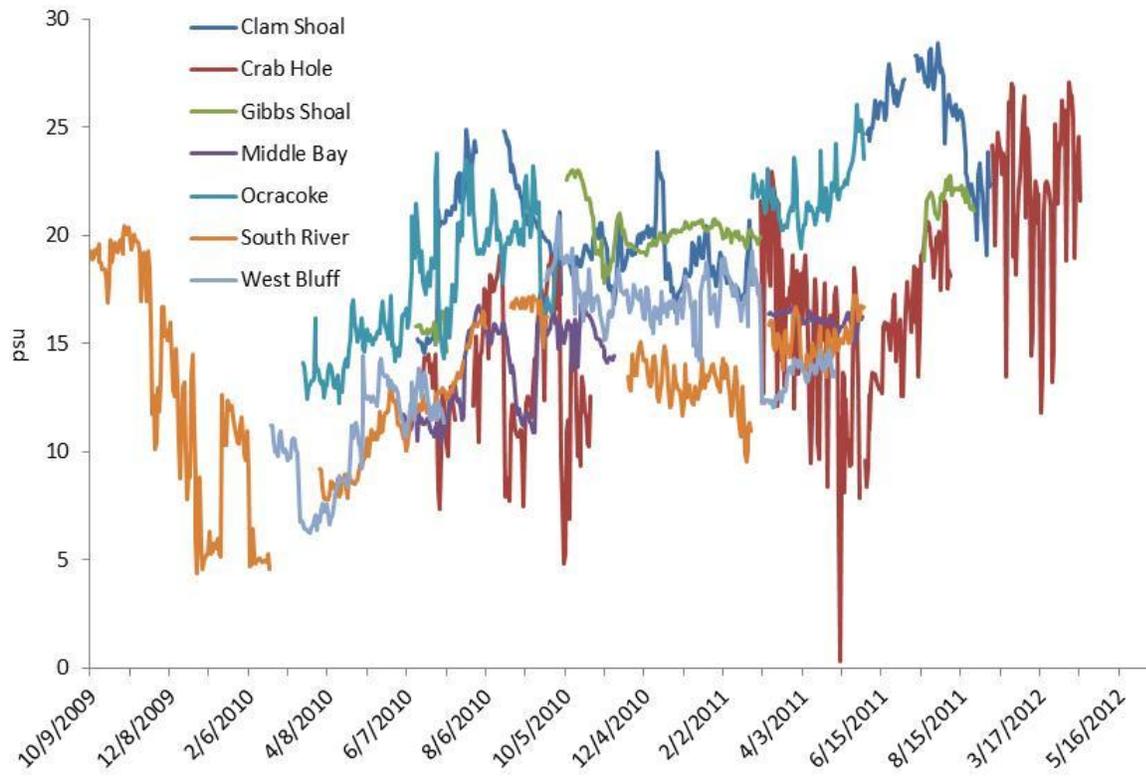


Figure 4. Box plots of salinity at 7 oyster sanctuaries in Pamlico Sound. Boxplots show inner 2 quartiles within box and whiskers extent to 1.5 times the respective inner quartile. The line through the box, asterisks, and circles indicate median, mean and data points outside of whiskers respectively.

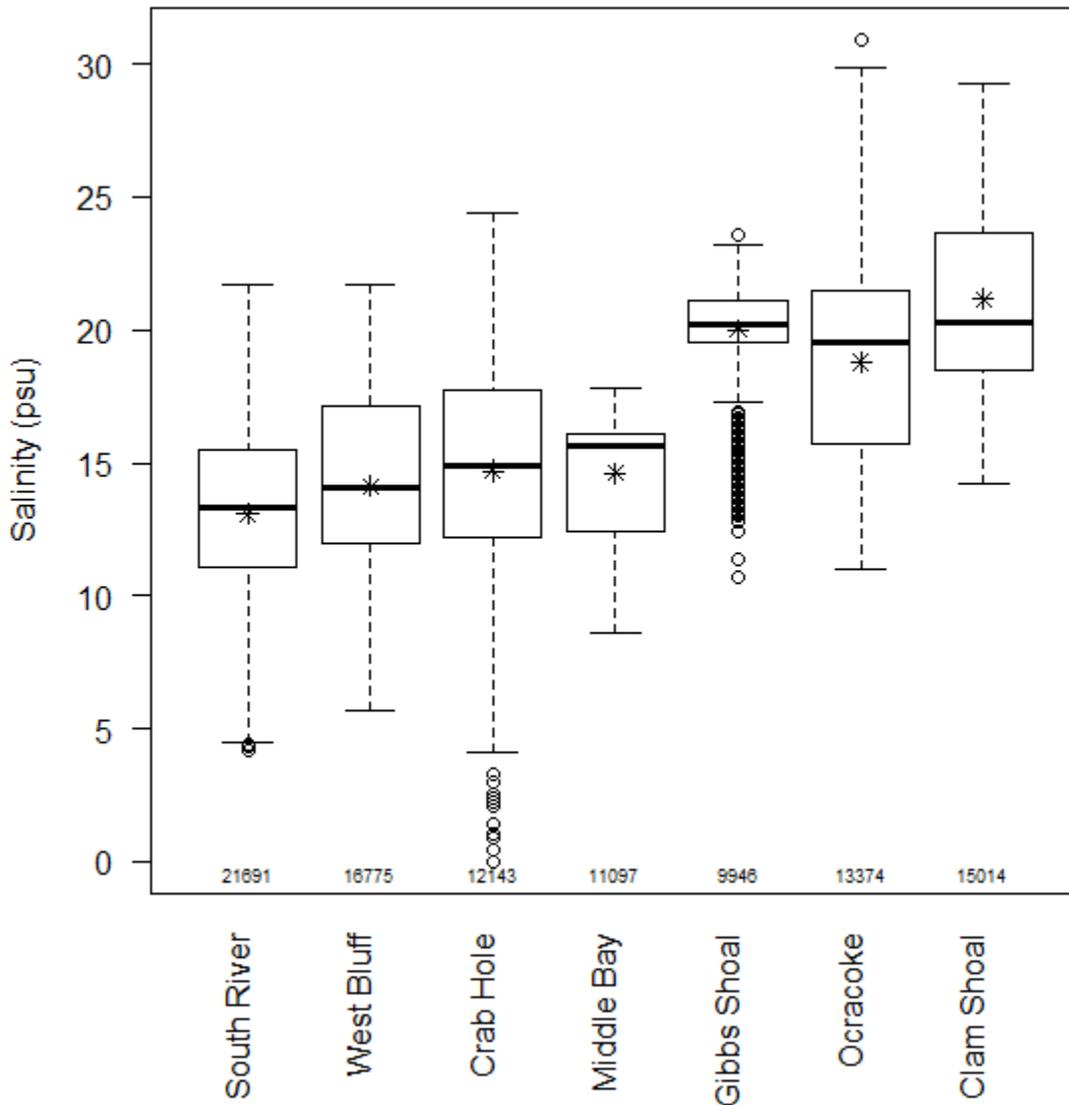


Figure 5. Currents at each of the monitored sanctuaries July-Aug. Currents were measured every 30 seconds by a S4 meter anchored at each site for 17-66 days (number above x-axis indicates number of days measured). Boxplots show inner 2 quartiles within box and whiskers extent to 1.5 times the respective inner quartile. The line through the box, asterisks, and circles indicate median, mean and data points outside of whiskers respectively.

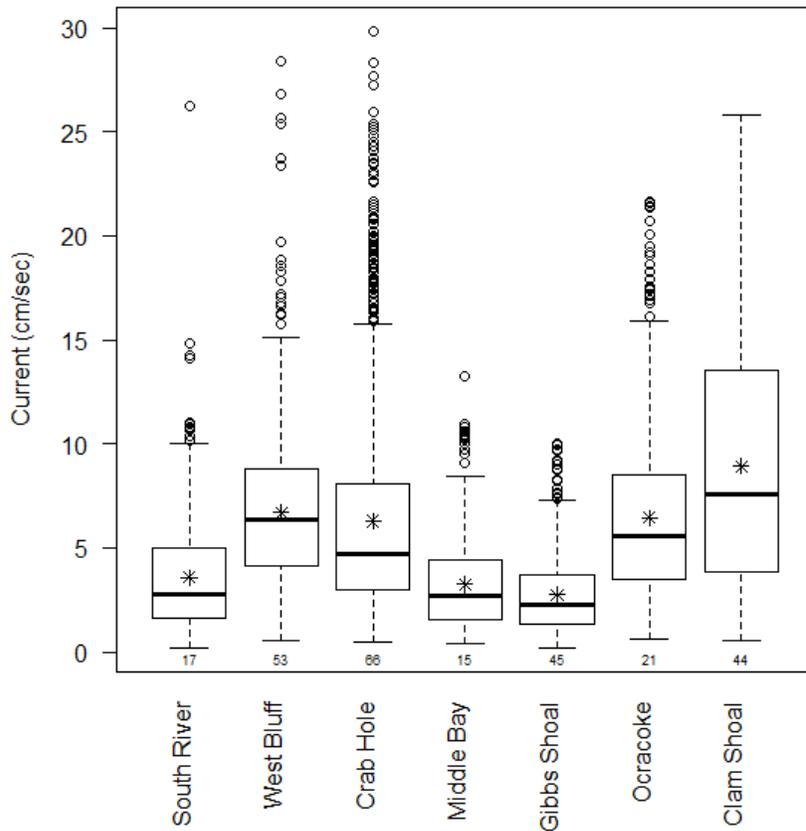


Figure 6. Number of oysters per shell (mean \pm 1 SE) before shells were deployed in the summer of 2010 (A), after deployment in the fall Oct. 2010 (B) and the following year in Sept. 2011 (C).

Number of shells sampled is noted above the x-axis.

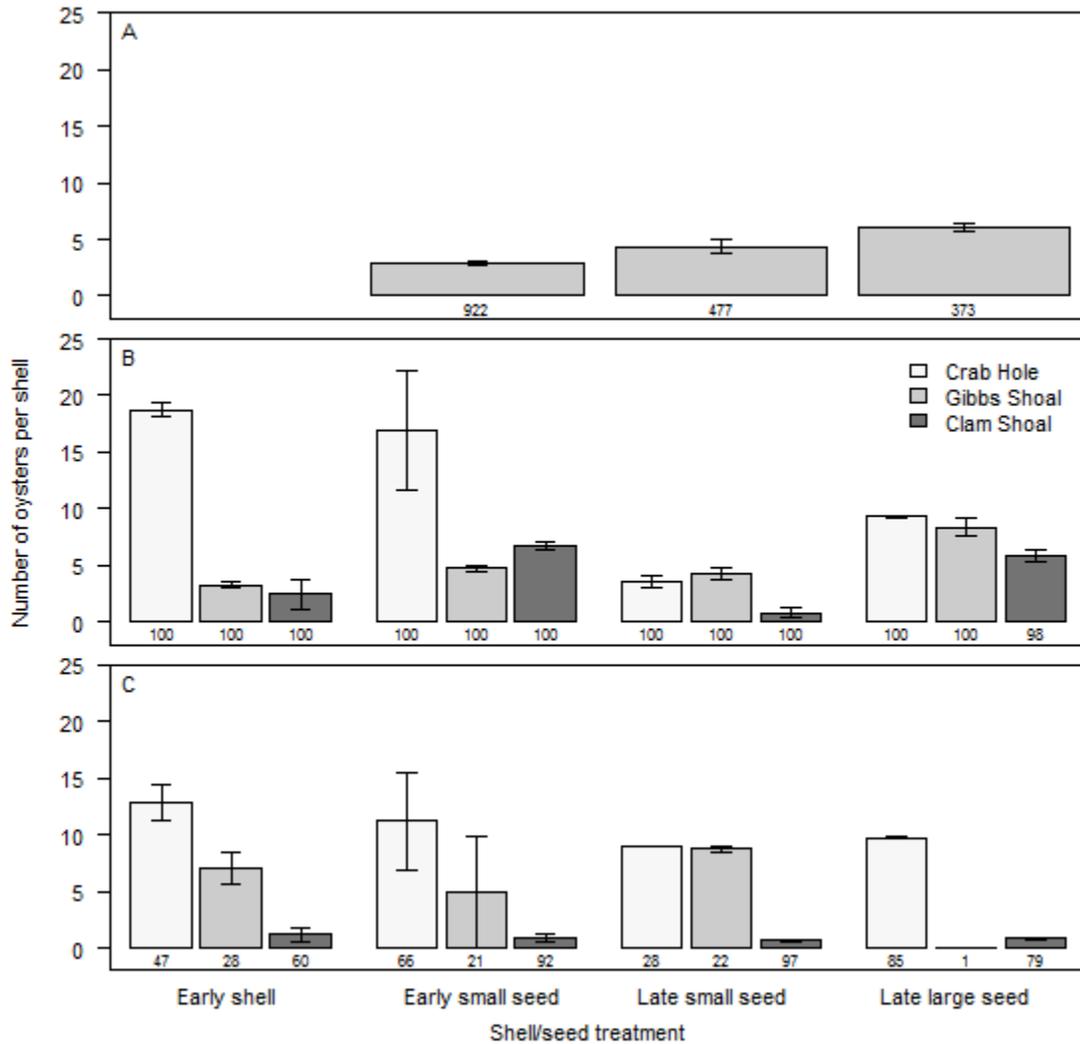


Figure 7. Size of oysters (mean±SE) per shell before shells were deployed during the summer of 2010 (A), after deployment in the fall Oct. 2010 (B) and the following year in Sept. 2011 (C).

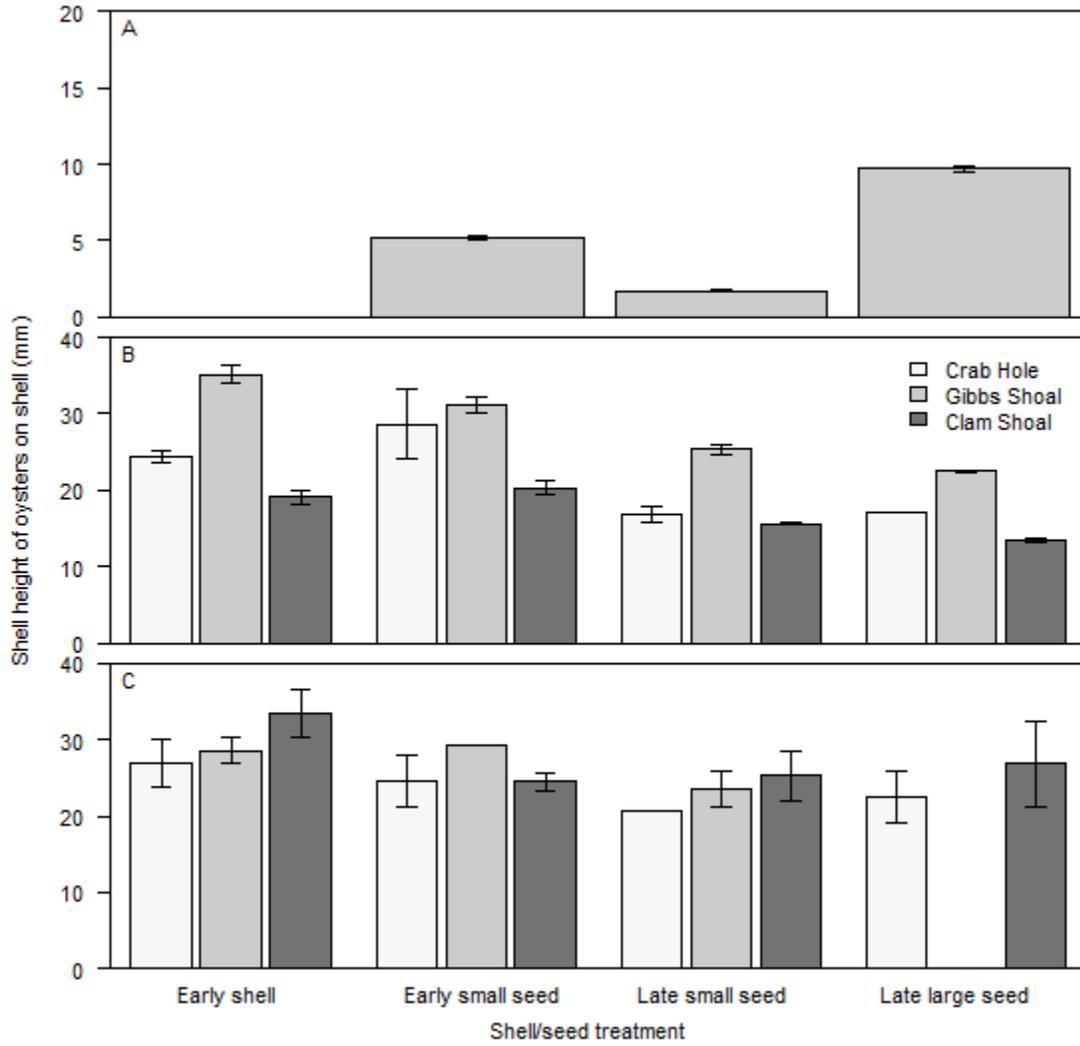


Fig. 8. The density of oysters (m^{-2} ; mean \pm SE) on sampled marl in fall 2010 (A), spring 2011 (B), and fall 2011 (C). The density of oysters on mounds created in 2005-2006 were included in the figure as a baseline for successful restoration (Established), but were not included in the analysis.

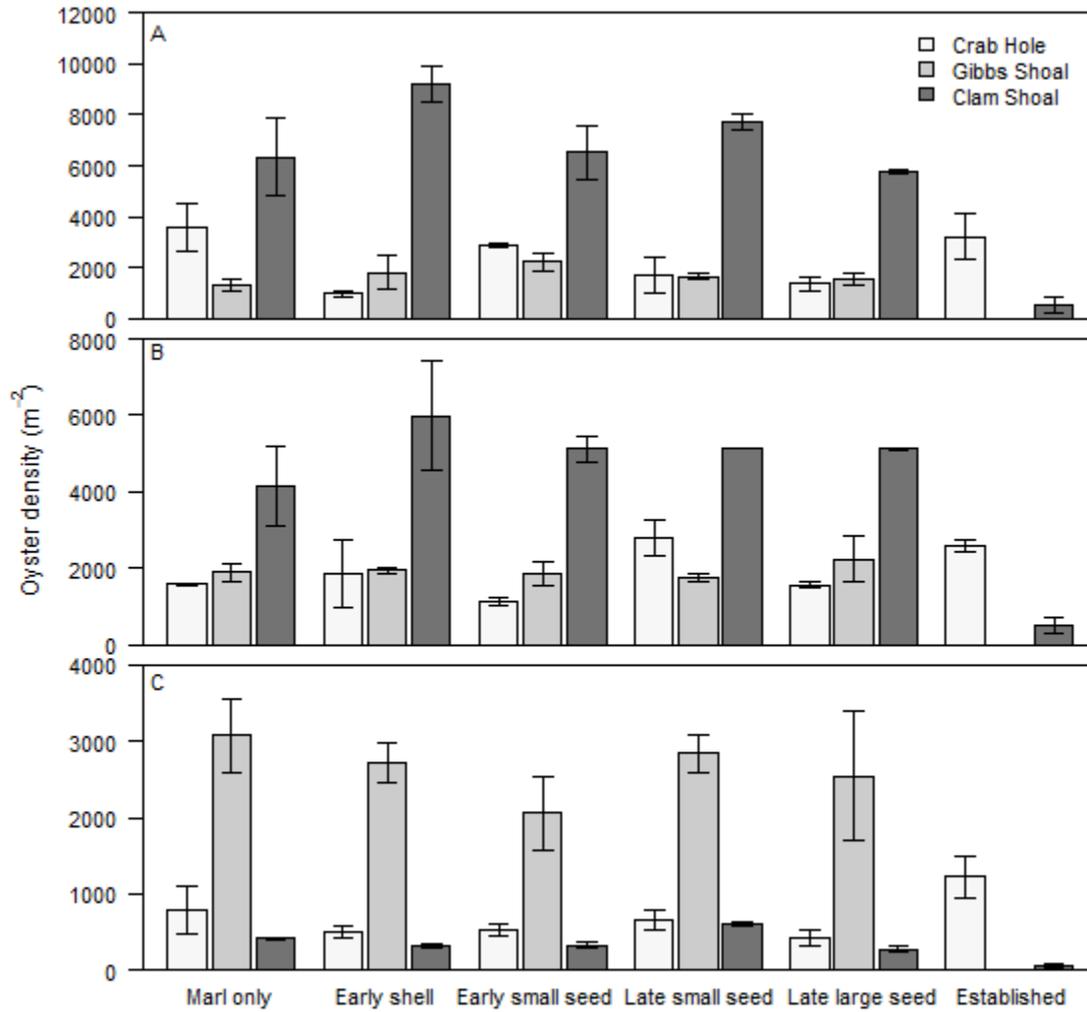


Fig. 9. The density of oysters (m^{-2} ; mean \pm SE) on sampled marl from mounds seeded in 2006 in South River (A), 2008 in South River (B), 2008 in West Bluff (C) and 2006 in Ocracoke (D).

Number of mounds sampled is noted above the x-axis.

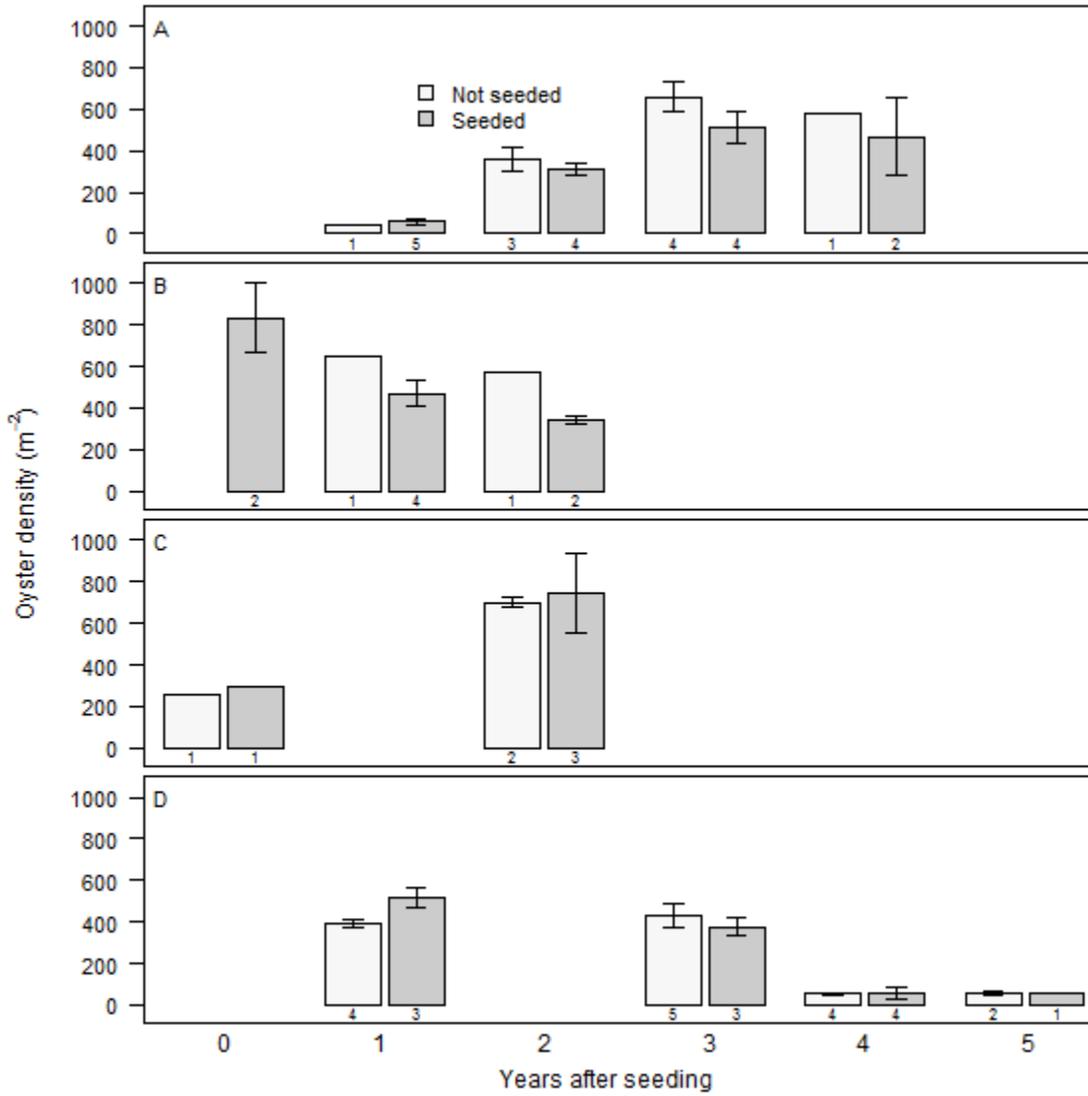


Figure 10. Number of spat collected at 3 oyster sanctuaries on 3 different collector types. Number of collectors sampled is indicated above the x-axis. Boxplots show inner 2 quartiles within box and whiskers extent to 1.5 times the respective inner quartile. The line through the box, asterisks, and circles indicate median, mean and data points outside of whiskers respectively.

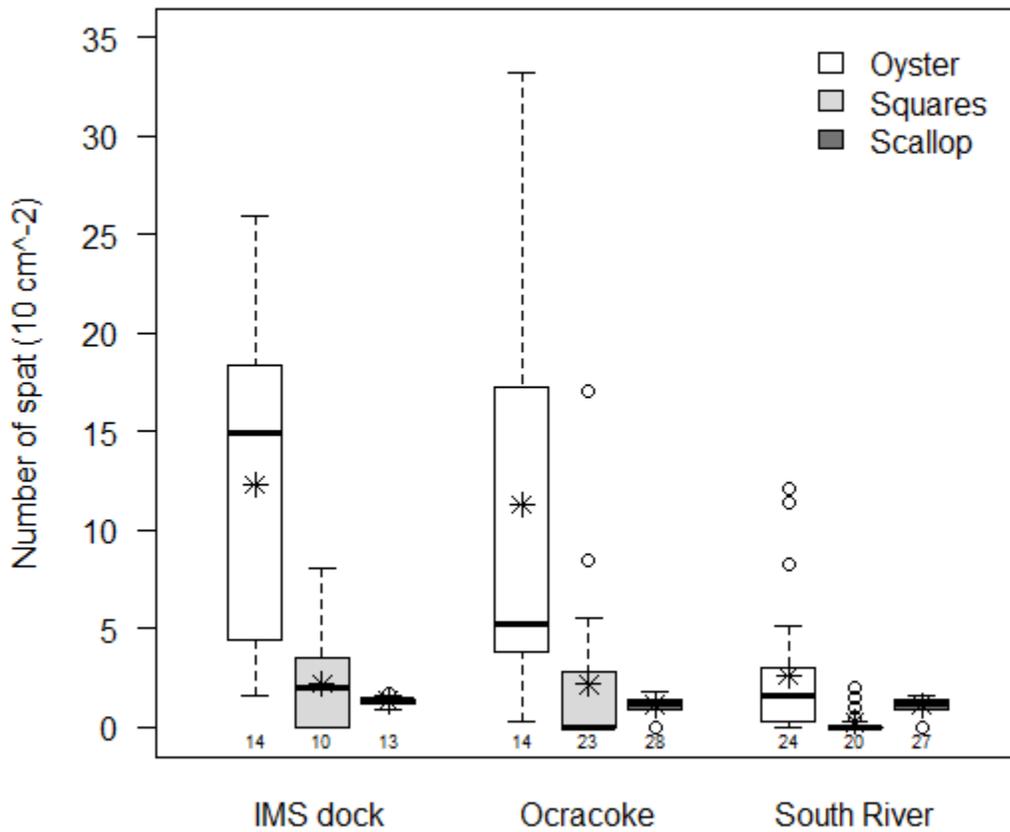


Figure 11. Number of oyster recruits to 10 x 10 cm square collectors (200 cm² total area) at all sites in 2009 and 2010 for all data points (A) and only points less than 10 (B). Number of squares samples is indicated above the x-axis. Boxplots show inner 2 quartiles within box and whiskers extent to 1.5 times the respective inner quartile. The line through the box, asterisks, and circles indicate median, mean and data points outside of whiskers respectively.

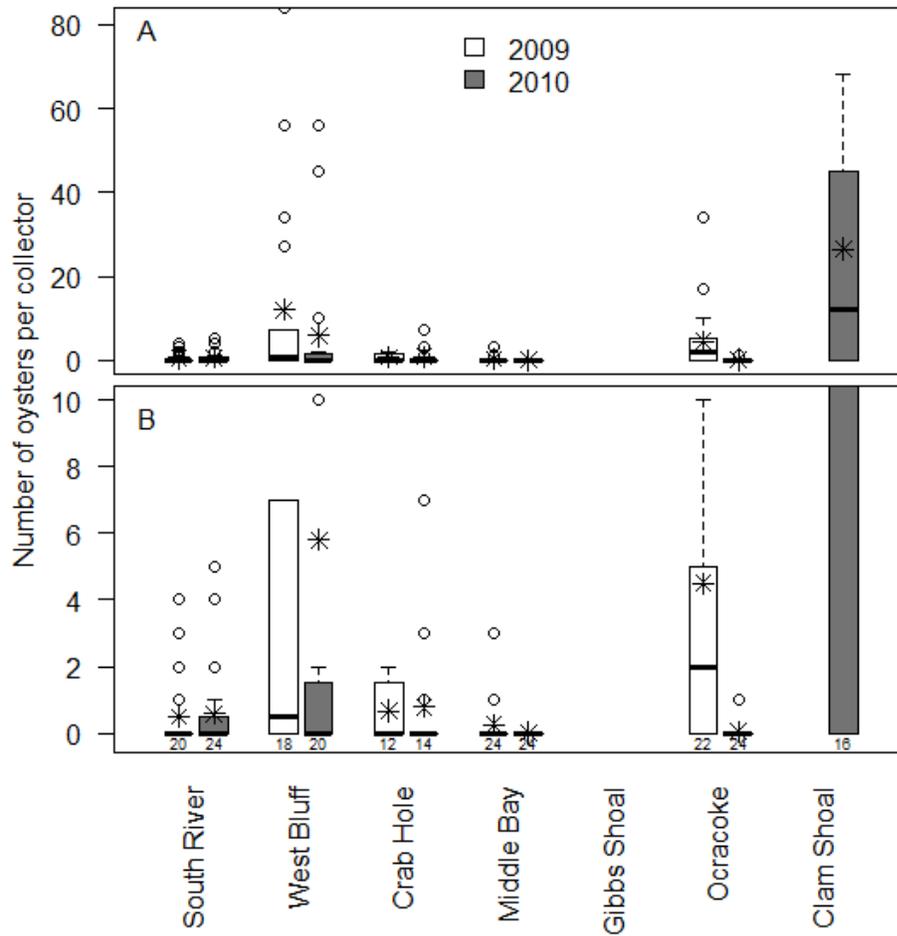


Figure 12. Most frequently caught species (mean±SE) in crab traps at 7 oyster sanctuaries.

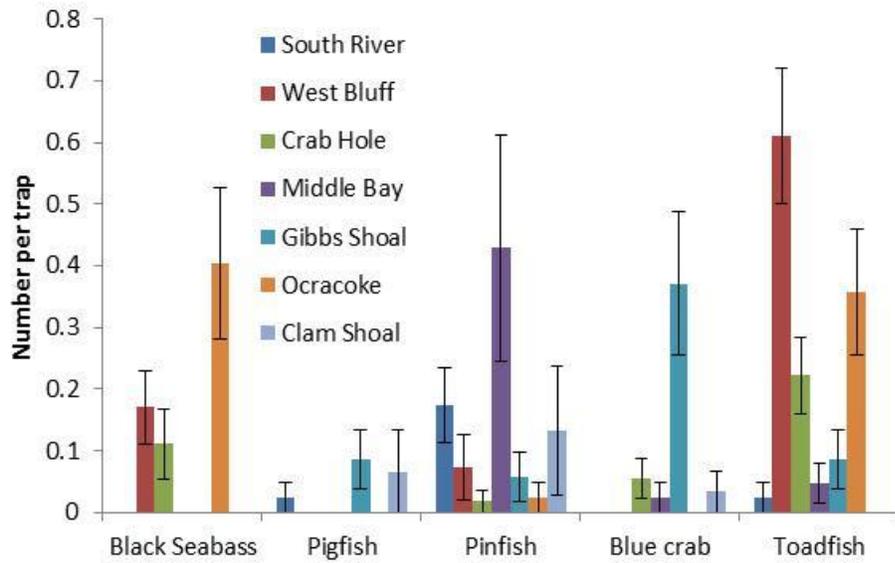


Figure 13. Number of fishes caught per gill net (mean±SE) at 7 oyster sanctuaries.

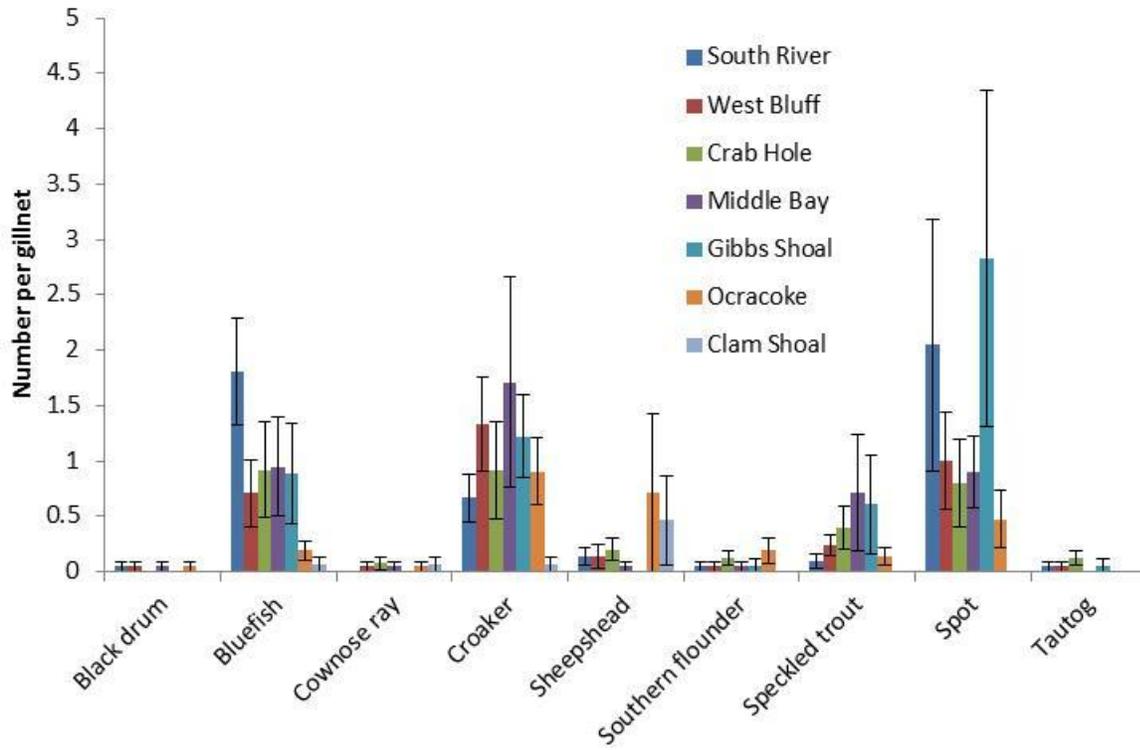


Figure 14. Size frequency of Sheepshead caught in gillnets.

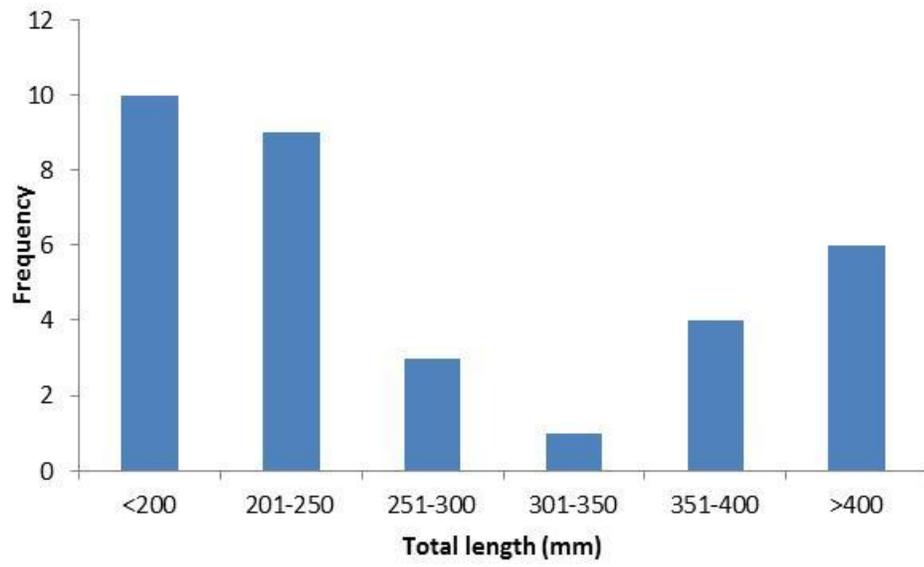


Figure 15. Density of *E. depressus* and *P. herbstii* at 7 different oyster sanctuaries. Boxplots show inner 2 quartiles within box and whiskers extent to 1.5 times the respective inner quartile. The line through the box, asterisks, and circles indicate median, mean and data points outside of whiskers respectively.

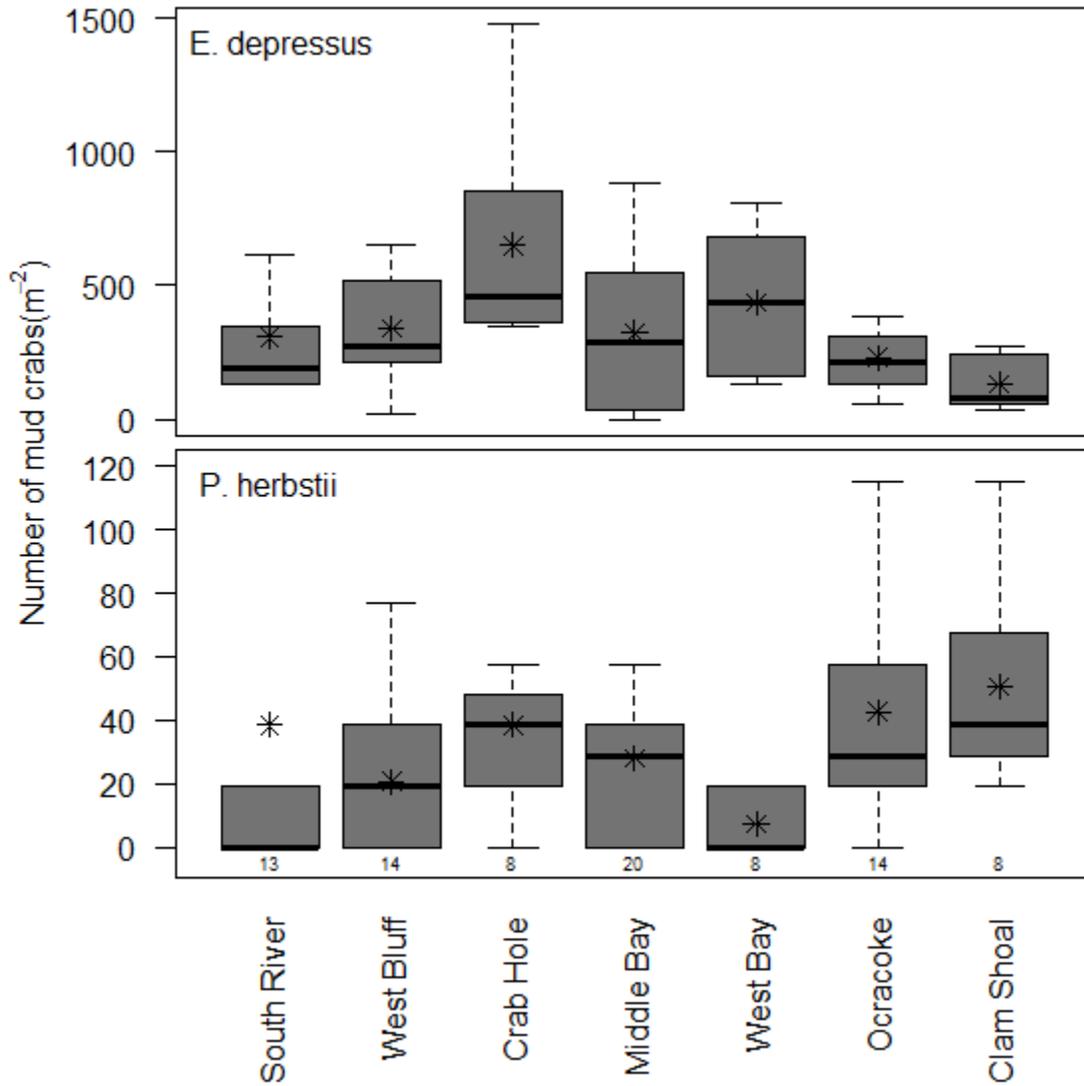


Figure 16. Mud crabs m^{-2} within size bins at 6 different oyster sanctuaries.

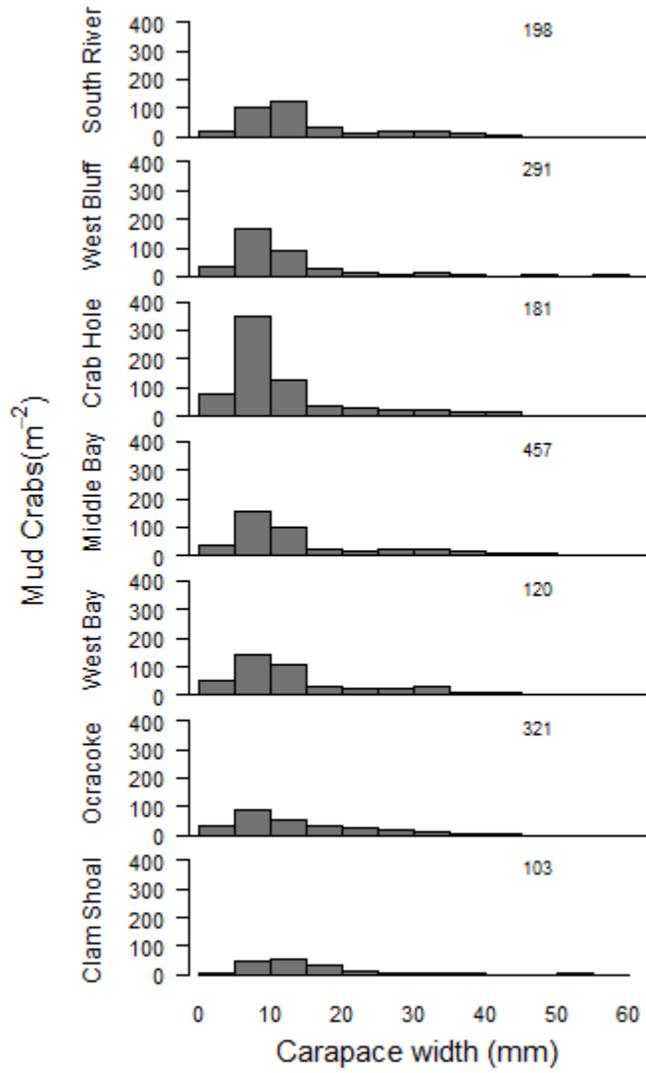


Figure 17. Changes in oyster abundance on shells within 5 different cage treatments at three different oyster sanctuaries. The number above the x-axis indicate the number of cages sampled over time. Boxplots show inner 2 quartiles within box and whiskers extent to 1.5 times the respective inner quartile. The line through the box and asterisks indicate median and mean respectively.

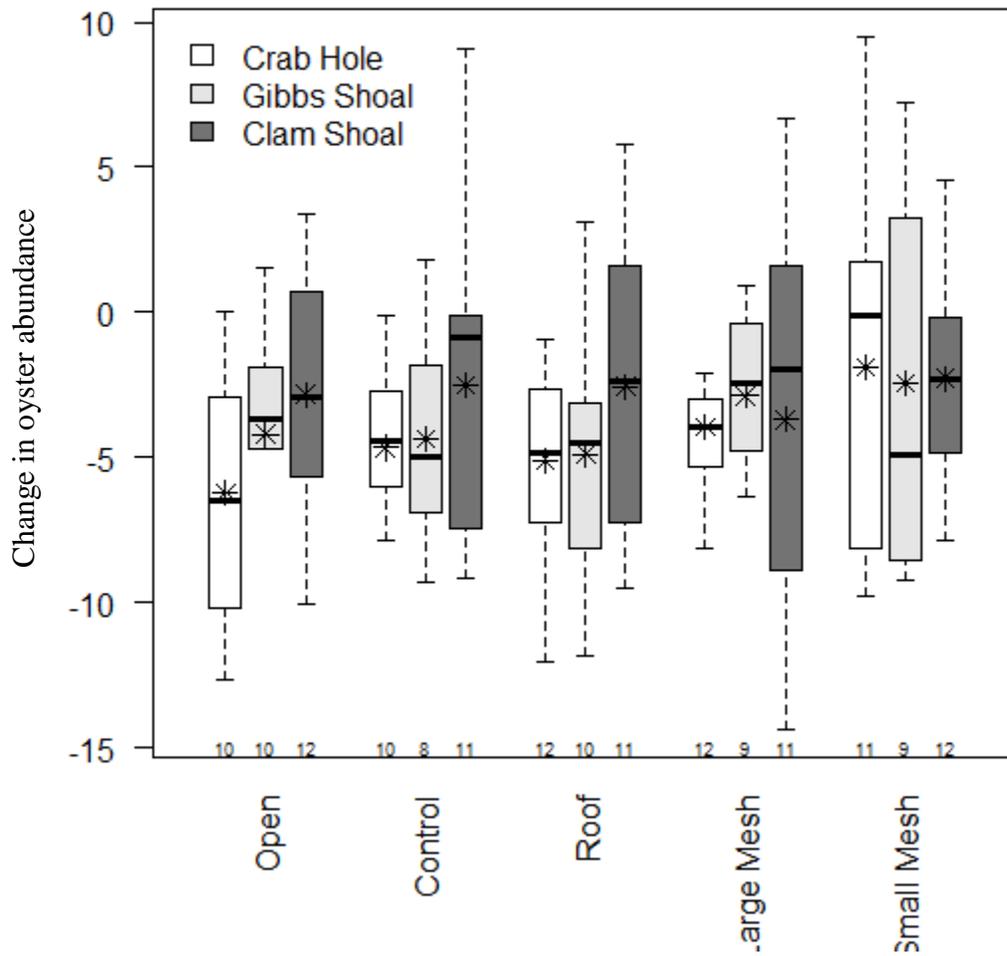


Figure 18. Number of bivalves consumed by sheephead (mean±SE). Sheephead were offered different sizes of oysters and mussels.

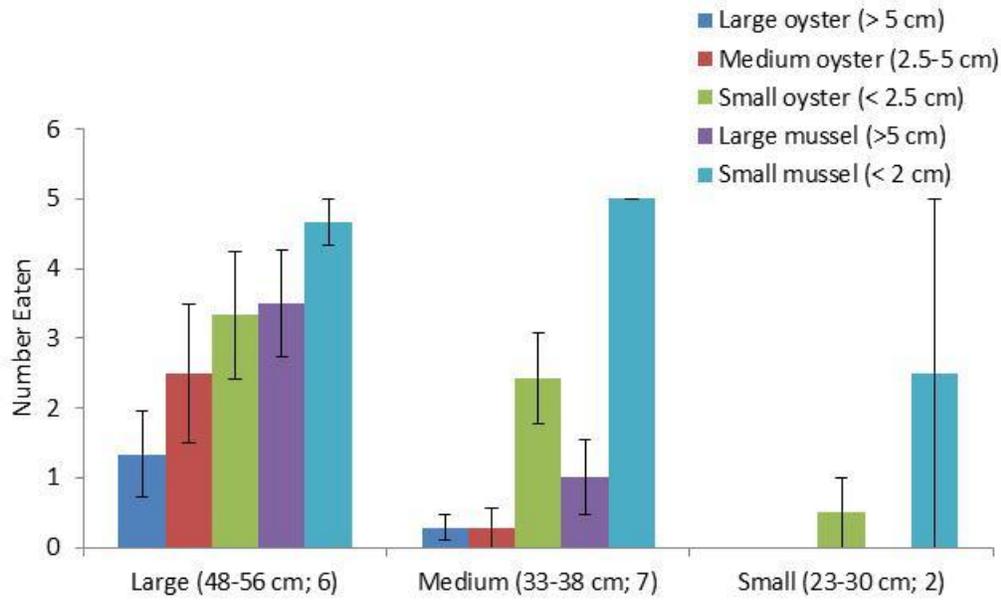


Figure 19. Number of bivalves consumed by black drum (mean±SE). Black drum were offered different sizes of oysters and mussels.

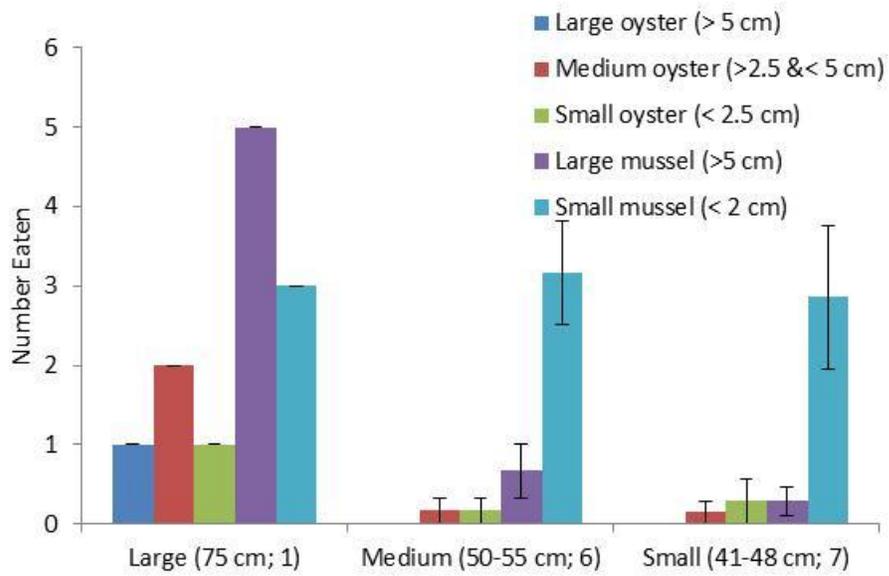


Figure 20. Percent oysters consumed by mud crabs (mean±SE). Crabs were offered three shells each with one category of oysters, small-8 oysters, medium-4 oysters, large -2 oysters

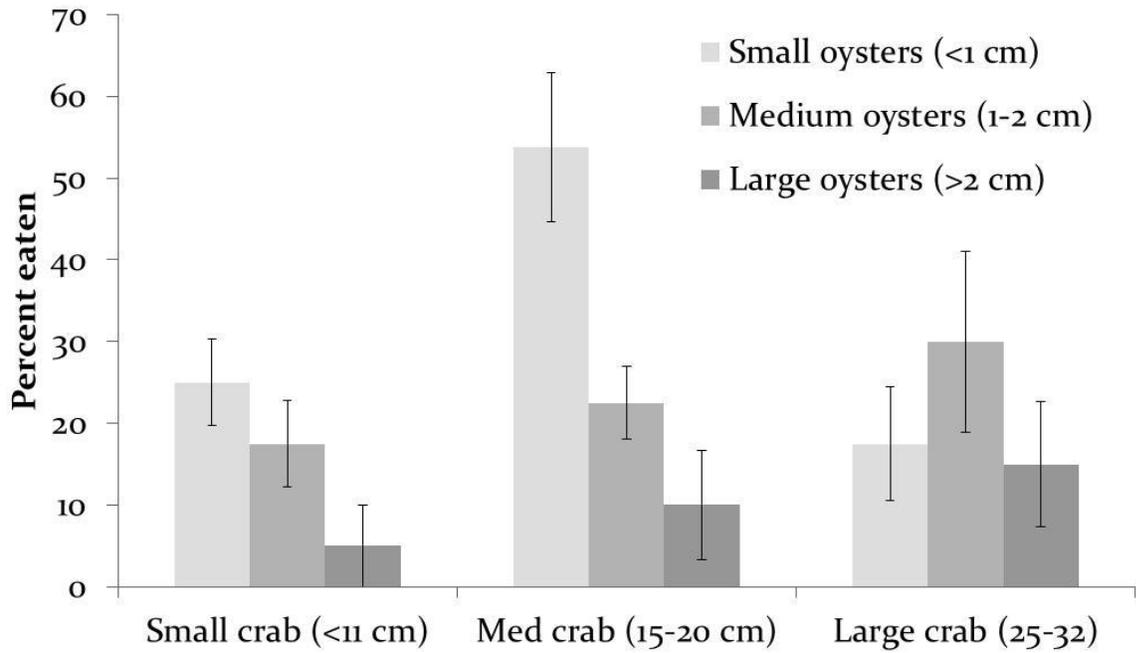
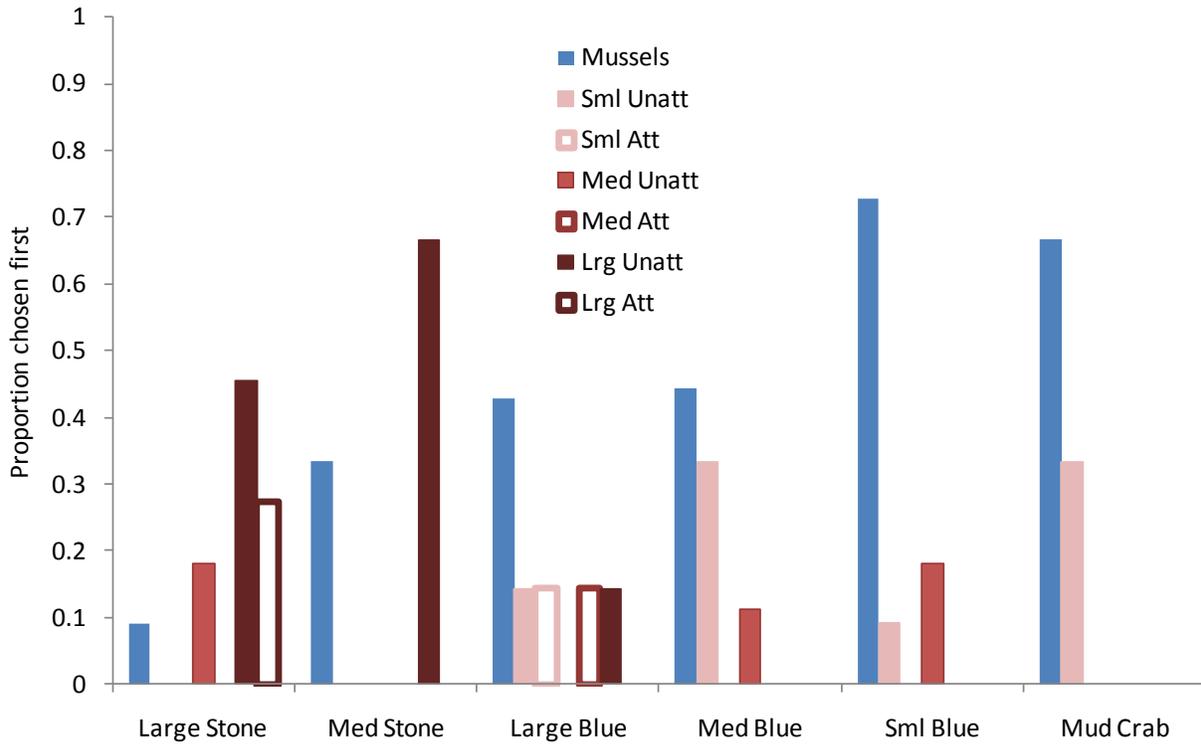


Figure 21. Feeding preference of crabs offered mussels and three different sizes (small < 2.5 cm, medium >2.5 and < 5 cm, large > 5 cm shell height) of oysters that were each either attached or not attached to adult shell, as well as mussels (2-3 cm shell height).



Supplemental Material-Appendices

Appendix A. The number of oysters per shell fit to mixed effect-generalized linear models. Models are listed from the simplest to the most complex for each model family. Best model (lowest AIC) is bolded and NA indicated model would not run because of lack of replication.

Model	Family	df	AIC
# oysters=treatment, random=mound	poisson	5	12757.96
# oysters=treatment*site, random=mound	poisson	13	12724.48
# oysters=treatment+site+year, random=mound	poisson	8	12605.14
# oysters=treatment*site*year, random=mound	poisson	NA	NA
# oysters=treatment+site+year+treatment:site+treatment:year+site:year, random=mound	poisson	19	11688.92
# oysters=treatment, random=mound	negative binomial	6	9646.92
# oysters=treatment*site, random=mound	negative binomial	14	9613.64
# oysters=treatment+site+year, random=mound	negative binomial	9	9586.02
# oysters=treatment*site*year, random=mound	negative binomial	NA	NA
# oysters=treatment+site+year+treatment:site+treatment:year+site:year, random=mound	negative binomial	20	9264.5

Appendix B. Summary of results for the number of oysters per shell fit to a negative-binomial mixed effect model. Factors included were treatment, site, year sampled and the two-way interactions. The intercept estimate is the estimated mean and estimates for all of the factor levels are changes relative to the intercept estimate. The pr is the estimated probability that the listed factor level or interaction is significantly different from the factor level that is the control (not listed). Pair-wise comparisons are significant if the standard errors relative to the respective means do not overlap.

	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	2.8438	0.154	18.47	< 2e-16
Treatment-Early small	-0.0733	0.2172	-0.34	0.7358
Treatment-Late small	-1.5322	0.222	-6.9	5.20E-12
Treatment-Late large	-0.5349	0.2194	-2.44	0.0148
Site-Gibbs Shoal	-1.6461	0.2219	-7.42	1.20E-13
Site-Clam Shoal	-1.93	0.2246	-8.59	< 2e-16
Sampling-fall 2011	-0.0509	0.1089	-0.47	0.6403
Early small seed:Gibbs Shoal	0.5157	0.3097	1.67	0.0959
Late small seed:Gibbs Shoal	1.6711	0.3136	5.33	9.90E-08
Late large seed:Gibbs Shoal	1.4598	0.3143	4.64	3.40E-06
Early small seed:Clam Shoal	1.0103	0.3102	3.26	0.0011
Late small seed:Clam Shoal	0.5394	0.3228	1.67	0.0947
Late large seed:Clam Shoal	1.2972	0.312	4.16	3.20E-05
Early small sees: Sampling-fall 2011	-0.3834	0.128	-2.99	0.0027
Late small seed: Sampling-fall 2011	0.8649	0.1488	5.81	6.10E-09
Late large seed: Sampling-fall 2011	-0.0647	0.1391	-0.47	0.6417
Gibbs Shoal: Sampling-fall 2011	0.5969	0.1344	4.44	9.00E-06
Clam Shoal: Sampling-fall 2011	-1.3622	0.1106	-12.32	< 2e-16

Appendix C. Summary of results for the number of oysters per shell fit to a negative-binomial mixed effect model. Analyses were run with data from fall of 2011 for each site separately, with treatment (fixed) and mound (random) as independent factors. The late large seed treatment is missing for Gibbs Shoal because only 1 shell was found on the two mounds.

Crab Hole

	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	2.554	0.166	15.350	<0.001
Treatment-Early small seed	-0.230	0.232	-0.990	0.320
Treatment-Late small seed	-0.355	0.286	-1.240	0.210
Treatment-Late large seed	-0.279	0.229	-1.220	0.220

Gibbs Shoal

	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	1.774	0.159	11.150	<0.001
Treatment-Early small seed	0.465	0.237	1.960	0.050
Treatment-Late small seed	0.403	0.234	1.720	0.086

Clam Shoal

	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	-0.084	0.261	-0.320	0.750
Treatment-Early small seed	-0.069	0.351	-0.200	0.840
Treatment-Late small seed	-0.325	0.353	-0.920	0.360
Treatment-Late large seed	-0.116	0.358	-0.320	0.750

Appendix D. The size of oysters on deployed shell fit to mixed effect-general linear models. Models are listed from the simplest to the most complex for each model family. Best model (lowest AIC) is bolded and NA indicated model would not run because of lack of replication.

Model	df	AIC
oyster size=treatment, random=mound	6	10462.84
oyster size=treatment+site, random = mound	8	10438.37
oyster size=treatment+year, random = mound	7	10421.75
oyster size=treatment*site, random = mound	14	10419.52
oyster size=treatment*year, random = mound	NA	NA
oyster size=treatment*year*site, random = mound	NA	NA

Appendix E. Summary of results for the size of oysters on deployed shell fit to a negative-binomial mixed effect model. Factors included were treatment, site, year sampled and the two-way interactions.

	Value	Std.Error	df	t-value	p-value
(Intercept)	25.278493	1.416065	1457	17.851226	0
Treatment-Early small seed	1.289854	1.989079	12	0.648468	0.5289
Treatment-Late small seed	-7.455579	2.040513	12	-3.65E+00	0.0033
Treatment-Late large seed	-5.490506	1.979502	12	-2.77E+00	0.0168
Site-Gibbs Shoal	7.943172	2.035137	12	3.90E+00	0.0021
Site-Clam Shoal	-0.825575	2.05927	12	-4.01E-01	0.6955
Early small seed:Gibbs Shoal	-3.721463	2.868715	12	-1.297258	0.2189
Late small seed:Gibbs Shoal	-0.521924	2.900534	12	-0.179941	0.8602
Late large seed:Gibbs Shoal	-5.296975	2.877028	12	-1.841128	0.0904
Early small seed:Clam Shoal	-3.882938	2.867001	12	-1.354355	0.2006
Late small seed:Clam Shoal	3.33342	2.967718	12	1.123227	0.2833
Late large seed:Clam Shoal	-2.210245	2.8818	12	-0.766967	0.4579

Appendix F. The number of oysters on marl fit to mixed effect-generalized linear models. Models are listed from the simplest to the most complex for each model family. Best model (lowest AIC) is bolded.

Model	Family	df	AIC
# oysters=site, random=mound	poisson	4	624110
# oysters=treatment*site, random=mound	poisson	16	624108
# oysters=treatment+site+sampling, random=mound	poisson	8	11035.98
# oysters=site*depth*sampling, random=mound	poisson	19	208554
# oysters=treatment+site+sampling+treatment:site+treatment:sampling+site:sampling, random=mound	poisson	19	10555.72
# oysters=treatment, random=mound	negative binomial	7	6442.42
# oysters=treatment*site, random=mound	negative binomial	17	6403.38
# oysters=treatment+site+depth+sampling, random=mound	negative binomial	12	6322.78
# oysters=site*depth*sampling, random=mound	negative binomial	20	6032.46
# oysters=site+depth+sampling+site:depth+site:sampling+depth:sampling, random=mound	negative binomial	16	6032.68
# oysters=treatment+site+sampling+treatment:site+treatment:sampling+site:sampling, random=mound	negative binomial	31	6118.26

Appendix G. Summary of results for the number of oysters on marl fit to a negative-binomial mixed effect model. Factors included were site, depth, and sampling.

	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	7.0524	0.121	58.26	< 2e-16
Site-Gibbs Shoal	-0.0298	0.1526	-0.2	0.85
Site-Clam Shoal	1.7317	0.1581	10.95	< 2e-16
Depth-Bottom	0.8913	0.1331	6.7	2.10E-11
Sampling-spring 2011	-0.1558	0.1485	-1.05	0.29
Sampling-fall 2011	-0.8256	0.1455	-5.67	1.40E-08
Gibbs Shoal:Bottom	-0.2157	0.1451	-1.49	0.14
Clam Shoal:Bottom	-0.7308	0.148	-4.94	7.90E-07
Gibbs Shoal: Sampling-spring 2011	0.1904	0.1773	1.07	0.28
Clam Shoal: Sampling-spring 2011	-0.215	0.1815	-1.18	0.24
Gibbs Shoal: Sampling-fall 2011	1.6727	0.1751	9.55	< 2e-16
Clam Shoal: Sampling-fall 2011	-1.7427	0.1792	-9.72	< 2e-16
Bottom: Sampling-spring 2011	0.1173	0.1464	0.8	0.42
Bottom: Sampling-fall 2011	-0.6485	0.1441	-4.5	6.80E-06

Appendix H. Summary of results for the density of oysters on marl fit to a negative-binomial mixed effect model. Analyses were run with data from fall of 2011 for each site separately, with treatment (fixed) and mound (random) as independent factors.

Crab Hole				
	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	8.199	0.213	38.440	<0.001
Treatment-Early shell	0.351	0.302	1.160	0.250
Treatment-Early small seed	0.085	0.305	0.280	0.780
Treatment-Late small seed	0.209	0.302	0.690	0.490
Treatment-Late large seed	0.022	0.302	0.070	0.940
Gibbs Shoal				
	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	8.199	0.213	38.440	<0.001
Treatment-Early shell	0.351	0.302	1.160	0.250
Treatment-Early small seed	0.085	0.305	0.280	0.780
Treatment-Late small seed	0.209	0.302	0.690	0.490
Treatment-Late large seed	0.022	0.302	0.070	0.940
Clam Shoal				
	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	8.199	0.213	38.440	<0.001
Treatment-Early shell	0.351	0.302	1.160	0.250
Treatment-Early small seed	0.085	0.305	0.280	0.780
Treatment-Late small seed	0.209	0.302	0.690	0.490
Treatment-Late large seed	0.022	0.302	0.070	0.940

Appendix I. The number of oysters on marl from sites seeded and sampling by NCDMF fit to mixed effect-generalized linear models. Models are listed from the simplest to the most complex for each model family. Best model (lowest AIC) is bolded and NA indicated model would not run because of lack of replication.

Model	Family	df	AIC
# oysters=seeded, random=mound	poisson	3	125478.2
# oysters=seeded, random=mound	negative binomial	4	8924.94
# oysters=seeded*depth, random=mound	negative binomial	8	8926.42
# oysters=seeded*year created, random=mound	negative binomial	6	8919.94
# oysters=seeded*site, random=mound	negative binomial	8	8921.02
# oysters=seeded*mound age, random=mound	negative binomial	6	8831.04
# oysters=seeded+mound age+year created, random=mound	negative binomial	6	8825.18
# oysters=seeded*mound age*year created, random=mound	negative binomial	10	8813.02
# oysters=seeded*mound age*year created+seeded:mound age+seeded:year created+moundage:year created, random= mound	negative binomial	9	8811.16
# oysters=seeded*mound age*year created+seeded:mound age+ moundage:year created, random=mound	negative binomial	8	8809.5
# oysters=seeded*mound age*site, random=mound	negative binomial	14	8723.9
# oysters=seeded+mound age+site+year created, random=mound	negative binomial	8	8820.02
# oysters=seeded*mound age*site*year created, random=mound	negative binomial	NA	NA

Appendix I. Summary of results for the number of oysters on marl from sites seeded and sampling by NCDMF fit to a negative-binomial mixed effect model. Factors included were seeded, mound age, and site.

	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	5.19E+00	0.2443	21.24	2.00E-16
Seeded-yes	-1.03E+00	0.2891	-3.55	0.00038
Mound age	3.92E-01	0.0933	4.2	2.60E-05
Year created-2008	1.88E+00	0.3832	4.89	9.90E-07
Seeded-yes:Mound age	3.63E-01	0.1137	3.19	0.00143
Mound age:Year created-2008	-6.28E-01	0.1984	-3.16	0.00156

