OYSTER REEF ECOLOGY AND RESTORATION: FINDINGS FROM FIELD AND MESOCOSM STUDIES

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ABSTRACT

Nathan Geraldi: OYSTER REEF ECOLOGY AND RESTORATION: FINDINGS FROM FIELD AND MESOCOSM STUDIES

(Under the direction of Charles Peterson)

Habitat degradation and invasive species are two of the most rampant threats to marine ecosystems worldwide. The causes of degradation include coastal development and destructive harvesting practices. My thesis focuses on how to best invest resources to restore lost habitat and the unexpected consequences of shoreline hardening. I examined the efficacy of a costly and widespread restoration practice of adding juvenile oysters to reefs to enhance reef restoration. The results from my experimental field manipulations of juvenile oysters at three oyster sanctuaries throughout the Pamlico Sound indicate that the addition of oysters does not enhance reef development because natural recruitment of oysters is not limiting and efforts should focus on deploying substrate to restore oyster reefs. In addition to restoration, understanding biotic and abiotic interactions in oyster reefs is necessary to understand population dynamics. I ran multiple mesocosm experiments on food web interactions in oyster reefs and found that movement and size of mud crabs influence oyster survival. In addition, natural oyster reefs and shoreline hardening structures were surveyed for native and invasive macroalgae. *Codium fragile*, a nonnative species, was primarily found on artificial structures while the native *Codium decorticatum* was found almost exclusively on intertidal oyster reefs. *C. fragile* also had significantly higher nitrogen removal rates and although

shoreline hardening was habitat for nonnative species, this species could mitigate excess nutrient loading. Results from my research will increase the efficacy of habitat restoration and increase our understanding of interactions between habitat alteration and invasive species.

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INTRODUCTION

Anthropogenic impacts such as global warming and consequent sea-level rise, overfishing, habitat degradation, introduction of non-native species, and increased nutrient inputs have accelerated ecological change and are altering the structure and function of many coastal ecosystems (Vitousek et al. 1997, Lotze et al. 2006, Halpern et al. 2008). The ability to mitigate and even reverse ecosystem alteration depends on understanding how these impacts change ecological processes. In my dissertation I use both applied and basic ecology to understand the effects of anthropogenic changes on oyster reefs, with the goal of using this knowledge to help restore oyster reefs to historic abundances.

Oyster reefs are one of the most depleted and degraded marine habitats worldwide (Beck et al. 2011). To reverse the current trend of oyster reef declines, governmental and private organizations have invested substantial resources into oyster restoration. Specifically, 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 increase oyster survival. I experimentally manipulated

mounds at three sanctuaries which were seeded during summer 2010, and varied recycled shell and seed presence, seed size, and deployment date of shell and seed. Findings from this study can be used to maximize the resources invested into oyster restoration.

Overfishing on oyster reefs alters the abundance of target species, which are usually predators, and can change the structure of oyster reef ecosystems (O'Connor et al. 2008). Predators can affect prey populations and, via trophic cascades, indirectly impact resource populations (2 trophic levels below the predator) through consumption of prey (density-mediated indirect effects; DMIEs) and by inducing predator-avoidance behavior in prey (trait-mediated indirect effects; TMIEs). TMIEs are usually calculated based on a single predator-avoidance response, either dispersal or reduced foraging activity of the prey, even though prey may employ multiple predator-avoidance behaviors. My third chapter quantifies direct and indirect predator effects in a mesocosm experiment using an oyster reef ecosystem consisting of a predator (toadfish – *Opsanus tau*), prey (mud crab - *Panopeus herbstii*) and resource (ribbed mussel – *Geukensia demissa*). In this chapter, I manipulated the presence and absence of the predator and whether prey could or could not disperse into a predator-free area, and then calculated the relative importance of indirect effects based on multiple predator-avoidance responses used by the prey.

Overfishing not only affects species abundance but also the size structure of populations (Garcia et al. 2012). The size of an organism can change by orders of magnitude during its lifespan, which can alter whether the individual is a competitor, a prey, or a predator for other individuals of the same or different species. The effect of size on intra- and interspecific interactions likely alters trophic cascades through mechanisms such as predation, cannibalism, interference competition, and predator-

avoidance behavior. In the fourth chapter I measured the effect of prey and predator size on resource survival by setting up a mesocosm experiment using a tri-trophic food chain in oyster reefs (predator-toadfish; prey-mud crab; resource-oyster) to measure the effect of prey and predator size on oyster survival.

One of the biggest threats to ecosystems worldwide is invasive species (Mack et al. 2000), including oyster reefs. In addition, artificial substrates in areas naturally devoid of hard substrate might increase the invasive potential of exotic organisms (Tyrrell and Byers 2007). Although researchers have made strides in understanding and predicting the effects of anthropogenic impacts, less is known about the potential interactive effects between human-induced changes. In the fifth chapter I measure the abundance of native (*C. decorticatum*) and non-native (*C. fragile*) *Codium* species on natural hard substrates (oyster reefs) and artificial substrates (bulkheads and rock revetments) in North Carolina estuaries and assess the effect of each *Codium* species on local nitrogen cycling using continuous flow microcosms. Results from this chapter will increase our knowledge of how multiple anthropogenic impacts can alter ecosystem processes.

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TESTING THE EFFICACY OF A WIDESPREAD HABITAT RESTORATION PRACTICE: ADDITION OF JUVENILE OYSTERS FAILS TO ENHANCE OYSTER REEF DEVELOPMENT

Introduction

The extent of historic loss and degradation of biogenic marine habitats caused by humans is staggering (Lotze et al. 2006). Although remaining threatened habitats may be maintained through conservation efforts, habitat restoration will be needed to regain lost habitat and associated ecosystem services. Restoration ecology may become one of the most important scientific endeavors of this century and is an essential component of conservation and management (Hobbs et al. 2011). To maximize the benefits of how limited resources for restoration are utilized, restoration techniques must be based upon rigorous ecological experimentation (Blankenship and Leber 1995).

Eastern oyster, *Crassostrea virginica*, populations have been a target of decades of restoration efforts (Brumbaugh and Coen 2009, Kennedy et al. 2011) because only 15% of historic oyster populations persist worldwide (Beck et al. 2011). Unlike other marine biogenic habitats such as seagrass meadows or coral reefs, oysters are harvested for human consumption thus increasing risk of degradation and decline. In addition to supporting a bivalve fishery, oyster reefs also provide other ecosystem services including enhanced production of fishes, crabs, and shrimps (Lenihan et al. 2001, Peterson et al. 2003) and improved water quality through filtration and denitrification (Grabowski and

Peterson 2007, Coen et al. 2007, Piehler and Smyth 2011). The economic value of ecosystem services provided by non-harvested reefs is greater than the value of oysters obtained from harvesting the reefs (Peterson et al. 2003, Grabowski and Peterson 2007, Coen et al. 2007).

To restore the services provided by lost oyster reefs, governmental and private organizations have invested substantial resources into oyster restoration, but the knowledge necessary to maximize the effectiveness of money spent on restoration is limited. Restoration efforts include providing hard substrate for oyster to grow on such as oyster shell, clam shell, or marl rip-rap (Lenihan 1999, Coen and Luckenbach 2000, Mann and Powell 2007). Where oyster recruitment, the number of oysters that settle and survive to a size that can be visually sampled (approximately 5mm shell height), is thought to limit oyster reef creation, the new reefs maybe seeded with juvenile oysters. Seeding consists of spawning oysters in captivity, allowing the larvae to settle on recycled shell, raising the juvenile oysters in captivity to a size at which it is thought mortality from predation is reduced and then deploying the seeded shells on natural substrate or onto constructed reefs. This practice occurs over the entire range of the eastern oyster (see review by Brumbaugh and Coen 2009) including New Hampshire (Grizzle et al. 2006), Virginia (Associated Press 2007), Maryland (Rodney and Paynter 2006), Alabama (Wallace et al. 2002), Louisiana (Supan et al. 1999), and North Carolina. North Carolina has devoted public resources to building multiple oyster hatcheries ($4.3 million for North Carolina in 2008) and deploying seeded shells on harvested areas (Ortega and Sutherland 1992) and oyster sanctuaries. Remarkably, even though oyster seeding is a costly and widespread restoration practice, no published information exists

supporting the notion that seeding is necessary to generate or enhance an adult oyster population.

Seeding newly deployed reefs might be necessary if oyster recruitment is limiting. Recruitment of oysters may be limiting because adult oysters could be locally extinct or so depleted that larval supply is low and settlement onto hard substrate does not occur in densities that result in sufficient recruitment to develop an oyster reef. If settlement is limiting reef restoration, it may be necessary to restore a network of reefs to ensure sufficient larval supply to maintain oyster populations because oysters have a 2-3 week pelagic larval stage (Kennedy et al. 1996) and usually do not recruit to the same reef on which they were spawned. However, it is difficult to determine whether settlement is limiting recruitment because settlement varies in space and time (Michener and Kenny 1991, Ortega and Sutherland 1992, Austin et al. 1996). An area that is settlement-limited one year may not be the next. Devoting resources to deploy seed oysters to create functional oyster reefs may or may not be necessary for oyster reef restoration depending on oyster settlement and subsequent recruitment in space and time.

In addition to bottom-up factors limiting recruitment, post-settlement mortality can also make seeding necessary to restore oyster reefs. High oyster mortality from predation could result in few oysters reaching maturity (Wallace et al. 2002, Kraeuter et al. 2003). Shellfish seed survival varies strongly with seed size and date of deployment (Leber et al. 2005, Peterson et al. 2005) and predation risk and oyster size are negatively correlated (Newell et al. 2000, Kulp et al. 2011). Predation can vary in time as predators migrate with season (Brown et al. 2008), recruit to oyster reefs and grow to sizes that consume oysters (McDonald 1982), or are indirectly affected by higher trophic levels

(Abbe and Breitburg 1992, Grabowski 2004). Oyster mortality also varies spatially because of physiological constraints of oyster predators (Wallace et al. 2002). Knowledge of how seed survival is affected by size at deployment, date of deployment, location of deployment, and the interaction of these factors is necessary to maximize resources invested in seed production (Peterson et al. 1995).

In this study we tested the effect of adding seeded recycled shell onto marl mounds within three different oyster sanctuaries in Pamlico Sound, NC. Specifically, recycled oyster shell and recycled oyster shell with small seed oysters attached were deployed in early summer, and shells with two sizes of seed oysters (small and large) were deployed in mid-summer. Deployed shell and mound surface (marl) were monitored to determine the effect of seeding on oyster abundance and reef development (abundance and size of oysters on deployed shell, and oyster density on the surface of the marl boulders of different treatments over time). To expand our empirical findings in both space and time, we analyzed survey data from the North Carolina Division of Marine Fisheries (NCDMF) from three additional sanctuaries seeded in two different years. The goal of this study was to guide future restoration efforts so that the limited resources devoted to oyster reef restoration can be maximized by determining if seed oysters are necessary for oyster restoration and if so, when and at what size the seed oysters should be deployed to minimize seed mortality.

Methods

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. 2.1). 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-cm^2 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 2.2).

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).

Results

Salinity was recorded for an average of 194 days at each sanctuary where we conducted our experiments (Fig. 2.2). All three sites only had 60 days of contemporaneous data. Crab Hole, Gibbs Shoal and Clam Shoal, experienced salinities

(mean ±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±SE), followed by early small seed (4.4±0.6) and late small seed (2.9±0.2) before deployment (Fig. 2.3A). 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. 2.4A).

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 Appendix 2.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. 3C. 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. 2.3, Appendix 2.B). Posthoc comparisons were based on variables standard errors from the model not overlapping . 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 2.B) and additional models

were run for each sanctuary separately with data from the fall 2011 sampling. In the fall of 2012 the shell/seed treatment was not significant ($p>0.012$) when analyses were run for each site separately (Fig. 2.3C, Appendix 2.C).

The size-frequency of oysters on deployed shell was analyzed using a parametric model with shell/seed treatment and site as factors (Appendix 2.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. 2.4C, Appendix 2.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 (Appendix 2.D).

The density of oysters on marl was best described by a negative binomial model with site, depth, and sampling date as factors (Appendix 2.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. 2.5, Appendix 2.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. 2.5); 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. 2.5). 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. 2.5, Appendix 2.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. 2.5C, Appendix 2.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 2.I). Seeded mounds had a lower density of oysters than unseeded mounts (Appendix 2.J, Fig. 2.6). Mounds created in 2008 had a higher density of oysters than mounds created in 2006 and the density of oysters increased with mound age.

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) exist throughout Pamlico Sound (Chesnut 1955, Rindone and Eggleston 2011, NRG unpub.), but recruitment seemed to exceed the effect of predators because the density of oysters remained above $400m^{-2}$ regardless of shell/seed treatment. 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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RESTRICTING PREY DISPERSAL OVERESTIMATES THE IMPORTANCE OF PREDATION IN CAUSING INDIRECT INTERACTIONS

Introduction

The importance of predation in community dynamics is exemplified by trophic cascades (Carpenter and Kitchell 1988, Estes et al. 1998, Pace et al. 1999, Myers et al. 2007). Studies on trophic cascades have usually focused on the indirect effect of predators that are generated by consumption of prey, but there is a growing number of studies that have found that behavioral responses of prey to avoid predators are also important to understanding both direct and indirect effects of predators in food webs (Schmitz et al. 2004, Preisser et al. 2005, Okuyama and Bolker 2007). For example, studies of tri-trophic food chains (predator-prey-resource) that include assessment of behavioral effects have shown that resource persistence is influenced as much by prey predator-avoidance behaviors (trait-mediated indirect effects; TMIEs) as by predators consuming prey (density-mediated indirect effects; DMIEs) (Werner and Peacor 2003, Preisser et al. 2005, Trussell et al. 2006). For instance, in grassy meadows, spiders feeding on grasshoppers had a similar positive effect on grasses as when non-feeding spiders were present (Schmitz et al. 1997). Hence, the inclusion of prey behavior in food web models is an important step towards developing a holistic understanding of ecological processes (Ings et al. 2009, Beckerman et al. 2010).

TMIEs often result from more than one predator-avoidance behavior in nature (Wirsing and Ripple 2011). For instance, elk, under the risk of predation by wolves, increase vigilance time and decrease foraging (Laundré et al. 2001, Childress and Lung 2003), which results in increasing willow heights (Ripple and Beschta 2006, Beschta and Ripple 2007). In addition, elk can also alter habitat selection in the presence of wolves and move away from their preferred resource in open grasslands to safer coniferous forests with lower-quality resources (Creel et al. 2005). Even though animals use multiple behaviors in response to predators, few studies consider more than one predator-avoidance behavior, especially when determining the relative importance between TMIEs and DMIEs (Abrams 2007, Wirsing and Ripple 2011).

Quantifying the relative importance of TMIEs and DMIEs is necessary in order to include behavior in food chain models that have until recently included only the effects of consumption. In a recent meta-analysis on the relative importance of prey behavior and predation in indirect interactions, all 20 examples of studies that measured TMIEs and DMIEs were conducted in mesocosms (Preisser et al. 2005). Researchers rely on mesocosm experiments to determine TMIEs and DMIEs because prey mortality and the consumption of resources by prey must be measured (Schmitz 2005, Okuyama and Bolker 2007). Generally mesocosm designs limit how prey can respond to predators. Three different quantified predator avoidance behaviors of prey, in addition to prey mortality, have been studied; reduced activity, changes in habitat, and immigration, but only one predator avoidance behavior has been measured at a time (Preisser et al. 2005; Appendix A). This practice of measuring one predator-avoidance behavior at a time may lead to overestimating the importance of the measured behavior in indirect effects when

compared to prey behavior natural settings.

One of the most frequently studied predator avoidance behaviors in indirect effect studies is reduced prey activity, but studies on prey activity that are conducted in mesocosms usually restrict the ability of prey to disperse to locations where the threat of a predator is diminished (Schmitz et al. 1997, 2008, Grabowski 2004, Grabowski and Kimbro 2005). For instance, the studies that measured the effect of reduced prey activity on indirect effects in Preisser et al. (2005) had mesocosm boundaries that limited dispersing prey to distances that we estimate prey could move in less than a minute (Appendix A). Restricting prey to an area that is small compared to the area that they use in nature (home range) inhibits the prey's ability to disperse from predators. The ability of prey to move away from the threat of predation can depend on the density of predators and the distance at which prey are able to detect a predator. Even if mesocosm size does not alter the ability of prey to disperse, mesocosms boundaries can alter detection and capture of prey (Englund 1997). Furthermore, minor changes in predator-prey interactions can have major impacts on resources (Preisser et al. 2005). Thus, mesocosm experiments measuring the effects of predators on prey activity could overestimate DMIEs because prey are unable to disperse from the threat of predation and mesocosm boundaries increase predator capture success.

The indirect effect of prey dispersal on the resource has been tested in enclosure studies in streams (Cooper et al. 1990, Sih and Wooster 1994, Forrester 1994, McIntosh et al. 1999) and grasslands (Cronin et al. 2004; see review by Preisser et al. 2005). But, these studies did not assess how resource survival was affected by predator consumption of prey and reductions in prey foraging because of difficulties in determining the number

of prey eaten versus the number of prey that dispersed (Preisser and Bolnick 2008, Orrock et al. 2008). One mesocosm study did estimate the effect of prey dispersal and reduced activity on algae in streams but did not measure the number of prey eaten by predators and the relative importance of TMIE and DMIE was not calculated (Diehl et al. 2000).

Our study system consisted of a tri-trophic food chain with toadfish (*Opsanus tau*; predator), mud crabs (*Panopeus herbstii*; prey), and ribbed mussels (*Geukensia demissa*; resource). Past experiments have been conducted with these same species in 1.7 m diameter mesocosms and found that the relative importance of TMIEs, resulting from reduced prey activity in the presence of a predator, was much greater than DMIEs (Grabowski 2004, Grabowski and Kimbro 2005). However, a study on the mobility of the same species of mud crab found that marked crabs released in the wild were not found within 5m of the release point after 48h (Stachowicz and Hay 1999). Consequently, we designed a relatively large-scale experiment that manipulated the presence and absence of a predator within mesocosms that either prevented or allowed prey, but not a predator, to disperse out of the mesocosm. The importance of reduced activity of prey, prey dispersal, and predation of prey were each quantified to assess the indirect effects of the predator on the resource.

Methods

Calculating indirect effects

Assessments of the importance of prey behavior in ecological processes must isolate behavioral effects from consumptive effects (Peacor and Werner 2001, Schmitz et

al. 2004, Okuyama and Bolker 2007). Our experiment accomplished this by parsing different indirect effects via counting the numbers of the resource species eaten per day by prey in the absence (M) and presence (m) of a predator, the daily per-prey consumption of the resource in absence (C) and presence (c) of a predator, and the number of prey eaten by a predator (p; Table 1). Following the labeling convention used by Okuyama and Bolker (2007), capital letters indicate absence of a predator and lower case letters indicate presence of a predator. The DMIEs were the amount of resources surviving because of prey mortality (c·p). The actual release (AR) was the difference between resources consumed by prey in the absence and presence of a predator (M-m). The activity resource release (AyR), or the amount of resources that were not eaten because prey reduce activity and foraging in the presence of a predator, was the difference between the AR and the DMIEs (Grabowski 2004), when dispersal was prevented. Thus, if the change in the numbers of resources and prey are known, indirect effects can be estimated (Table 1). We took this construct one step further by calculating the dispersal resource release (DR), the positive effect of a predator on resource survival resulting from prey dispersal, by multiplying the per-prey consumption of resources by the number of prey that dispersed and then subtracting the number of resources not eaten because of dispersal in the presence (c·e) and absence (C·E) of a predator. The increase in resource survival resulting from reduced prey activity was then calculated in mesocosms that allowed prey dispersal (AyR=AR-DR-DMIE; Table 3.1).

Our mesocosm design was based on a combination of past research on prey dispersal, which has primarily been measured in stream mesocosms (referred to as emigration in those studies), and research on changes in prey activity, which has

primarily been conducted in closed mesocosms mimicking marine or terrestrial environments. Crab movement out of the mesocosm was considered dispersal and not refuge-seeking behavior because we refer to dispersal as the movement out of a hostile environment while refuge seeking behavior is hiding within a hostile environment. Refuge seeking is a reduction in crab activity when crabs hide deeper within the oyster shell to escape predation (Grabowski and Kimbro 2005).

Experimental Setup

Experiments were conducted in 21 m^2 outdoor cement ponds (7 x 3m) at the University of North Carolina's Institute of Marine Sciences (Morehead City, NC, USA). Animals were collected by hand or trap in Bogue Sound and held in flow-through tanks supplied with raw seawater (1 Ls^{-1}). Toadfish were fed chunks of frozen fish and crabs were fed mussels (> 1 cm shell height) *ad libitum* every 2 days before experiments started.

The experimental design consisted of 2 crossed factors: predator (present or absent) and mesocosm design (open - prey could leave the mesocosm or closed - prey could not leave mesocosm). The 2 mesocosm designs were created by dividing each cement pond in half with one of 2 alternate sizes of Vexar mesh, one of which allowed crab dispersal (open – 5 cm mesh) while the other did not (closed – 1 cm mesh). Depending on the mesh size crabs could move out of the mesocosm (3.5 x 3m) into a predator-free sanctuary (other side of the cement pond; Fig. 3.1). The sanctuary in the closed treatment was used as a control to measure mussel mortality not attributable to crab consumption.

Oyster habitat was created by adding cleaned adult oyster shells (37.9 L bucket full of shells) to each mesocosm and sanctuary and spreading it out to cover a 0.56 m^2 area. The oyster shells were approximately 15 cm deep. The oyster habitat was placed 0.5 m from the mesh barrier so that oyster habitat in open mesocosms was 1m apart from oyster habitat in sanctuaries (Fig. 3.1). The size of the oyster habitat and the distance between patches are commonly found in natural oyster reefs (Eggleston et al. 1998, Macreadie et al. 2011; Appendix 3.A). Eight oyster shells each had 7 mussels (resource) attached to them and were haphazardly placed within the oyster habitat. Mussels are commonly found in interstitial spaces in oyster reefs and are eaten by mud crabs (Toscano and Griffen 2012). Mussel shell height ranged from 8 to 24 mm (17 ± 0.04, mean ± standard error, n=60). Mussels were placed on the shell 24 hours before the beginning of the trial and they naturally attached to the oyster shell via their byssal threads.

Five mud crabs (range=10 - 39 mm *carapace width*, 26 ± *0.4 mm, n=119*) were placed in the oyster habitat in each mesocosm (Fig.3.1). Crab density within the oyster habitat in mesocosms (8.9 crabs m^{-2} of oyster habitat) was selected from the lower end of the natural range of crab density for individuals with 20 - 40 mm *carapace widths* within intertidal oyster reefs in South Carolina (4 - 20 m^{-2}, McDonald 1982) to reduce density-dependent movement and interference competition. After crabs had acclimated for 30 min in the mesocosm, a single adult toadfish (range=230 - 320 mm total length, 278 ± *1.0* mm, n=8) was added to the predator-present treatments. Each trial of the experiment consisted of a single replicate of each of the 4 treatments (predator-present or absent crossed with mesocosm-open or closed). Replication was gained through successive trials

(n = 6) and treatment was haphazardly assigned to mesocosms before each trial. Trials were conducted from July to Sept in 2009. Trial time was based on keeping resources above 50% to minimize crab dispersal resulting from resource depletion and to minimize a decrease in prey feeding rate because of resource depletion (Murdoch 1969), which was measured in pilot trials and took 2 - 3 days. Mesocosms were completely drained of seawater at the end of each trail, which took approximately 15 min, to allow crabs and mussels to be accurately counted.

Observations of crab location were conducted 3 – 4 times during each trial. The observations were conducted from 8 am – 8 pm. Each mesocosm and sanctuary was searched for 1 min and the location of each visible crab was recorded. The locations of crabs were grouped into 4 categories: within oyster habitat, closer than 5 cm to mesocosm walls, in mesocosm corners, or in the open (between oyster reef and mesocosm boundaries).

Experimental design justification and caveats

The experimental design allowed us to quantify the number of prey that dispersed out of the mesocosm in the absence and presence of a predator. Although this design may also have experimental artifacts, we tried to mimic natural conditions by allowing mud crabs to disperse while still measuring changes in prey and resource abundance resulting from consumption and dispersal. The mesh barrier restricted movement of predators to remain within the mesocosm, but the barrier probably did not affect toadfish predation on mud crabs because a toadfish's ambush attack is characterized by a quick and sudden strike and toadfish are sedentary ambush predators that occupy dens (tin cans or piles of oyster shell) for 3 - 5 weeks (Gray and Winn 1961). Hence, the potential artifacts

introduced by mesh barriers are probably minimal in toadfish-mud crab interactions in this experiment. A treatment in which toadfish could move into the sanctuary was not included in this study because it would have a different shape and twice the area as the mesocosms we used and changes in prey and resource consumption could not be directly attributed to the presence of the mesh barrier because of concurrent changes in mesocosm area and predator density.

Past studies calculate TMIEs (Griffin and Thaler 2006, Trussell et al. 2006, Okuyama and Bolker 2007) by using a "risk" or "cue" treatment. Risk treatments usually consisted of a predator that is caged within mesocosms or water flowing through a tank containing a predator before flowing into the study mesocosm. We did not include a risk treatment because risk treatments can underestimate predator-avoidance behaviors because prey never have an opportunity to escape the predator (Abrams 2007) and the reduction in prey foraging resulting from predator presence is calculated from per prey consumption of resources when the predator can consume the prey. However, the risk treatment does keep prey density constant and removes any artifact resulting from crabs altering their feeding rates with changes in crab density. We acknowledge that this could bias our results if crabs increased feeding when crab density decreased from either predation or dispersal, but the experiment was designed to minimize changes in density-dependent crab feeding rates and intraspecific interactions by using crab densities from the lower end of natural densities and with resources that were distributed throughout the oyster habitat.

Statistical Analysis

Response variables were analyzed in factorial ANOVAs with mesocosm design (open or closed) and predator (present or absent) as fixed factors and trial (1-6) as a blocked factor. Dependent variables were: % crabs consumed (crabs eaten /{[initial # of crabs-final #of crabs]/2}), % crabs remaining in the mesocosm (final # of crabs/{initial # of crabs- # crabs eaten}) and % mussels consumed in the mesocosm. ANOVAs were also run for results in the sanctuary (open mesocosms only) with predator in the mesocosm (present or absent) as a fixed factor and trial (1-6) as a blocked factor. Dependent variables for ANOVA's run with results from sanctuaries were number of crabs in the sanctuary at the end of the trial and % mussels consumed in the sanctuary. All data were first tested for normality and homoscedasticity by the K-S normality test and the Levene's test, respectively, and passed both tests without transformation unless stated otherwise.

Crab behavior was analyzed using a 2-way MANOVA with predator mesocosm design and trial as independent factors. The numbers of crabs observed per trial along the sides, in corners, and in oyster habitat were the dependent variables. The number of times a crab was observed in each trail was divided by the average number of crabs present and the number of observations conducted during that trail to account for differences in the number of crabs and observations among trials. Only 1 crab was observed in a sanctuary and only observations in mesocosms were used in the observation analysis. A crab was never observed in the open so this category was not used in the analysis. To elucidate which observation category was driving the significant MANOVA results, separate three-way ANOVAs, with predator (fixed), mesocosm design (fixed), and trial (blocked) as independent factors, were run with each location category as the dependent variable.

Indirect Effect Calculations

To determine the effect of mesocosm design, prey activity within the mesocosm, and prey dispersal on the relative strength of DMIEs and TMIEs, we used calculations similar to Grabowski (2004). Variables and equations are shown in Table 1; lower case variables indicate predator presence and upper case variables indicate predator absence (Okuyama and Bolker 2007). The mean number of crabs eaten by a predator during a trail (p) was calculated for closed and open mesocosms. We determined the mean number of crabs that dispersed out of open mesocosms during a trial with (d) and without predators (D), as well as the per-prey rate of resource consumption for open and closed mesocosms with (c) and without (C) a predator. All calculations were carried out independently for open and closed mesocosms. The rate of resource consumption per prey was calculated by dividing the number of resources consumed per day by the average number of crabs present during the trail. The average number of crabs was calculated by dividing the initial plus the final number of crabs by 2

DMIEs, or the number of mussels surviving because of predation of mud crabs, was calculated for predator treatments ($p \cdot c$). Actual resource release (AR), or the number of mussels not eaten because of the presence of a predator, was calculated by subtracting the mussel consumption without and with a predator (M-m). Dispersal resource release (DR), or the number of mussels not eaten because of crab dispersal out of the mesocosm and away from the predator, was calculated by subtracting the number of mussels not consumed because of crab dispersal without a predator present ($C \cdot D$) from the number of mussels not consumed because of crab dispersal in the presence of a predator ($c \cdot d$) in the mesocosm. Dispersal resource release was only calculated for open mesocosms. The

activity resource release (AyR), or the number of mussels not eaten because of mud crabs reducing activity in the presence of a predator, was calculated for closed mesocosms (AyR=AR-DMIE). The calculation for AyR in open mesocosms included the number of resources not eaten because of crab dispersal (AyR=AR+DR –DMIE, Table 1). TMIEs or the total indirect effects resulting from predator-avoidance behaviors were calculated for closed (AyR) and open mesocosms (AyR+DR). Finally, the relative magnitude of TMIEs compared to the total indirect effect of the predator on the resource was calculated for open and closed mesocosms by dividing TMIEs by the sum of indirect effects (DMIE+TMIE; Table 1).

The contribution of the DMIE can be calculated by subtracting the TMIEs from 1. Standard errors were not calculated for the indirect effect percentages because one trial in both AyR and DMIEs calculations had a negative number, which resulted from more mussels being consumed in the presence of a predator for those trials. The negative number greatly skewed the calculations by reducing the mean even when transformations were conducted. Thus, the means of the resource release were used and error was not calculated.

Results

The proportion of crabs consumed by toadfish was eight times higher in closed (0.35 ± 0.11 crabs per trial; mean ± standard error; n=6 for all analyses) than in open mesocosms (0.041 ± 0.041 crabs per trial; $F_{1, 11} = 6.64$, $p = 0.030$; Fig. 3.2A; Appendix 3.C). Predator presence did not affect the proportion of crabs remaining in mesocosms (final # of crabs/(initial # of crabs- # crabs eaten); $F_{1,15} = 0.03$, $p = 0.857$; Fig. 3.2B;

Appendix 3.D), but mesocosm design did affect the proportion of crabs remaining in the mesocosm with more crabs remaining in the closed mesocosms, although only marginally significant ($F_{1,15} = 3.66$, $p = 0.075$; Fig. 3.2B; Appendix 3.D). Thus, predator presence did not affect crab dispersal, but crabs did disperse when in open mesocosms. The closed mesocosms did not have all of the crabs remaining in the mesocosm because 3 crabs in no predator trials and 2 crabs in the predator trials managed to get under the small mesh barrier and moved into the control. This should not have affected our results because so few crabs escaped from closed mesocosms. Toadfish presence did not affect the number of mud crabs that were in the sanctuary at the end of the trial ($F_{1,5} = 0.19$, $p = 0.679$; Fig. 3.2C; Appendix 3.E).

All mussel mortality was assumed to be from mud crab consumption because mussel mortality in the control sanctuary was negligible (0.6 ± 0.25 mussels per trial) and toadfish did not eat mussels (N. Geraldi pers. obs.). Toadfish presence reduced mussel mortality by half ($F_{1,15} = 11.38$, $p = 0.004$), but there was no difference in mussel mortality between open and closed mesocosms ($F_{1,15} = 0.50$, $p = 0.490$; Fig. 3.3A; Appendix 3.F). Percent mortality of mussels in the sanctuary was reduced from 17 to 5% when the mesocosm had a predator ($F_{1,5} = 4.32$, $p = 0.092$; Fig. 3.3B; Appendix 3.G). Neither the trial factor nor the interaction term had an effect ($p > 0.05$) in any of these statistical tests.

Although the ability to observe crabs was limited by variable water turbidity; observations of all treatments during trials were conducted 21 times (3-4 observations during each trial) and 33 crabs were observed during the entire experiment. The majority of crabs was observed along the edges of the mesocosms (20), and these crabs were

moving in 75% of the observations. A total of 10 crabs was observed in the corners, and these corner crabs were inactive in 90% of the observations. Three crabs were observed in the oyster habitat. There was a significant interaction between predator and mesocosm type ($F_{1,20} = 4.059$, $p = 0.023$), and predator was marginally significant ($F_{1,20} = 2.445$, $p = 0.097$) when observations of crabs in corners, in oyster reef, and along edges were analyzed using a MANOVA (Appendix 3.H). The proportion of crabs was not normally distributed among the three dependent variables and crabs along edges did not have homogeneous variances. When transformation did not improve normality or heteroscedasticity, the variables were left untransformed. Non-parametric tests were not run because they cannot analyze mixed-effect models. Although variance tests are robust to non-normal data (Underwood 1997), caution should be taken in interpreting the ANOVA for proportion of crabs along edges because this dependent variable did not have homogeneity of variance. Neither predator nor the type of mesocosm had a significant effect on the proportion of crabs observed in corners ($p > 0.40$; Appendix 3.I). The proportion of crabs observed along edges was significantly affected by predator ($F_{1,15} = 6.37$, $p = 0.023$; Fig. 3.2D; Appendix 3.J) and mesocosm type ($F_{1,15} = 5.54$, $p = 0.033$). The interaction between these 2 factors was also significant ($F_{1,15} = 5.95$, $p = 0.028$). Neither predator nor the type of mesocosm had a significant effect alone on the proportion of crabs observed in the oyster habitat ($p > 0.40$; Appendix 3.K), but the interactions between these two independent variables was marginally significant ($F_{1,15} = 3.24$, $p = 0.088$).

The contributions of predator-avoidance behaviors and consumptions of prey on resource survival are summarized in Table 1. The presence of toadfish reduced the

number of mussels eaten per crab per day by half. On average there was a small effect of mesocosm design and open mesocosms had 8% higher levels of per-prey consumption. The DMIE was almost 3-times greater in the closed (1.31 ± 0.80) than in open mesocosms (0.46 ± 0.46). This resulted from the significantly higher predation on mud crabs in closed mesocosms. The average number of crabs that dispersed out of an open mesocosm was the same for no-predator (1.33± 0.21) and predator treatments (1.33 ± 0.42). Although prey (crab) density remained unchanged in closed no-predator treatments, reduction in prey density resulting from predation and/or dispersal was similar between closed predator, open no-predator, and open predator treatments (1.33±0.42, 1.33±0.21, and 1.50±0.59 crabs respectively. The DR, or the number of mussels not eaten because of changes in crab dispersal induced by predator presence, was -2.21± 0.70. This negative number indicates that the DR (number of mussels "saved") was lower in the presence of a predator than in the absence of a predator, an outcome that resulted from higher consumption of resources per prey in no-predator treatments. The activity resource release (AyR) was lower in the closed (4.19 ± 2.06) than open (7.58 ±3.46) mesocosms. Finally, the activity (AyR) and dispersal (DR) resource release were combined for open mesocosms to calculate the number of resources not eaten resulting from both of these prey behaviors (5.37± 3.13).

The contribution of TMIEs as compared to DMIEs was calculated for closed and open mesocosms. The TMIEs from activity reduction in the closed mesocosm accounted for 76.2% of the indirect effect. The TMIEs in the open mesocosm, or the increase in resource survival resulting from changes in prey foraging activity and dispersal, accounted for 92.1% of the effect of the predator on the resource. The difference in

indirect effects between the treatments was primarily driven by the significantly higher predation on mud crabs in closed mesocosms.

Discussion

We found that the magnitude of the TMIEs compared to DMIEs was dependent on how many predator avoidance behaviors were measured. Our results add to the growing body of evidence that fear of predation can have a greater influence on food chain dynamics than predation. The evidence includes experiments in grass meadows (Schmitz et al. 1997, Schmitz and Suttle 2001, Schmitz 2008), freshwater streams (Huang and Sih 1991, Peckarsky 1996, Peacor and Werner 1997, McIntosh et al. 1999), and intertidal pools (Trussell et al. 2002, 2006). But, unlike these past studies we measured multiple predator avoidance behaviors. When prey were unable to disperse (closed mesocosms), TMIEs on mussel survival were 3 times higher than the DMIEs. When crabs were allowed to disperse, the TMIEs on mussel survival increased to 11 times the DMIEs. This increase in TMIEs resulted from rates of mud crab consumption by toadfish (the sole source of DMIEs) that were 9 times higher in closed mesocosms than in mesocosms where crabs could disperse. Crabs were observed moving along mesocosm edges more often in closed mesocosms than in open mesocosms, probably because they were trying to disperse. This left prey more vulnerable to predation and increased prey mortality and estimation of DMIEs. Open mesocosms had only 1 of 4 sides permeable to crabs, and yet prey consumption by a predator was significantly reduced as compared to closed mesocosms. Predation resource release (DMIEs) could be even lower in natural settings because no mesocosm boundaries exist, but this is dependent on predator density

because prey could inadvertently move into an area with predators. Mesocosm experiments on indirect effects could be overestimating DMIE because of mesocosm artifacts, especially when mesocosm size restricts the distance prey can move in relatively short time periods (< 1 minute), a limitation that is common in previous indirect effect experiments (Appendix 3.A). However, the magnitude of the potential bias resulting from mesocosms is context dependent and is probably affected by the predator-avoidance behaviors of the prey, the forage area of the prey and predator (home range), and whether the predator actively searches for prey or ambushes prey.

Prey can reduce predator encounters by dispersing away from the predator (Cooper et al. 1990, Fraser and Gilliam 1992, Forrester 1994, McIntosh et al. 2002, Creel et al. 2005). We found that the percent of mud crabs remaining in the mesocosm was not affected by toadfish presence, which is supported by a smaller body of literature that shows no effect of predators on prey dispersal (Sih and Wooster 1994, Orrock et al. 2008, Winkelmann et al. 2008). While the number of dispersing crabs did not change, crabs that remained in a predator mesocosm ate fewer mussels than crabs that remained in the predator-free mesocosms, and as a result the number of mussels surviving in a mesocosm because crabs dispersed was less when a predator was present than when a predator was absent. This resulted in a negative dispersal resource release because the predator had a negative effect on resource survival. The effect of dispersal on resource survival was four times greater in magnitude than the effect of prey mortality. Our results bring up interesting scenarios in which the cascading effect of dispersal is not intuitive, such as when a predator does not alter prey dispersal, but does decrease TMIEs. This could occur when the per-prey consumption of resources is lower in the presence of a predator. Or, a

dispersal resource release could be negligible even though predators increased dispersal, because per-prey consumption decreased in the presence of a predator.

Our experiment allowed us to estimate the importance of multiple predator-avoidance behaviors in trophic interactions. We found that prey foraging and dispersing each had a greater effect than prey mortality on resource survival. Past experiments usually determined indirect effects by measuring prey activity in closed mesocosms or prey dispersal in open mesocosms, but to our knowledge no study has directly measured both. Excluding dispersal as an antipredator behavior underestimated the activity resource release (AyR). The opposite could happen as well; excluding dispersal could overestimate the AyR if predator presence increased dispersal and increased resource survival. Calculations of indirect effects are based on the difference between resource consumption in the absence and presence of the predator. Thus, the magnitude of different indirect effects is dependent upon each other. Measuring only one predator-avoidance behavior, when prey utilize more than one, biases conclusions and thereby misrepresents indirect effects.

Unlike prey that reduce activity in the presence of a predator, prey that disperse probably affect resources in the area where the prey disperse to. This is known as 'remote effects' of predators (Orrock et al. 2008) and is seldom quantified. We found that a predator has a disproportionately larger effect on resource survival in sanctuaries, where resource consumption was 5 times greater when there was no predator, as opposed to when there was a predator in the mesocosm. This was probably a consequence of both chronic predator effects (Bolnick and Preisser 2005), in which prey that were recently under threat of predation remain vigilant, and a consequence of prey continuing to detect

the predator in the mesocosm (e.g. chemical and/or visual cues). Remote predator effects are not only dependent on whether prey alter dispersal rates in the presence of a predator, but also the distance from a predator in which the prey resume foraging without 'fear'. Although such effects are dependent on the spatial scale, incorporating the effect of dispersing prey on the resource outside of the study area is important in understanding the overall effect of predator-avoidance behavior on resource populations.

The indirect effects of prey mortality and reduced prey activity were previously investigated in a tri-trophic food chain with toadfish, mud crabs, and juvenile oysters (Grabowski 2004, Grabowski and Kimbro 2005). Grabowski (2004) found a TMIE that was larger than what we found in the comparable, closed mesocosm (TMIE was ≥94% compared to our finding of 76.2%). Several factors may explain these differences. First, the effect of a predator on avoidance behavior is dependent on prey density (all prey are inhibited regardless of their density; Okuyama and Bolker 2007, Belovsky et al. 2011) and indirect effect calculations are based on the change in resource consumption by all the prey. Thus, indirect effects can change depending on the prey density, and if feasible, it is best to measure indirect effects as a function of prey density (Abrams 2007). The prey to predator ratio that Grabowski (2004) used was double ours, which probably resulted in a larger TMIE. But, consumption of mud crabs per toadfish in Grabowski's study was similar to ours in closed mesocosms (0.5 vs. 0.6 crabs·day^{-1}) but not in open mesocosms (0.06 crabs·day^{-1}), which suggests that mesocosms used in Grabowski's study could have overestimated the relative importance of DMIEs compared to more natural conditions. Finally, our experiment had a patch of oyster habitat surrounded by open substrate, whereas Grabowski (2004) had oyster reef covering the entire mesocosm.

Many habitats, including oyster reefs, exist in a continuum of patch sizes. Prey dispersing from habitat patches are often more vulnerable to predation (e.g. Micheli and Peterson 1999). Although patch size and configuration of habitat has not been included in indirect effect experiments, it too may alter indirect effects (Macreadie et al. in review). The effects of prey to predator ratio and habitat layout probable contributed to the differences between this study and that of Grabowski (2004). However, our open mesocosm may be closer to natural conditions because prey could move distances closer to distances they move in nature.

While the limited spatial and temporal scales of indirect effect experiments are cited as reasons why the results may not be scalable to natural food webs (Schmitz 2007, Abrams 2008), the number of large-scale studies finding that predator-avoidance behaviors are just as important as prey mortality in indirect effects is growing (Laundré et al. 2001, Dill et al. 2003, Stallings 2008, Laundre 2010, Madin et al. 2010). A major goal in conservation is to protect and restore ecosystems that have been altered by humans, which often includes restoring apex predator populations through creating national parks or marine reserves. Animal behavior is at the interface between selection pressure and population dynamics (Beckerman et al. 2010) and thus integral to our ability to understand and predict changes in ecological communities. Our findings show that complex prey behavior is important in determining the effect of a predator on local resources and ignoring particular predator-avoidance behaviors can overestimate the importance of predators consuming prey on indirect effects of predators

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NATURAL SIZE RANGES OF PREDATOR AND PREY DIMINISH CASCADING EFFECTS THROUGH AN INCREASE IN PREY CANNIBALISM AND INTERFERENCE COMPEITIION

Introduction

Populations contain individuals which can range in size by orders of magnitude and span multiple trophic levels. Community ecology traditionally uses species as the basic building block of food webs and often ignores the different functional roles that the same species can play at different times of their life span (Raffaelli 2007). Theoretical models that incorporate species body size as a component of natural predator-prey dynamics are able to reproduce the stability of the natural community better than models that treat species as static entities (Berlow et al. 2008, Petchey et al. 2008, Ings et al. 2009, Rall et al. 2011). Beyond theoretical predictions, only a few experimental studies have incorporated species size variation into food web interactions (Rudolf 2007a, Miller and Rudolf 2011).

The specific nature among species interactions change as individuals grow (Werner and Gilliam 1984, Woodward and Hildrew 2002, Yang and Rudolf 2010) because size often determines who eats or outcompetes whom (Shine 1991, Sousa 1993, Mittelbach and Persson 1998, Aljetlawi et al. 2004, Costa 2009). Interaction strength or the relative effect of one species on another through time, affects population dynamics

(De Roos et al. 2003, Emmerson and Raffaelli 2004, Brose 2010), which influences community structure (Werner and Gilliam 1984, Vucic-Pestic et al. 2010). Hence, including a natural size range of species to evaluate changes in the interaction strength between predators and prey is necessary to model community dynamics.

The relative sizes of predator and prey affect whether a predator consumes the prey and if a prey has effective predator-avoidance behavior. Predators are often limited in the size of prey they can consume which determines their predation window. The maximum size of prey a predator can consume is limited by the ability to capture the prey and ingest the prey. The minimum size of prey a predator can consume is determined by the ability of the predator to detect and capture the prey. Optimal foraging theory predicts that predators with a choice of prey size should choose a specific size range of prey within their predation window that maximizes capture success and energy uptake (Berlow et al. 2008, Petchey et al. 2008, Vucic-Pestic et al. 2010). Behavior also affects predator-prey interactions (Lima and Dill 1990, Lima 1998). Similar to the predation window, only a certain size range of the prey population reacts to a predator with predator-avoidance behavior (Turner and Mittelbach 1990, Peacor and Werner 2000), which will be referred to here to as the predator-avoidance window. Predator-avoidance behavior is trigger by the threat of being consumed by a predator and the predator-avoidance window is often similar to the predation window (Turner and Mittelbach 1990, Crumrine 2010). However, consumption and behavior of prey need to be measured simultaneously to determine the size range of prey affected by predation and predator-avoidance behaviors.

Predator-prey interactions, whether behavioral or consumptive, cascade down to resources in tri-trophic food chains (predator-prey-resource). The predator indirectly affects the resource by consuming prey (DMIE) and causing prey predator-avoidance behavior (TMIE). Predator-avoidance behavior can be as important, if not more important, than consumption of prey in indirect effects (Peacor and Werner 2001, Preisser et al. 2005). Predator-prey interactions that have a minimal effect on prey can be magnified in indirect effects and significantly affect the resource (Preisser et al. 2005). The effect of predator and prey size on DMIEs and TMIEs is not well studied (Rudolf 2008b). A range in prey size adds horizontal complexity to food chain dynamics because larger size differences between individuals of the same species can lead to an increase in negative intraspecific interactions such as cannibalism and interference competition (de Roos et al. 2006). Increases in cannibalism and interference competition in the absence of predators can counterbalance the indirect effect that predators have on resources (Persson 1999).

This study measured the effect of predator and prey size on intra- and interspecific interactions and the cascading effect on the resource within a tri-trophic food chain (predator-prey-resource; toadfish-mud crab-juvenile oyster). I altered predator size and presence, prey size class (3 different sizes and all three sizes together), and measured prey and resource survival. Specifically, this study quantified the effect of 1) prey and predator size on prey and resource survival (TMIEs and DMIEs) and 2) predator presence and size on the intraspecific interactions, TMIEs, and DMIEs when all sizes of prey were present.

Methods

Experiment 1

The study system consisted of a tri-trophic food chain with toadfish (*Opsanus tau*; predator), mud crabs (family *Xanthidae;* prey) and juvenile oysters (*Crassostrea virginica;* resource). The experimental design had 2 crossed factors, each with 4 levels: predator (absent, small, medium, and large) and prey size class (small, medium, large, and all three size classes). Predator treatments that contained toadfish had 1 toadfish in the predetermined size class (<250, 250-300,>300mm total length). The prey treatments contained either 12 small, 7 medium, 5 large or 24 (12 small, 7 medium, and 5 large) mud crabs. These densities were the mean number of mud crabs per m^2 of oyster reef in South Carolina within the size range of carapace widths (CW; 22 crabs 10-15mm, 13 crabs 16-25mm, and 9 crabs >26mm CW for the 3 size classes respectively; McDonald 1982). Two species of mud crabs (family *Xanthidae*) were used, *Panopeus herbstii* and *Eurypanopeous depressus*, which co-occur in oyster reefs (Menendez and Abele 1983, Meyer 1994). Predator size and prey size classes (Table 4.1; see Appendix 4.A for picture) were chosen for multiple reasons. First, these sizes span the range of these species caught on oyster reefs with minimum mud crab size (10mm carapace width) restricted by the mud crab's ability to consume the size of juvenile oysters used in the experiment (Kulp et al. 2011, Toscano and Griffen 2012; NRG pers.obs). Second, our large mud crabs should be outside of the predation window of the small toadfish because toadfish do not consume prey greater than $1/10^{th}$ their length (Bisker et al. 1989). Finally, the average biomass of individuals (both predator and prey) was approximately doubled with each successively larger size class (Table 4.1) to increase the occurrence of

size-dependent interactions because this has been hypothesized as a large enough difference in weight (2 times) that will allow species to coexist (Schoener 1974, Bowers and Brown 1982).

Experiments were conducted in sixteen outdoor stainless steel tanks (2m long × 1m wide × 0.6m deep, with a 1cm layer of sand on the bottom), supplied with unfiltered seawater (at a flow rate of ~0.2L/s) from nearby Bogue Sound, at the University of North Carolina's Institute of Marine Sciences (Morehead City, NC, USA). Crabs and fish were collected by hand and trap in Bogue and Pamlico Sounds (NC, USA) and held separately in flow-through tanks that were supplied with raw seawater. The use of unfiltered seawater in experiments allowed recruitment of crab prey items, such as mussels, during the trials. This mimicked natural reef conditions in which resources other than oysters are available for crabs. Toadfish were fed chunks of frozen fish and crabs were fed oysters (< 1 cm shell height) *ad libitum* every 2 days before experiments started.

Oyster habitat was created in each mesocosm by adding cleaned adult oyster shells (37.9L) to the mesocosm and spreading them out to cover a 0.56m^2 area (0.75 x 0.75m square). The oyster shells created oyster habitat that was approximately 15cm deep. This patch size of oyster habitat is commonly found within natural oyster reefs (Eggleston et al. 1998, Macreadie et al. 2011). Juvenile oysters were produced from hatchery raised larvae and settled on cleaned adult shell in flow through tanks. Eight cleaned adult *oyster shells*, each with 5 juvenile oysters (see Table 4.1 for sizes; a total of 40 oysters per treatment) *were haphazardly placed within the oyster habitat.*

Treatments were haphazardly assigned to tanks. Mud crabs were placed in the oyster habitat in each mesocosm. After crabs acclimated for 10 minutes in the mesocosm,

a single adult toadfish of the treatment-appropriate size was added to the predator-present treatments. Each trial of the experiment consisted of a single replicate of each of the 16 treatments, and replication was gained through successive, replicated trials (n = 6). Trials were run from July to October in 2010. Trials were ended before 50% of oysters were consumed, which was 6-8 days as determined in pilot trials, to minimize a decrease in prey feeding rate because of resource depletion (Murdoch 1969). At the end of each trial, all mesocosms were drained of seawater, the oyster shells were removed and searched thoroughly, and the sand was removed and sieved through 1-mm mesh so that all crabs were counted. Individual toadfish and mud crabs were used once and released after each trial.

Small mud crab treatments were the only single size class in which cannibalism occurred. To ensure this was a result of mortality and not a result of failure to recover surviving individuals because of their small size, I ran a control experiment. Twelve small crabs were added to a tank with shell at 5 pm and recovered at 9 am the next day following the procedures previously described. Six replicates were run simultaneously in October 2010 and 71 out of 72 crabs were recovered. Because of the 99% crab recovery, it was assumed that all crabs not recovered at the end of trials were eaten. To estimate mortality of oysters from causes other than predation, I added 40 oysters (5 per shell) to a Vexar bag and placed two bags of those oysters in separate tanks not being used for trials. This control was conducted during trials 3-5 (n=6). Three oysters died out of 240. Because of the high survival in these controls, oyster mortality within the experimental tanks was attributed to predation.

Experiment 2

Cannibalism occurred in the 'small' and 'all prey' treatments of Experiment 1 (Exp 1) (Appendix 4.C), so a second experiment (Exp 2) was run to parse intra- and interspecific predation. Exp 2 was conducted in the same mesocosms following the same procedures as Exp 1 with the following changes. The experimental design consisted of 2 crossed factors: predator (absent, caged, and free), and prey size classes (small and all; see Table 1 for sizes). The mean size of toadfish fell within the medium size class of Exp 1, although the size range was greater (Table 4.1). All treatments had a Vexar mesh bag (90cm x 50cm x 10cm high, 0.5cm mesh) in a haphazardly chosen corner of the mesocosm, which over-laid $1/4^{th}$ of the oyster habitat. A toadfish was put in the bag in the caged treatment and added directly to the tank in the free treatment. The experiment was run from July through August of 2011. Two replicates of the 6 treatments were run simultaneously 3 separate times for a total of 6 replicates. Trials continued for 5 days. To estimate mortality of oysters from causes other than predation, 40 juvenile oysters (5 juvenile oysters per shell) were put in Vexar bags and placed in tanks not used for the experiment. This was repeated twice during each trail (n=6) and all oysters survived. During the second experiment, individual crabs were weighed to estimate the total biomass of each mud crab size treatment and the crab biomass eaten during each trial for Exp 1 and Exp 2 (Table 4.1).

Indirect effect and multiple predator calculations

Assessments of the importance of prey behavior in ecological processes must isolate behavioral effects from consumptive effects (Peacor and Werner 2001, Schmitz et al. 2004, Okuyama and Bolker 2007). I parsed different indirect effects by counting the number of the oysters eaten per day by prey in the absence (M) and presence (m) of a

predator (M=initial # of oysters – final # of oysters), the daily per-prey consumption of the resource in absence (C) and presence (c) of a predator (C=M/[initial # of crabs-final # of crabs/2]), and the number of prey eaten by a predator (p; p=initial # of crabs-final # of crabs; Appendix B). The expected resource release (ER) is the amount of resources surviving because of prey mortality (c·p) resulting from intra- and interspecific predation. The actual resource release (AR) is the difference between resources consumed by prey in the absence and presence of a predator (M-m). The behavioral resource release (BR), or the amount of resources that are not eaten because prey reduce activity and foraging in the presence of a predator, is the difference between the AR and ER (AR-ER; Grabowski 2004). Thus, if the change in the numbers of resources and prey are known, indirect effects can be estimated (Appendix 4.B). All calculations were conducted on individual replicates and error was calculated among replicates.

Additional calculations were made to quantify the number of resources not eaten because of interference competition by comparing the number of resources eaten in each of the individual size class treatments to the number of resources eaten in the 'all prey' treatment with no predator. First, I calculated the resources expected to be eaten if there was no intraspecific interactions using the multiplicative rule (Soluk and Collins 1988, Soluk 1993), which estimates multiple predator effects (the sum of the proportion of oysters consumed in each individual size class trial minus the product of the proportion of oysters consumed in each individual size class trial). Subtraction of the product of the proportion of oysters eaten accounts for the fact that an oyster eaten by one size class cannot be eaten by another. This proportion of individuals expected to be eaten was then multiplied by 40 (number of oysters in each trial) and divided by the length of the trial

(days) to standardize the expected number of oysters consumed per day when all size classes were present. The estimated number of resources eaten with all size classes present was subtracted from the actual number of oysters consumed per day in 'all prey' treatments to determine the number of resources released because of intraspecific interference. The calculated number of oysters per day released because of interference competition is included in figures as BR in the 'all prey-no predator' treatment. It was used to compare interference competition to both a predator-avoidance behavior (BR) and the expected release resulting from cannibalism in 'all prey' treatments (ER).

To estimate the importance of behavior in species interactions and food chain dynamics, the relative strength of behavior is compared to consumption, which is the traditional measure of interaction strength. The strength of BR relative to ER was calculated by dividing the BR by the sum of BR and ER. The contribution of the ER can be calculated by subtracting the BR from 1. The overall means of BR and ER were used to calculate the ratios because multiple trials had negative BRs, which resulted from more oysters being consumed in the presence than in the absence of a predator for those trials. The negative number greatly skewed the calculations by reducing the mean even when transformations were conducted. As a result, the standard error was calculated by error propagation. Errors were propagated using the formulas for addition and division respectively; $[(\delta x)^2 + (\delta y)^2]^{1/2}$, $R[(\delta x/x)^2 + (\delta y/y)^2]^{1/2}$. R is the product of the means, x and y are the means, and δ indicates the standard error of the respective means.

Statistical analysis

Dependent variables were analyzed in a series of 2-way ANOVA's with fixed crossed factors. Interactions were run initially but were removed if they were not

significant. Trial was included in ANOVAs as a random factor, but did not significantly alter findings and it was removed from all analyses. Because ANOVAs with balanced designs are robust against errors introduced by non-normal distributions (Underwood 1997), I did not test for normality. Bartlett's test was used to test heteroscedasticity. If the dependent variable had heterogeneous variance it was log-transformed and all dependent variables passed Bartlett's test (p-value > 0.05) after transformation. Tukey's test was used to determine significant pair-wise comparisons when the main effect was significant.

The effect of predator size and prey size class on crab mortality was analyzed in two separate ANOVAs with crab biomass consumed as the dependent variable. Biomass was used as the dependent variable instead of individuals consumed because number of crabs differed for each individual size class, but biomass was similar (Table 4.1). The first ANOVA was run with predator (4 levels) and prey (3 levels) as independent factors. The mud crab treatment with all size classes was not included in this ANOVA because it had 3 times the biomass available for predator consumption and was run in a separate ANOVA with predator (4 levels) as the independent factor.

Total oysters consumed and oysters consumed per crab were analyzed in separate ANOVAs with predator (4 levels) and prey size class (4 levels) as crossed fixed factors. The ER and BR were each analyzed as dependent factors in 2 separate ANOVAs to keep crab biomass constant. The first ANOVA for the ER had prey size class (3 levels) and predator (4 levels: absent, small, medium, and large) as independent variables and the second had predator (4 levels) as the independent variable. For the BR, the first ANOVA

had prey size class (3 levels) and predator (3 levels: small, medium, and large) as independent variables and the second had predator (4 levels) as the independent variable.

In Exp 2, analysis of consumed crab biomass was conducted using two separate ANOVAs: one ANOVA for small crabs and a second for all crab size classes. The dependent variable was biomass of crabs consumed, and predator (3 levels; absent, caged, and free) was the independent variable. Oysters consumed and oysters consumed per crab from Exp 2 were analyzed as dependent factors in two separate 2-way ANOVAs with predator (3 levels) and prey size (2 levels) as independent factors. The dependent variables from Exp 2 had homogeneous variances and were not transformed.

Results

Experiment 1

No crabs were consumed in the medium or large crab treatments without a predator present (Appendix 4.C). On average 2 crabs were consumed in the 'no predator-small crab' treatment. Within the 'all prey' treatment, large crabs were only consumed by small and medium toadfish, while small and medium crabs were consumed by all toadfish sizes and in the absence of toadfish (Appendix 4.C). The biomass of crabs consumed in individual crab size class treatments was higher for the small crab than the large crab treatment ($F_{2,66}$=3.87, p=0.026; Fig. 4.1A) and greater for the medium toadfish treatment than when the toadfish was absent ($F_{2,66}$=3.80, p=0.014; Fig. 4.1B). The biomass of crabs consumed was marginally significant among toadfish treatments when all crab size classes were present ($F_{3,20}$=2.75, p=0.069; Fig. 4.1C). Crab biomass consumed was higher in the small toadfish treatment than the large toadfish treatment (Tukey's test, p=0.080). When a predator was present, differences in crab biomass

consumed among treatments did not cascade to alter resource survival. The total number of oysters consumed was not affected by mud crab treatment ($F_{3,89}$=1.51, p=0.219; Fig. 4.2A), but was higher when a toadfish was absent compared to when a toadfish of any size was present ($F_{3,89}$=6.27, p<0.001; Fig. 4.2B). The number of oysters consumed per crab was higher in medium and large crab treatments than in the small and all prey treatments ($F_{3,89}$=23.37, p<0.001; Fig. 4.2C). In addition, more oysters were consumed per crab in 'toadfish-absent' treatments then when a toadfish of any size was present ($F_{3,89}$=5.97, p<0.001; Fig. 4.2D).

The expected resource release (ER) or oysters not consumed because of crab mortality was not affected by crab size class ($F_{2,89}$=1.01, p=0.394; Fig. 4.3A) or predator treatment ($F_{3,89}$=1.19, p=0.310; Fig. 4.3B) when crab treatments had a single size class. When all crab size classes were present, the expected release of oysters was not affected by predator treatment ($F_{3,20}$=1.02, p=0.40; Fig. 4.3C). The behavioral resource release (BR) or the number of oysters not consumed because of both anti-predator and intraspecific behavior (interference competition) when individual prey size classes were present was not affected by prey size class ($F_{2,49}$=0.34, p=0.996; Fig. 4.3D) or predator treatment ($F_{2,49}$=0.63, p=0.537; Fig. 4.3E). Furthermore, the BR was not affected by predator treatment when all mud crab size classes were present ($F_{3,20}$=0.28, p=0.839; Fig. 4.3F). A comparison of BR strength relative to the overall resource release indicated that the BR was stronger than the ER (Fig. 4.3G-I) and behavior caused an average of 70-96% of oyster survival resulting from predator-prey and prey-prey interactions.

Past studies on DMIEs and TMIEs primarily have very low or no prey mortality when the predator is absent; therefore ER and BR are equal to DMIE and TMIE,

respectively. Although this was the case when only one crab size class was present in this study, there were similar levels of crab mortality with and without a predator when all crab size classes were present. In Exp 2, cannibalism was minimal in the presence of a predator, and because cannibalism and interspecific predation were not additive, I did not perform calculations to partition intra- and interspecific predation (i.e. ER with a predator minus ER without a predator). All dependent variables from Exp 1 had homogeneous variance after log-transformation and there were no significant interactions.

Experiment 2

When only small crabs were present in the mesocosm, the biomass of crabs consumed was not different among toadfish treatments ($F_{2,15}$=1.63, p=0.228; Fig. 4.4A). However, when all size classes of crab were present an average of 0 crabs were eaten in caged toadfish treatments, 2 crabs were eaten when the toadfish was absent, and 3 crabs were consumed when a toadfish was present and able to consume mud crabs (Appendix 4.D). Small crabs entered mesocosms via the unfiltered seawater during Exp 2 because pipes in the water delivery system were probably fouled with organisms. This resulted in negative crabs consumed and I was unable to determine which crabs entered through the water system. Because trials were haphazardly assigned to mesocosms and crabs dropping into tanks from the inflow pipes could not have been biased by the treatment in the tank, crabs entering the tanks during the trials was random and should not have affect the results. Therefore, all crabs recovered at the end of trials were included in the analysis. In 'all prey' treatments, there was a marginally significant difference in crab biomass consumed among predator treatments ($F_{2,15}$=3.34, p=0.063; Fig. 4.4B), with

greater biomass consumed in the free than caged predator treatments (Tukey's test, p=0.05).

The number of oysters consumed was affected by predator ($F_{2,30}$=8.15, p=0.001) and mud crab treatments ($F_{1,30}$=101.75, p<0.001), and the interaction was significant ($F_{2,30}$=7.96, p=0.002; Fig. 4.5A). Fewer oysters were consumed in the small crab treatments than the 'all prey' treatments, regardless of predator treatment. When all mud crab sizes were present, fewer oysters were consumed in the free predator treatments than when the toadfish was absent. Oyster consumption per crab was also affected by both predator ($F_{2,30}$=6.52, p=0.004) and mud crab treatments ($F_{1,30}$=52.50, p<0.001), and the interaction was significant ($F_{2,30}$=6.05, p=0.006; Fig. 4.5B).

Discussion

Predator presence indirectly increased resource survival through prey mortality and predator-avoidance behavior. Neither predator size nor prey size class significantly affected resource survival despite significant effects of prey and predator size on the amount of crab biomass consumed. Stability in resource survival in this food chain across treatments may have resulted from multiple factors: 1) consumption of resources was constant across individual prey size classes because the treatments had similar prey biomass; 2) intraspecific competition and cannibalism among prey increased when a range of prey sizes was present; and 3) behavioral effects were stronger than intra- and inter-specific predation. In addition, I found that intraspecific interference competition was just as important as predator-avoidance behavior when a broader range of prey size

was present. These experimental findings unravel some of the mechanisms behind the effect of size structure and intra- and interspecific behavior in the stability of populations.

Although there was no significant effect of predator or prey size on resource survival, there were significant effects of size on both prey-resource and predator-prey interactions. Crab size was positively correlated with per-capita crab consumption of oysters, which is common among metazoa because body size and metabolic demand are positively correlated (Brown et al. 2004). But, abundance and size are inversely related and individual size classes of mud crabs had similar crab biomasses and each size class consumed an equal amount of resources. Hence, the effect of prey size on per individual consumption of resource was negated by the relationship between size and abundance.

Similar to the findings on prey size, there was no effect of predator size on crab biomass consumed when only 1 size class of prey was available. A small predator consumed more prey biomass than a large predator when all sizes of prey were present. This could have resulted from larger toadfish being more sedentary than smaller ones leading to a lower energetic demand. When all crab sizes were available to the predator, more small crabs than large crabs were consumed by all predator sizes. In half of all 'small predator-all prey' treatments, a large crab was consumed by a small toadfish. Thus, and contrary to past studies, toadfish consume mud crabs that are greater than $1/10^{th}$ their size (Bisker et al. 1989). Large toadfish did not consume large mud crabs in 'all prey' treatments and preferred, consistently, small prey, indicating the predation window for larger predators was narrower than for small predators. Neither the wider predation window nor the greater consumption of prey biomass by small predators compared to large predators cascaded to affect resource survival. But, predator-avoidance

behavior did and the predator-avoidance window was similar for all predator-prey size combinations (BR was similar for all treatments). Thus, if measured by the cascading effect on the resource, the predator-avoidance window has a greater affect than the predation window on predator-prey interactions.

Recreating natural size ranges and abundances of crabs increased cannibalism. Cannibalism is ubiquitous in nature (Fox 1975, Polis 1981) and increases with species density and size range (Rudolf 2008a). Many experimental studies use only a small size range compared to what exists in nature (Miller and Rudolf 2011) and probably underestimate the importance of intraspecific interactions in food web dynamics (Rudolf 2007b, 2008b). No cannibalism occurred in treatments with medium or large mud crabs alone. Cannibalism did occur in small crab treatments, which may have resulted from relative thickness of the exoskeleton of small sized crabs which leaves them vulnerable to similar sized crabs. When all crab size classes were together cannibalism also occurred. In the absence of a predator, cannibals consumed as much crab biomass as when a predator was present. The inclusion of a caged predator reduced cannibalism by an average of 92%, which indicates that a predator consumes the majority of crabs when a predator is present and drastically reduces cannibalism. Although overall biomass consumed did not change in the absence or presence of a predator, the mortality shifted from smaller to larger crabs in the presence of a predator. Similar findings have been found in lakes where removal of predators resulted in increased abundance of very large prey and decreased abundance of small prey because of cannibalism (Wahlstrom et al. 2000). Presence of predators may not have a net effect on overall prey biomass, but loss of the predator can alter prey size frequency which may cascade to lower trophic levels.

A growing body of evidence indicates that predators indirectly affect resources by consuming prey (DMIE) and causing predator-avoidance behavior (TMIE), and that predator-avoidance behavior is equal to or greater than direct consumption in tri-trophic food chains (Peacor and Werner 2000, Preisser et al. 2005). Many of these studies use only a narrow size range in each trophic level. When Law and Rosenheim (2011) included 2 size classes of prey in a terrestrial food chain, they found that predator presence did not have an effect on prey density because cannibalism was as important as interspecific predation on prey abundance. I also found that cannibalism was similar to interspecific predation when a range of prey sizes were present and both intra-and interspecific predation resulted in similar increases in resource survival. In addition, this study measured the effect of behavior in food chain dynamics and found that intraspecific behavior was equal to predator-avoidance behavior when a nature range of prey size was present. Including a natural range of prey size was not only important for consumptive effects, but also for behavioral effects, which were on average 96% of the intraspecific interaction. Similar to TMIEs and DMIEs, behavior was also more important than consumption in intraspecific interactions.

The indirect effects of prey mortality and reduced prey activity have been previously investigated in a tri-trophic food chain with toadfish, mud crabs, and juvenile bivalves (Grabowski 2004). Grabowski (2004) used medium-sized crabs and small toadfish as compared to this study and found minimal mortality of crabs when a predator was absent. Although prey consumption by a predator differed between experiments the TMIEs were similarly important in indirect effects (mean 95% vs. 80% in Exp 1). Although the additive design in my study could not determine whether the intraspecific

interactions in all size class treatments were a result of size class or density (Griffen 2006), relative importance of size vs. density in intraspecific interactions of mud crabs can be drawn by comparing past experiments that altered crab density. Grabowski and Powers (2004) used 3 different densities of large mud crabs, as compared to this study, and measured the change in consumption of juvenile clams. They found that clam consumption increased slightly from low to medium crab densities but resource mortality did not change among the medium and high crab density. Crab mortality was approximately 2.4%, but they did not report if mortality changed with crab density. The density of large mud crabs used by Grabowski and Powers was up to 5 times the average natural densities of crabs this size (McDonald 1982) and it is important to keep in mind that an increase in density in nature will usually include all sizes, skewed towards small sizes. In addition, Toscano and Griffen (2012) used a substitutional design and found that 3 crab sizes in isolation consumed a similar amount of bivalves as when together. These findings, in combination with our study, indicate that interference competition is primarily density dependent, while cannibalism is primarily driven by size.

Understanding the effect of size in predator-prey interactions could increase our understanding of how selective fishing can decrease stability in the target species population and indirectly impact marine food webs. Garcia et al. (2012) found that fisheries selecting for a specific species or size within a species caused greater population instability than harvesting fish regardless of species or size. They conclude that switching from selective to nonselective fishing would result in more sustainable fisheries. The present study concurs and maintaining a wide range of sizes could result in food chain stability because of the importance of intraspecific interactions.

My study corroborates the importance of intraspecific interactions in food chains from diverse ecosystems (Chase et al. 2002, Rudolf 2006, de Roos et al. 2006, Andersson et al. 2007, Law and Rosenheim 2011) and reiterates the long-standing notion that both competition and predation shape communities. Recent studies have highlighted the importance of predator-avoidance behavior in trophic cascades (Peacor and Werner 2001, Preisser et al. 2005, Peckarsky et al. 2008), but the inclusion of intraspecific behavior is necessary to build food web models that replicate natural systems. The disparity between population stability found in natural food webs and the instability in experimental studies could result from the increased horizontal complexity that natural size ranges of species add to food webs (Chase et al. 2002, Woodward et al. 2005, Rall et al. 2011). These findings indicate that even relatively simple food webs can be stable to changes in predator abundance but trophic interactions could be sensitive to human alteration of population size frequencies through size selective harvesting.

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UNEXPECTED CONSEQUENCE OF ARTIFICIAL STRUCTRUES: THE POSITIVE EFFECT OF NON-NATIVE SPECIES ON NITRGEN REMOVAL

Introduction

Human activities are directly and indirectly altering coastal ecosystems (Vitousek et al. 1997, Lotze et al. 2006, Halpern et al. 2008). Major changes resulting from human activities include global warming and associated sea-level rise (FitzGerald et al. 2008), coastal development (Vitousek et al. 1997), spread of invasive species (Mack et al. 2000, Schaffelke et al. 2006), and eutrophication resulting from increased inputs of nitrogen (Nixon 1995). Interactions among human impacts have the potential to mitigate or exacerbate environmental change. Because human impacts seldom occur in isolation, the ability to efficiently manage the impacts depends on a mechanistic understanding of their causes and interactions (Cloern 2001, Schaffelke et al. 2006, Didham et al. 2007).

Global climate change will result in both inundation of low-lying coastal land from rising sea level (IPCC 2007) and increased erosion from more intense and frequent storms (FitzGerald et al. 2008). Land owners and communities will have to make important decisions on whether to artificially harden shorelines or lose low-lying property (Kittinger and Ayers 2010, Chapman and Underwood 2011). However, shoreline-hardening structures such as bulkheads, revetments, and jetties have negative impacts on the surrounding ecosystem (Titus et al. 1991), including loss of natural habitat and reductions in associated fishes (Peterson et al. 2000, Seitz et al. 2006, Bilkovic and

Roggero 2008). In addition, artificial hard structures that are installed in environments dominated by soft substrate contain a higher percentage of non-native species than surrounding soft substrates (Wasson et al. 2005), and when both natural and artificial hard substrates are present, artificial substrates can have a higher percent cover of non-native species than natural hard substrates (Tyrrell and Byers 2007).

Codium fragile subsp. *tomentosoides* (*C. fragile*), a siphonaceous green alga native to Asia, dominates artificial structures in soft substrate-dominated environments along the Italian coast (Bulleri and Airoldi 2005, Vaselli et al. 2008). *C. fragile* is considered one of the most invasive macroalgae in the world because of its trans- and inter-oceanic introductions (Trowbridge 1998), its high rate of dispersal and successful establishment, and its ecological capacity to displace native species (Nyberg and Wallentinus 2005). It has outcompeted native kelps for space on natural hard shoreline in the Northwest Atlantic (Harris and Jones 2005, Scheibling and Gagnon 2006) and coexists with native *Codium spp.* in the British Isles (Trowbridge 2001, Trowbridge and Farnham 2004), New Zealand, and eastern Australian shores (Trowbridge 1995, 1998, Schaffelke and Deane 2005). In North Carolina, where *Codium decorticatum* is native, *C. fragile* was first documented in 1979 attached to jetties, seawalls, and shells and may compete with the native species for the relatively-scarce hard substrate in the lower intertidal and shallow subtidal within North Carolina estuaries and sounds (Searles et al. 1984). Given the difficulty in distinguishing species of *Codium* and the consequent uncertainty in individual species distributions in North Carolina, we use "*Codium"* from this point forward to refer to both species within the *Codium* genus.

Changes in the species composition and abundance of *Codium* could affect the amount and type of biologically available nitrogen in coastal ecosystems (Rosenberg and Paerl 1981). Increased nitrogen concentrations can increase eutrophication, which can result in hypoxia, fish kills, and harmful algal blooms (Nixon 1995). *Codium* is associated with nitrogen-fixing bacteria that convert N_2 gas to ammonium, a biologically available form of nitrogen (Head and Carpenter 1975). Rates of nitrogen fixation vary by *Codium* species (Dromgoole et al. 1978). In New Zealand, *C. fragile* has higher rates of nitrogen fixation than native *C. adhaerene* (Dromgoole et al. 1978). In North Carolina, nitrogen fixation was not detected in *C. isthmocladum*, a native deepwater species, while *C. decorticatum* fixed 1.2µg of N_2 fixed per g of dry wt per h (Rosenberg and Paerl 1981). Two additional studies in the northwest Atlantic found nitrogen fixation rates in *C. fragile* averaging from 0.03-3.2 (Gerard et al. 1990) and 0.6-1.0 µg of N_2 fixed per g of dry wt per h (Head and Carpenter 1975). A small portion of the newly fixed nitrogen is used for growth (Gerard et al. 1990), while the remainder enters the surrounding water. Thus, *Codium* can alter local nitrogen pools; however, the magnitude would be dependent on the abundance of the different *Codium* species (Rosenberg and Paerl 1981). The identification of the nitrogen-fixing bacteria and their mode of association (epi- or endophytic) with *Codium* are inconclusive. Therefore, "*Codium"* will refer not only refer to the two shallow *Codium* species in North Carolina, but also to the macroalgae and associated organisms unless otherwise stated.

The opposing process to nitrogen fixation is denitrification, which converts nitrate to N_2 gas thereby removing it from the pool of available nitrogen. These two processes are the major factors determining whether nitrogen is limiting or not in coastal

ecosystems (Howarth et al. 1988). Denitrification has also been associated with macrophytes in both marine and freshwater ecosystems (Eriksson and Weisner 1999, Bastviken et al. 2003, Tall et al. 2011) and it is possible that both nitrogen fixation and denitrification could be associated with *Codium*. However, our understanding of how macroalgae mediate denitrification is limited (McGlathery et al. 2007, Goecke et al. 2010) and the cumulative effects of *Codium* on coastal nitrogen dynamics are unknown. If denitrification rates associated with *Codium* are greater than rates of nitrogen fixation, *Codium* could provide a valuable ecosystem service of removing usable nitrogen (Costanza et al. 1997).

To assess the abundance and distribution of native and non-native *Codium* and possible effects on nitrogen dynamics in coastal North Carolina, we sampled natural (oyster reefs) and artificial (bulkheads, revetments, jetties) shallow-water hard substrates to estimate percent cover, species composition, and biomass of *Codium* in selected North Carolina estuaries once each season from 2009-2011. We also employed continuous-flow microcosms to examine the effects of *Codium* on nitrogen transformations during three seasons (spring, summer, and fall): very little *Codium* was present during winter. Finally, we calculated the net change in N_2 flux attributable to *Codium*.

Methods

Codium sampling

We sampled natural hard substrates (oyster reef and oyster shell) and shoreline stabilization structures around Wilmington and Morehead City, North Carolina to determine the abundance and distribution of non-native *C. fragile* and native *C.*

decorticatum (Fig 5.1). Mean tidal range in Wilmington was 116.1cm and 94.8cm in Morehead City (NOAA stations 8658163 in Wrightsville Beach, NC and 8656483 in Beaufort, NC, respectively). Shoreline stabilization structures sampled included: bulkheads, which were vertical walls of different materials (plastic and treated wood) built along the shoreline; revetments, which were granite boulders piled on a steep slope (20-40 º from the vertical) oriented along the shoreline; and jetties, composed of piled granite boulders (20-40º from the vertical) running perpendicular to the shoreline.

Morehead City-area sampling sites included Radio Island (a jetty; 0.3 to 10m below mean sea level (MSL); Fig. 5.1A); Taylor's Creek (two separate revetments: 1m above to 3m below MSL; Fig. 5.1B); Rachel Carson Reserve (part of NOAA's National Estuarine Research Reserve; four intertidal oyster reefs; 0.8 m above and below MSL; Fig. 5.1C), and Pine Knoll Shores (a revetment, a treated wood bulkhead and a plastic bulkhead; 2 m above to 0.6 m below MSL; Fig. 5.1D). Sampling near Wilmington encompassed the Masonboro Inlet jetty (5m above to 2m below MSL; Fig. 5.1E), an intertidal oyster reef, and a tidal creek with intertidal oyster shell (both 0.5 m above and below MSL; Fig. 5.1E). Sites were chosen because they had continuous hard substrate (>10m long) within the tidal range in which *Codium* occurs (0.25-1 m below MSL; Thomsen et al. 2007). Sites around Morehead City were sampled once each season (season, sampling months, water temperature range in ºC: winter, Feb.-Mar., 6.8-12.3; spring, April-May, 17.2-24.4; summer, June-July, 27.3-31.2;fall, Nov.-Dec., 9.3-20.5). Sites near Wilmington were sampled in summer and winter (same date and similar temperature range as above). Sampling began in May 2009 and ended in May 2011. Mean water temperature was calculated from weekly measurements taken in Bogue

Sound at UNC's Institute of Marine Sciences during sampling dates from surface water using a Sontec YSI.

Sampling consisted of estimating the percent cover of visible taxa (not including taxa under algal canapy) and bare substrate within replicate 25x25cm quadrats (0.0625 m^2). Depending on the length and depth of the hard structure, 10 to 15 quadrats were taken at one or two tidal heights (approximately 0.5 and 0.75 below MSL) at each site by aligning the bottom of the quadrat with the appropriate tidal height. Elevation of the top of fixed markers (50 cm PVC pipe driven into the sediment) at each site was measured using a Trimble GPS. Sampling depth relative to mean sea level was estimated by comparing the nearest NOAA tide height station measurement at low tide with the water's elevation on the fixed marker at the concurrent low tide. To determine the biomass of *Codium* per area, we divided sites that were 20m or more in length into percent cover and biomass sampling sections so that *Codium* removal for biomass measurements did not affect percent cover of *Codium* for subsequent samplings. To measure the biomass per area, the percent cover of *Codium* within the quadrat was recorded and all *Codium* was harvested within ten- 0.0625 m^2 quadrats. The wet weight of *Codium* was measured after spinning it in a salad spinner 20 times (Power et al. 2008).

Identification of *Codium* species was made by removing a 5 mm piece of thallus tip from each plant within percent cover and biomass quadrats. A cross section of each piece was then chopped to fine pieces using a razor blade, mounted on a slide, and observed under a Wild M20 dissecting microscope (100 x power). *C. fragile* was distinguished from *C. decorticatum* by presence of apiculate utricle tips (Searles and Schneider 1991, J. Fegley pers. demonstration).

The effect of artificial and natural substrates on community assemblage of sessile organisms was tested with a PERMANOVA (PRIMER, Clarke and Gorley 2006) with substrate and season as crossed factors. Dependent variables were the vectors of percent cover of individual taxa of each quadrat. Region (Morehead City and Wilmington) was not included in any analysis because Wilmington sites were only sampled in winter and summer. All analyses were rerun excluding Wilmington samples to determine if unequal sample sizes altered findings. Excluding Wilmington samples did not change significant results and all subsequent analyses include samples from Wilmington sites.

The factors affecting percent cover of *Codium* were analyzed using a generalized linear model (GLM) with binomial error and a logit canonical link, which fit the strictly bounded data set containing a majority of zeros (residual deviance < degrees of freedom; Crawley 2007). The initial model included 2 fixed factors: season (4 levels), and substrate type (2 levels: natural and artificial). Depth (0.50 and 0.75 m below MSL) was not included in the model because multiple sampling sites did not exist at both elevations. Because we could not accurately identify the difference between *C. fragile* and *C. decorticatum* in the field, the analysis of community assemblage of sessile organisms and percent cover were conducted with *Codium* as a genus and not as individual species. After determining the species of *Codium* in the lab, the number of *C. fragile* and *C. decorticatum* individuals were analyzed using the same model as percent cover, but the percent of each species at each sampling was used as the response variable (Crawley 2007). The model included 1fixed factor: substrate type (2 levels; natural and artificial). Season was not included because *Codium* was absent on natural substrate in fall and winter.

Codium nitrogen dynamics

The effects of *C. fragile* and *C. decorticatum* on nitrogen dynamics were measured in continuous flow microcosms (40-cm high x 7.6-cm diameter, 800ml water volume) using methods from Piehler and Smyth (2011). Three individuals of each species were collected from Bogue Sound. Within 2 hours of harvesting, a 15 g section of each individual was added to a microcosm. We employed a total of 7 microcosms with each species replicated three times and including a microcosm with water only to account for changes in nitrogen composition not attributable to the *Codium*. The 7 microcosms were run simultaneously.

Unfiltered water from Bogue Sound, which was aerated with an air stone, was pumped at 2ml per min into the top of the microcosm and flowed out of a tube positioned 5 cm above the bottom of the microcosm. This flow rate was determined to be the maximum flow rate achievable while still preventing washout of changes in nitrogen gas concentration (Miller-Way and Twilley 1996, MFP and ARS unpublished data). Microcosms were maintained in a temperature-controlled room (Baily, Inc.), set to match the water temperature of Bogue Sound at the time of the experiment. Microcosms were run for an acclimation period of approximately 18 hours prior to initial sampling to decrease oxygen concentration in the water that could interfere with measurements of denitrification (Eyre et al. 2002). To mimic natural light, grow lamps (50 µeinstiens) were cycled off/on every 12 hours starting after the 18-hour acclimation period. This light intensity is approximately equal to natural light at 1.8m depth in Bogue Sound (S.Thompson unpub. data). The light cycle was held constant throughout all seasons to maintain a consistent measurement schedule. After 18 hours, samples from an inflow

line, which bypassed the microcosms and flowed directly into 5ml sample vials, and the outflow line of each microcosm were taken at 0 (dark), 6 (dark), 18 (light) and 24 (light) hours. The samples were analyzed for dissolved gasses: N_2, Ar, and O_2 (Kana et al. 1994) by a Balzers Prisma QME 200 quadropole mass spectrometer (MIMS; Pfeiffer Vacuum, Nashua, NH, USA). Concentrations of dissolved gasses were determined as described in Ensign et al. (2008). Additionally, 50ml samples of water from the inflow and outflow line of each microcosm were collected for nutrient analysis at 6 (dark) and 24 (light) hours. Water was filtered through Whatman GF/F filters (25mm diameter, 0.7µm nominal pore size) and the filter was frozen until analysis. Filtrate was analyzed with a Lachat Quick-Chem 8000 (Lachat Instruments, Milwaukee, WI, USA) automated ion analyzer for nitrate + nitrite (collectively nitrate), ammonium and total dissolved nitrogen. Detection limits were 0.6µg/l for NO_x^-, 2.55µg/l for NH_4^+, and 35.4µg/l for total dissolved nitrogen. Dissolved inorganic nitrogen (DIN) was calculated as the sum of nitrate and ammonium. Dissolved organic nitrogen (DON) was calculated as the difference between total dissolved nitrogen and DIN. Microcosm experiments were conducted three times: summer and fall of 2010 and spring 2011 at 26ºC, 17ºC, and 22ºC, respectively. Experiments were not conducted in winter because of the low abundance of *Codium* and because denitrification rates are negligible in North Carolina during winter months (Piehler and Smyth 2011).

Water blank concentrations were subtracted from the microcosm-specific concentrations to exclude changes resulting from water column processes. Rate calculations were based on the assumption that concentration profiles of dissolved nutrients and gasses remained constant over time (Miller-Way and Twilley 1996). The

rates of change in nutrient and dissolved gases were calculated using the equation $J=(C_{out} - C_{in}) \times F/M$, where C_{out} and C_{in} are the outflow and inflow concentrations (µg), respectively, F is the peristaltic pump flow rate (2 ml hr^{-1}), M is the wet mass of *Codium* (15g), and J is the change in concentrations (µgC g*Codium*$^{-1}$ hr^{-1}). This method measures net fluxes (production — consumption) such that positive flux of nitrogen-gas (N_2) indicates denitrification in excess of nitrogen fixation. O_2 was calculated as biological oxygen demand (BOD) and positive numbers indicate O_2 uptake in the microcosm. Individual measurements from each microcosm over time were averaged separately for both light and dark periods to yield two microcosm-specific values. Denitrification rates were analyzed using a mixed-effects ANOVA with season, species, and light/dark as fixed factors and microcosm number as a random factor. Bartlett's test was used to check for homogeneity of variance for all ANOVA's and passed unless otherwise stated.

Nitrogen fixation

The microcosm experiments measured the net flux of N_2 and nitrogen fixation associated with *C. fragile* and *C. decorticatum* was measured using an acetylene reduction assay modified for macroalgae (Head and Carpenter 1975). *Codium* (5g wet weight) was added to 160ml serum vials with 100ml of unfiltered seawater form Bogue Sound. Each species was replicated 4 times in both clear (light) and opaque (dark) bottles for a total of 16 bottles. Acetylene was added to each bottle (15% acetylene by volume) and the bottles were placed in flowing seawater under full sunlight for 4 hours. After 4 hours, 5ml of gas was removed with a syringe and transferred to 3ml serum vials. Ethylene concentration was measured with a Shidmadzu GC-9A gas chromatograph and nitrogen fixation was determined following Stewart et al. (1967) and Hardy et al. (1968).

A 2-way fixed-factor ANOVA was conducted to determine the effects of *Codium* species and light on nitrogen fixation. A constant (2) was added to denitrification rate prior to running analysis so that all rates were greater than 1, allowing square root transformation. After transformation, the variance remained slightly heterogeneous (Bartlett's Test, $p=0.035$), but balanced parametric tests are robust against slight deviations from homoscedasticity (Underwood 1997). Nitrogen fixation assays were conducted in May of 2010 in 23 º C water and were not repeated because of the observed uniformly low rates of fixation compared to denitrification.

Additional Denitrification Experiments

Additional experiments were conducted to test the possibility that our results derived from artifacts in the continuous flow microcosms. The first experiment was designed to determine if cutting the *Codium*, to standardize weight, affected the concentration of dissolved nutrients in the water and thereby affected denitrification rates. Fifteen g of freshly cut *C. fragile and C. decorticatum* (n=3 for each species) were added to separate 1000 ml bottles with 800 ml of unfiltered seawater (23 º C) from Bogue Sound. Water samples (50ml) were collected from each bottle immediately after and 24 hr following the addition of *Codium* for nutrient analysis. Experiments were conducted in the dark to mimic the initial acclimation period in the continuous flow microcosm experiments. An additional sample was taken from the water before the addition of *Codium* to determine ambient nutrient concentrations. Concentrations of dissolved nutrients were determined following the methods described above.

The second experiment tested if the minimal mixing within the microcosms, because of the low supply rate of water (2ml per min), was artificially inflating

denitrification by increasing micro-anoxic regions (Eriksson 2001). Micro-anoxic regions are small regions of no oxygen which are necessary for denitrification to occur and could be increased artificially in the low mixing environment of the microcosm. We used methods identical to the continuous flow microcosms previously described except that microcosms contained stir bars spinning at three different rates: no mixing had a stationary, cylindrical stir bar (30 mm length x 8 mm diameter); low mixing had a stir bar spinning on the bottom of the microcosm at 17 revolutions per minute (same as original experiment); and high mixing had a stir bar spinning at 78 revolutions per minute. Each mixing intensity treatment was replicated 3 times with 15 g of *C. fragile* in each microcosm. The experiment was carried out for a total of 18 hr following an 18-hr acclimation period and only in the dark. The experiment was conducted once in the summer of 2011 with unfiltered water from Bogue Sound (27ºC). Differences in denitrification rates were analyzed with a one-way ANOVA with mixing intensity as the independent variable following the analysis described for the continuous flow microcosms.

Denitrification extrapolation

Our results from sampling *Codium* and the microcosm experiments were used to estimate the amount of nitrogen removed per m^2 of artificial structure with the measured biomass of *C. fragile.* It was assumed that only *C. fragile* colonized the artificial structures because greater than 99% of *Codium* found on artificial substrata was *C. fragile.* The biomass (mean ± SE) of *C. fragile* was calculated for each quadrat sampled on artificial structures from separate linear regressions of percent cover and biomass for each season. Linear regression was used, instead of measured biomass, because biomass

was not sampled at all sites. Biomass (mean ± SE) was calculated from percent cover quadrats for each season and divided by the area of the quadrat to calculate biomass per m^2. Measured rates of denitrification (N_2 g wet weight^{-1} hr^{-1}) were multiplied by biomass per m^2 (g wet weight per m^2) in order to obtain a denitrification rate per m^2 of artificial structure (N_2 m^{-2} hr^{-1}) during each season. Error was propagated using the formula; $R \cdot [(\delta x/x)^2 + (\delta y/y)^2]^{1/2}$. Where R is the product of the means, x and y are the means being multiplied, and δ indicates the standard error of the respective means. The time submerged was calculated from the number of hours the tide was higher than 0.4m below MSL, which is the upper bound of *Codium* distribution and is a conservative calculation because denitrification only occurs when *Codium* is submerged. Hours submerged during day and night were calculated by multiplying the total hours that *Codium* was submerged for each season by the mean percent of day and night for each respective season. The hours submerged during day and night for each season was multiplied by the light/dark denitrification rates to calculate diurnal denitrification per season. We assumed that low and high tides occurred equally during the day and night during each season. The total nitrogen removed per m^2 for each season was summed to calculate the amount removed per m^2 per year.

Results

Surveys

C. decorticatum and *C. fragile* both occurred from approximately 0.4 to 1.0m below MSL. Within this elevation *Codium* was a dominant macroalga on artificial substrates but not on natural substrate (Fig. 5.2). *Ectocarpus* sp. was, generally, the second most abundant taxon and covered approximately 6% of the surface on both

artificial and natural substrate. *Sargassum* sp. was, on average, more abundant on artificial (6 percent cover) than natural substrate (0.5 percent cover). Other genera covering greater than 1% of the surface included *Bryopsis sp.*, *Aurosiphonia arcta*, *Hypnea cornata*, and *Dictyota dichotoma*. Overall, 36 taxa were identified during sampling, 28 on artificial substrates and 24 on natural substrates. Natural and artificial substrates had significantly different community compositions (PERMANOVA, sample statistic=0.188, p=0.001, untransformed data), but the dispersion was also significantly different for untransformed data. Log transformation did not improve dispersion and untransformed data was used for the analysis. Dispersion is analogous to an ANOVA having heterogeneous variance. Although we cannot make definitive conclusions about the significance of observed differences in community assemblages on artificial and natural substrates, the artificial substrates had greater variability in composition (PERMDISP; f=166.5, p=0.001). Substrate type and season significantly affected the percent cover of *Codium* (Appendix 5.A). The interactions were not significant, and therefore removed from the model (Crawley 2007). A 2-season model (fall/winter and spring/summer) was not significantly different from the model with 4 seasons so the 2-season model was used because it had fewer levels (Crawley 2007). Substrate and season were significant in the final model. *Codium* on artificial substrates was dominated by non-native *C. fragile* (771 of 779 samples; Fig. 5.3), while natural substrates were dominated by *C. decorticatum* (63 of 73 samples). There was a significant difference in the abundance of *C. fragile* and C. *decorticatum* between natural and artificial substrates (t-value=2.696, df=38, p=0.007)

Nitrogen experiments

Denitrification rates were different between species, but there was a significant interaction between species and season (Appendix 5.B, Fig. 5.4). The different rates between species resulted from the high denitrification rates in *C. fragile* compared to *C. decorticatum* in summer, which also caused the significant interaction between species and season. Within microcosms, light and the interaction between season, light, and species were significant (Appendix 5.B, Fig 5.4). Post-hoc tests were not run because they are inappropriate for a mixed effect model so interactions were assessed using inspection of the figures. The significant 3-way interaction within microcosm resulted from the *C. fragile* in summer having high rates at night compared to *C. decorticatum*. Fluxes of DIN, DON, and BOD (mean± SE) are presented (Table 5.1) to infer mechanisms of denitrification and analyses were not conducted on these fluxes. On average, DIN uptake was greatest in the summer, with *C. fragile* taking up more than *C. decorticatum*. *C. decorticatum* had higher rates, on average, of DON uptake in the summer and in the dark compared to *C. fragile*. There was a trend for increased BOD by *C. fragile.*

Additional denitrification experiments

Nitrogen fixation rates were significantly different between light and dark samples but not between *Codium* species (Appendix 5.C). *Codium* fronds added to vials increased concentrations of DIN and DON at time 0, but DIN and DON concentrations were similar to their initial concentrations after 24 hours (Appendix 5.D). Mixing intensity did not significantly change denitrification rates, although highest rates were seen with the highest mixing ($f_{2,6}$=1.918, p=0.227; Appendix 5.E).

Extrapolation

The nitrogen removed by *C. fragile* per m^2 of artificial structure was calculated for day and night during three seasons (Table 5.3). Relatively high *C. fragile* biomass (Appendix 5.F) and high denitrification rates (Fig. 5.4) produced the highest estimates of nitrogen removal rates in the summer. The total amount of nitrogen removed per m^2 of artificial substrate (0.4-1m below MSL) by measured abundances of *C. fragile* was 5.8 ± 0.7g N m^{-2} $year^{-1}$.

Discussion

Artificial substrates have greater abundances of non-native species, especially in areas where natural, hard substrates are limited (Wasson et al. 2005, Tyrrell and Byers 2007, Vaselli et al. 2008). Our study supported this pattern: the non-native *C. fragile* was the dominant *Codium* species on artificial substrates (>99%), while the native *C. decorticatum* was the dominant *Codium* on natural substrate (~80% of *Codium*). In addition, *C. fragile* was the most abundant algae on artificial substrates during spring and summer within the sampled tidal range. This study demonstrated that the abundance of two similar species within the same genera, one native and one non-native, was dependent of substrate type.

The difference in *Codium* abundance on artificial and natural substrate could result from multiple factors including differences in environmental factors and *Codium* morphology. For instance, the intertidal oyster reefs (natural substrate) were in tidal creeks with low flow and low wave energy compared to the environmental setting of the shoreline hardening structures. Native *C. decorticatum* was usually tall (> 40cm) with flattened fronds (>1 cm), and was often isolated (>1m) from other *C. decorticatum* individuals (NRG per. obs.). The morphology of *C. decorticatum* seems better adapted

for the low-energy environment that has historically harbored the only natural, hard substrate in North Carolina. In contrast, *C. fragile* was shorter (<20 cm) and bushy with thin fronds (< 1 cm) and often existed in dense patches, which reduces the potential for dislodgement from the shoreline hardening structures that are in higher-energy environments (Denny et al. 1985, Kawamata 2001, Pratt and Johnson 2002, Thomsen and Wernberg 2005). Additionally, the shoreline hardening structures were made primarily of granite, a substrate which is not found naturally in North Carolina but is similar to the rocky shorelines that *C. fragile* has successfully invaded throughout the world (Trowbridge 1998). Because substrate type and amount of energy in the environment were confounded in this study, future experiments should to be conducted to elucidate the mechanisms that are shaping *Codium* distributions.

We found that differences in *Codium* distributions can have significant effects on nitrogen cycling. The potential for multiple *Codium* species to alter nitrogen pools through microbially-mediated nitrogen transformations has been studied (Head and Carpenter 1975, Dromgoole et al. 1978, Gerard et al. 1990), but past work focused only on nitrogen fixation. We found measured rates of nitrogen fixation similar to those reported previously in North Carolina measured by acetylene reductions assays (0.02-1.2μgN_2 fixed g dry wt^{-1} h^{-1}; Rosenberg and Paerl 1981). However, we found that the flux of N_2 gas was positive indicating that denitrification was greater than nitrogen fixation.

Net nitrogen gas production suggests that *Codium* species may be "hot spots" for denitrification (McClain et al. 2003). Denitrification requires a low-oxygen environment, availability of nitrate, and an electron donor (e.g., carbon). Previous work suggests that *C. fragile* uptakes DIN from the water column (nitrate and ammonium) at high rates (>25

μmol of N per g of dry wt per h; Hanisak and Harlin 1978). While a majority of this DIN is likely assimilated by the algae, a portion of the DIN may be utilized for denitrification which was not measured in previous studies. However, we found relatively low uptake of nitrate by *Codium.* Relatively low uptake of nitrate by *Codium* and low concentrations of ambient nitrate dissolved in the water suggest that the source of nitrate for denitrification is from nitrification of ammonium rather than from the water column (Eriksson and Weisner 1999, Krause-Jensen et al. 1999, An and Joye 2001). The coupling of nitrification and denitrification associated with *Codium* could result from the presence of epiphytes. Epiphytes have been shown to affect oxygen gradients associated with macroalgae such that aerobic processes occur in the surface layer of the algae and anaerobic processes occur in adjacent deeper layers of the thallus tissues (Nielsen et al. 1990, Krause-Jensen et al. 1999). The nitrate produced from ammonium by nitrification in the aerobic zone could be used for denitrification in the nearby anoxic zone. In addition, the epiphytes and macroalgae could serve as the energy source (electron donor) for the denitrifying bacteria by supplying large amounts of labile organic matter (Khailov and Burlakova 1969, Tyler et al. 2003). The simultaneous occurrence of these conditions on macroalgal surfaces probably results in microzones where conditions are favorable for denitrification (Law et al. 1993).

Previous work on nitrogen dynamics associated with macroalgae have focused on the effects of the algae on sediment microbial processes in the sediment such as competition for nutrients (Tyler et al. 2003, Hardison et al. 2011, Eyre et al. 2011). *Codium* on artificial structures is not directly associated with soft sediment and our study demonstrates that denitrification can be directly attributable to macroalgae and the

associated epiphytic community and not sediment microbial processes. The significantly higher rates of denitrification associated with *C. fragile* compared to *C. decorticatum* could result from different epiphytic communities on the two *Codium* species (Lutz et al. 2010). Although epiphytic denitrification has been associated with freshwater macrophytes (Eriksson 2001), this relationship for macroalgae remains unclear. Previous studies of nitrogen dynamics with macroalgae have examined algae that do not live long enough to develop epiphyte communities (Tyler et al. 2003, Hardison et al. 2011) or have chemical defenses limiting epiphytes (Eyre et al. 2011). Further research must be conducted to understand the mechanisms and factors that affect denitrification associated with epiphytes (Cornwell et al. 1999), *C. fragile* (Howarth et al. 2011), and other macroalgae (Rysgaard et al. 1995, Tall et al. 2011). Such information is necessary to determine whether *C. fragile* is unique in providing nitrogen removal or denitrification is often associated with macroalgae, in which case *C. fragile* may not have an overall ecosystem service benefit if *C. fragile* only supplants native macroalgae with similar properties.

We measured denitrification rates associated with *Codium*, but there were potential methodological artifacts that might have biased our results. Such artifacts include nutrients leaking from the *Codium* into the microcosm that resulted from the wound sustained during collection, and the creation of low oxygen microzones from minimal mixing in microcosms. First, nutrients did leach from sampled *Codium*, but nutrient concentrations returned to ambient levels within 24 hours and likely did not affect denitrification rates because nitrogen measurements were not taken until 24 hours after *Codium* was added to the microcosms. Second, increased mixing within microcosm

did not decrease denitrification and there was a trend for increased denitrification. This indicates that our measurements in microcosms are not inflating denitrification, but may be underestimating denitrification.

To extrapolate laboratory rates to the ecosystem, we assumed a positive linear relationship between biomass and denitrification. This assumption is based on past findings that denitrification associated with macrophytes is a function of surface area (Eriksson and Weisner 1999), which is correlated with biomass. Moreover, our findings are probably conservative for two reasons. First, there was a trend for higher denitrification in the high-mix treatment, which probably increased rates of exchanges across the water-alga interface and more closely resembles environmental conditions than the treatment that was used for our calculations. Second, granite boulders that are used to harden shorelines have a complex 3-dimensional structure and the amount of *Codium* per m^2 is greater than the amount in the planar m^2 used for our calculations, which would result in our estimate of denitrification being conservative.

We found that *C. fragile* enhances denitrification, an important ecosystem service that removes biologically available nitrogen, which could be substantial in areas where the coastline is dominated by hard substrate. *C. fragile* is now a dominant species in areas along the Gulf of Maine, where it is found from the intertidal to depths of 8 m and has a maximum biomass of 10.2 kg m^{-2} (Mathieson et al. 2003). In areas along the coast of Chile, *C. fragile* averages 22.9 kg m^{-2} (Neill et al. 2006). Assuming environmental conditions similar to North Carolina and given our measured rates of denitrification, *C. fragile* could remove a significant amount of nitrogen in these areas: 500m^2 of *C. fragile* during the four month warm season in Chile could remove 22kg N_2.

Recent studies on other habitat-forming organisms corroborate our findings that biogenic habitats can be denitrification hotspots. Sediment patches within seagrass beds and adjacent oyster reefs had similar annual rates of areal denitrification compared to *Codium* (Piehler and Smyth 2011). Few studies have measured denitrification rates of organisms (algae, oysters, or seagrass) and their accompanying communities. *In situ* rates of denitrification associated with seagrass beds, measured with large batch cores, are temporally and spatially variable, ranging from 476 to 4480 μg N- N_2 m^{-2} h^{-1} respectively (Eyre et al. 2010, 2011). However, these studies did not quantify seagrass density or biomass, or determine if denitrification occurred in sediments or on the seagrass, so the contribution of seagrass to the measured denitrification rates cannot be inferred. Understanding the mechanisms that affect nutrient removal within these habitats is a prerequisite if biogenic habitats are to be included in management schemes to mitigate nutrient pollution.

Nutrient loading in coastal waters is one of the greatest threats to conservation and restoration of coastal ecosystems (Conley et al. 2009). Excessive inputs of nutrients can lead to eutrophication, which includes low-oxygen events, fish kills, harmful algal blooms, and shellfish closures. Reducing nutrient loading by regulation alone is not feasible given that the majority of nutrient inputs cannot be traced to specific sources (Bricker et al. 1999) and the lack of political will to ratify appropriate management strategies. To reduce nutrient loading, management strategies must include increasing the denitrification capacity of the system by increasing areas with high rates of denitrification or hotspots (Brush 2008). The overall effect of *C. fragile* and artificial structures on coastal ecosystems is complex because altering habitats by adding shoreline hardening

structures can also have negative impacts. For instance, bulkheads and revetments alter and/or remove the natural transitional habitat from marine to terrestrial environments, salt marshes, which provide ecosystem services such as fish habitat (Peterson et al. 2000, Seitz et al. 2006, Bilkovic and Roggero 2008) and nitrogen removal (Piehler and Smyth 2011). But, revetments made of boulders can provide habitat, which likely replace some or all of the services provided by the natural habitat (Bilkovic and Roggero 2008). Jetties provide hard substrate in an environment that was previously shifting soft sediment and, although they alter geological processes, jetties can provide ecosystem services that did not exist previously, such as providing substrate for habitat forming species that in turn provide fish habitat (Hay and Sutherland 1988) and remove nitrogen. The overall gains or losses of ecosystem services provided by artificial structures is complex (Chapman and Underwood 2011), and will depend on quality and quantity of both original habitat and the habitat provided by the artificial structure.

Anthropogenic impacts change environments both locally and globally. The ability of coastal managers to mitigate such changes, which often occur simultaneously, depends on understanding mechanisms associated with multiple impacts (Cloern 2001). We found an unexpected positive effect emerging from the interaction between habitat alteration and non-native species. Although non-native species do have many negative impacts, positive aspects of invasions have been found (Posey 1988, Pejchar and Mooney 2009, Carroll et al. 2010). Pursuing eradication as the recommended reaction to invasive species has become a contentious issue, because of the high economic cost and low success rate of eradication (Gozlan 2008, Schlaepfers et al. 2011, Davis et al. 2011). Making decisions on how to manage non-native species without knowing if interactions

occur with other anthropogenic stressors could mitigate or exacerbate environmental impacts. This study reveals that not all non-natives may merit the same level of management concern and non-native species and shoreline hardening can interact to have a positive effect on coastal ecosystems.

Table 3.1. The variables and formulas used to calculate indirect effects (upper panel) and the experimental results (lower panel). Lower case letters denote presence of predator and upper case denotes absence of predator. Estimation of the resource release resulting from predators consuming prey (DMIE), the dispersal of prey (DR), the reduction in prey activity induced by a predator (AyR), and the percent contribution of predator-avoidance behaviors in indirect effects.

Mesocosm condition	Predator	No. crabs eaten	No. crabs dispersed	Mussel consumption (mussels·d^{-1})	Standardized mussel consumption (mussels·$crab^{-1}$·d^{-1})	Predation resource release (DMIE, mussels·d^{-1})	Actual resource release (mussels·d^{-1})	Dispersal resource release (mussels·d^{-1})	Activity resource release (mussels·d^{-1})	Dispersal & activity resource release (TMIE, mussels·d^{-1})	Indirect effects attributable to TMIE (%)
Closed	No			M	C	DMIE=p·c	AR=M-m		AyR=AR-DMIE	TMIE=AyR	(AR-DMIE)/AR
	Yes	p		m	c						
Open	No		E	M	C	DMIE=p·c	AR=M-m	DR=(c·e)-(C·E)	AyR=AR-DMIE-DR	TMIE=AyR+DR	(AR-DMIE)/AR
	Yes	p	e	m	c						
Closed	No			8.70±1.82	1.82±0.33	1.31±0.80	5.50±1.74	(0)	4.19±2.06	4.19±2.06	76%
	Yes	1.33 ±0.42		3.19±1.32	0.80±0.36						
Open	No		1.33±0.21	10.00±2.70	2.22±0.60	0.46±0.46	5.75±2.97	-2.21±0.70	7.58±3.46	5.37±3.13	92%
	Yes	0.17 ±0.17	1.33±0.42	4.25±1.83	0.97±0.43						

Table 4.1. Size measurements of oysters, mud crabs, and toadfish in Exp 1 and 2. The biomass of individual mud crabs was measured during Exp 2. The biomass per size class was calculated by multiplying the average biomass in each size class to the number of crabs in the respective size class treatment. Standard error (SE) and number of individuals measured (n) are included.

		Oyster	Mud crab			Toadfish		
			Small	Medium	Large	Small	Medium	Large
Experiment 1	Mean ± SE.	10.9±0.3	12.4±0.2	20±0.2	30.5±0.4	198.1±8.8	270.4±2.5	321.9±4.5
Size (mm)	n	249	135	134	137	21	22	21
	Range	4.4-23.4	9-15.4	15.3-24.8	25-44.3	95-248	250-286	294-358
Experiment 2	Mean ± SE	8.3±0.3	12.5±0.2	20±0.2	29.6±0.4		264.1±6.6	
Size (mm)	n	70	70	70	70		24	
	Range	4.4-13.4	9-14.9	16.4-24.6	23.4-39.5		195-313	
Overall	Mean ± SE		12.4±0	19±0.1	30.8±0.9	196.8±14.3	375.7±16.6	587±41.2
Biomass (g)	n		122	157	61	23.0	19.0	19.0
	Range		0.16-2	0.76-6.09	4.45-38.26	62-384	252-551.1	220-934
Experiment 1 Biomass (g)	Mean ± SE		148.8±0.3	133±0.7	154±4.6			

Table 5.1. Nutrient fluxes (mean±SE) from inflow and outflow of microcosms for native (*C. decorticatum)* and non-native (*C. fragile*) *Codium* during 3 seasons and light and dark sampling periods. For dissolved nutrients positive values indicate production in the microcosm and negative values indicate uptake. O_2 fluxes are presented as biological oxygen demand (BOD) where positive values indicate uptake of oxygen within microcosms.

Season	Light/Dark	Species	DIN ($\mu gN*g^{-1}*hr^{-1}$)	DON ($\mu gN*g^{-1}*hr^{-1}$)	BOD ($\mu gO*g^{-1}*hr^{-1}$)
Summer	Light	Native	-0.21±0.01	0.14±0.02	-0.32±0.18
		Non-native	-0.4±0.14	-0.04±0.13	9.7±4.41
	Dark	Native	-0.05±0.02	-0.87±0.03	6.27±0.43
		Non-native	-0.02±0.04	-0.33±0.26	10.86±3.12
Fall	Light	Native	-0.05±0.02	-0.07±0.03	3.18±0.59
		Non-native	0.05±0.09	-0.35±0.09	3.74±0.74
	Dark	Native	-0.02±0.02	0.34±0.07	4.48±2.19
		Non-native	-0.04±0.04	0.24±0.14	3.26±0.74
Spring	Light	Native	-0.09±0.04	0.19±0.11	4.48±0.49
		Non-native	-0.13±0.03	-0.03±0.05	4.11±1.08
	Dark	Native	-0.01±0.01	-0.3±0.14	6.33±1.17
		Non-native	0.86±0.86	0.4±0.38	18.41±8.16

Table 5.2. Nitrogen fixation resulting from native (C. decorticatum) vs. non-native (C. fragile) Codium (mean±SE). Native and non-native species were not significantly different, but light/dark was significantly different (2-way ANOVA, $p<0.05$).

Light/Dark	Species	N-fixation ($\mu gN*g^{-1}*hr^{-1}$)
Light	Native	0.71±0.25
	Non-native	0.48±0.3
Dark	Native	0.12±0.04
	Non-native	0.05±0.03

Table 5.3. Extrapolation of denitrification associated with C. fragile on artificial substrates. The denitrification rate from light and dark for each season was multiplied by the mean biomass per m^2 of *C. fragile* on artificial substrates to calculate the denitrification rate per m^2. The denitrification rate per m^2 was then multiplied by the hours submerged to determine the denitrification rate for light and dark in each season. Yearly denitrification was calculated by summing the seasonal denitrification for light and dark rates for all seasons.

Season	Light	Microcosm denitrification ($\mu gN\text{-}N_2*g$ *Codium*$^{-1}*hr^{-1}$)	Biomass of *Codium* ($g*m^{-2}$)	Denitrification ($\mu gN\text{-}N2*m^{-2}*hr^{-1}$)	Hours sub-merged ($hr*season^{-1}$)	Seasonal denitrification ($kgN\text{-}N_2*m^{-2}*season^{-1}$)	Yearly denitrification ($kgN\text{-}N_2*m^{-2}*year^{-1}$)
Fall	Dark	0.25±0.08	1088±0.1	276±4.8	1056	2.91E-4±5.06E-6	5.78E-3±7.32E-5
Fall	Light	0.08±0.13	1088±0.1	86±25.7	946	8.17E-5±2.44E-5	
Spring	Dark	0.85±0.53	1306±0.1	1111±10.0	852	9.47E-4±8.54E-6	
Spring	Light	0.15±0.18	1306±0.1	193±19.7	1008	1.94E-4±1.99E-5	
Summer	Dark	1.06±0.38	2784±0.1	2938±5.7	801	2.35E-3±4.57E-6	
Summer	Light	0.61±0.37	2784±0.1	1703±9.6	1124	1.91E-3±1.08E-5	

Fig. 2.1. Locations of sampled oyster sanctuaries in Pamlico Sound. Experimental sanctuaries had 5 shell/seed treatments. NCDMF seeded sanctuaries had either seeded or unseeded mounds and were sampled by NCDMF.

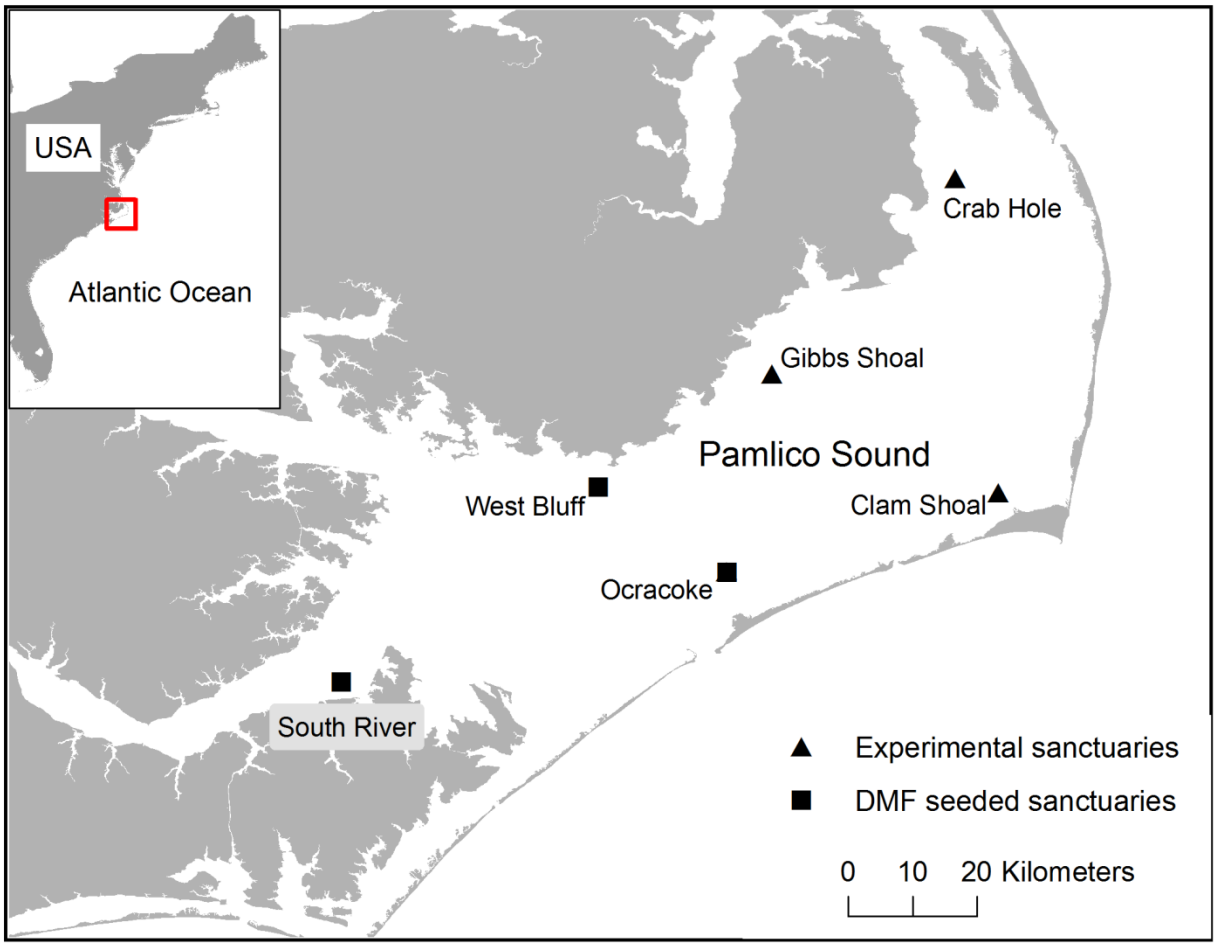


Fig. 2.2. Salinities through time at the three experimental sanctuaries. Salinities were recorded every 30 minutes by a logger placed on the top of a mound. The mean salinity for each day is shown.

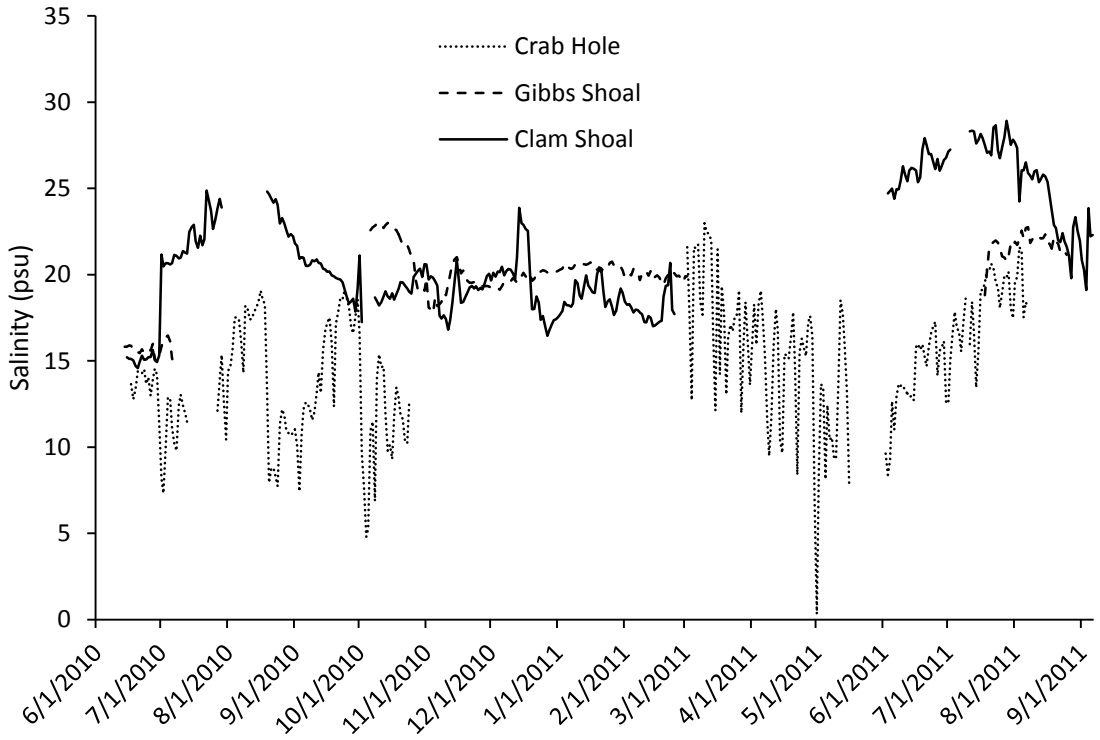


Fig. 2.3. Number of oysters per shell (mean ± 1 SE) before shells were deployed in the summer of 2010 (A), after deployment in the fall Oct. 2010 (B) and the following year in Sept. 2011 (C). Number of shells sampled are noted above the x-axis.

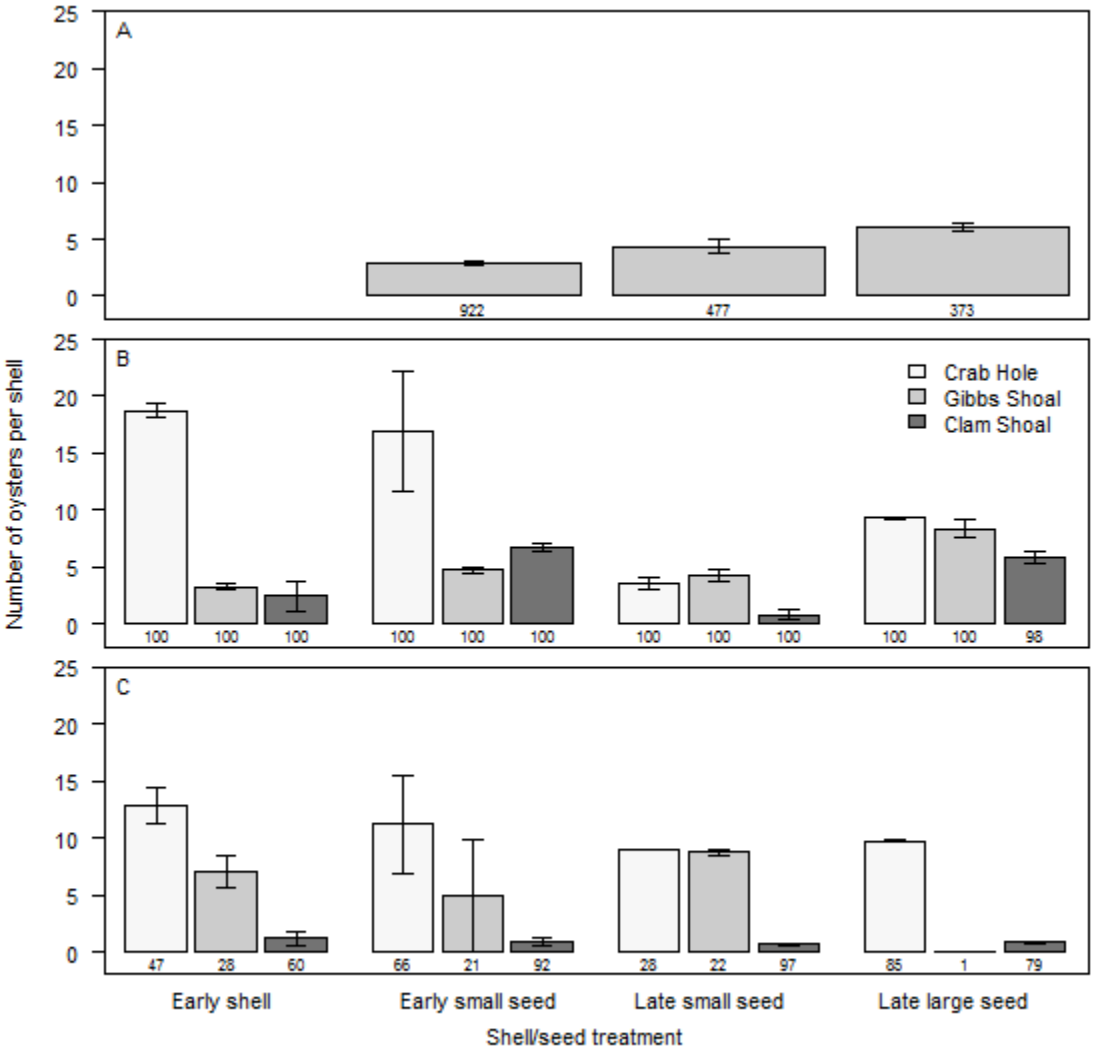


Fig. 2.4. Size of oysters (mean±SE) per shell before shells were deployed during the summer of 2010 (A), after deployment in the fall Oct. 2010 (B) and the following year in Sept. 2011 (C).

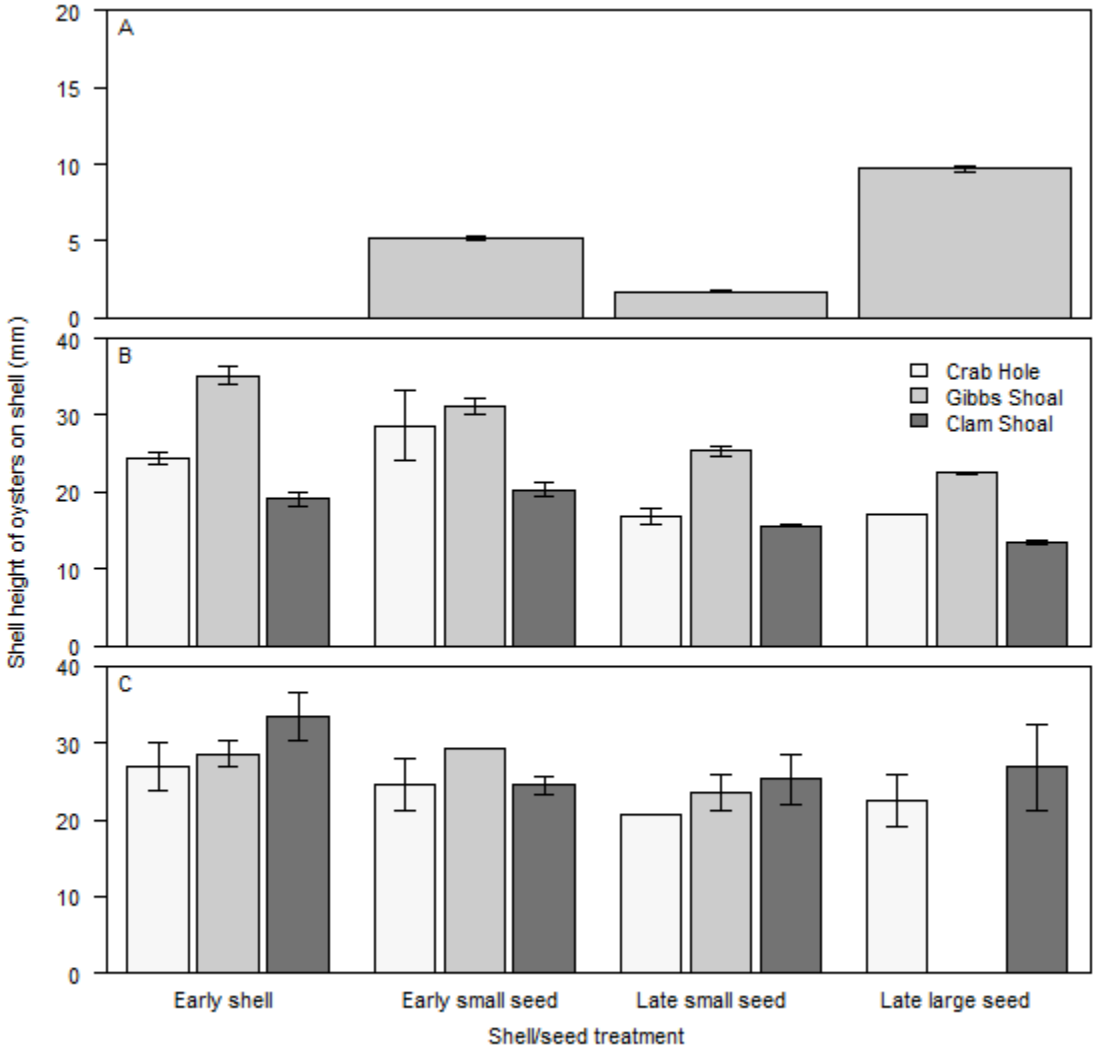


Fig. 2.5. The density of oysters (m^{-2}; mean±SE) on sampled marl in fall 2010 (A), spring 2011 (B), and fall 2011 (C). The density of oysters on mounds created in 2005-2006 were included in the figure as a baseline for successful restoration (Established), but were not included in the analysis.

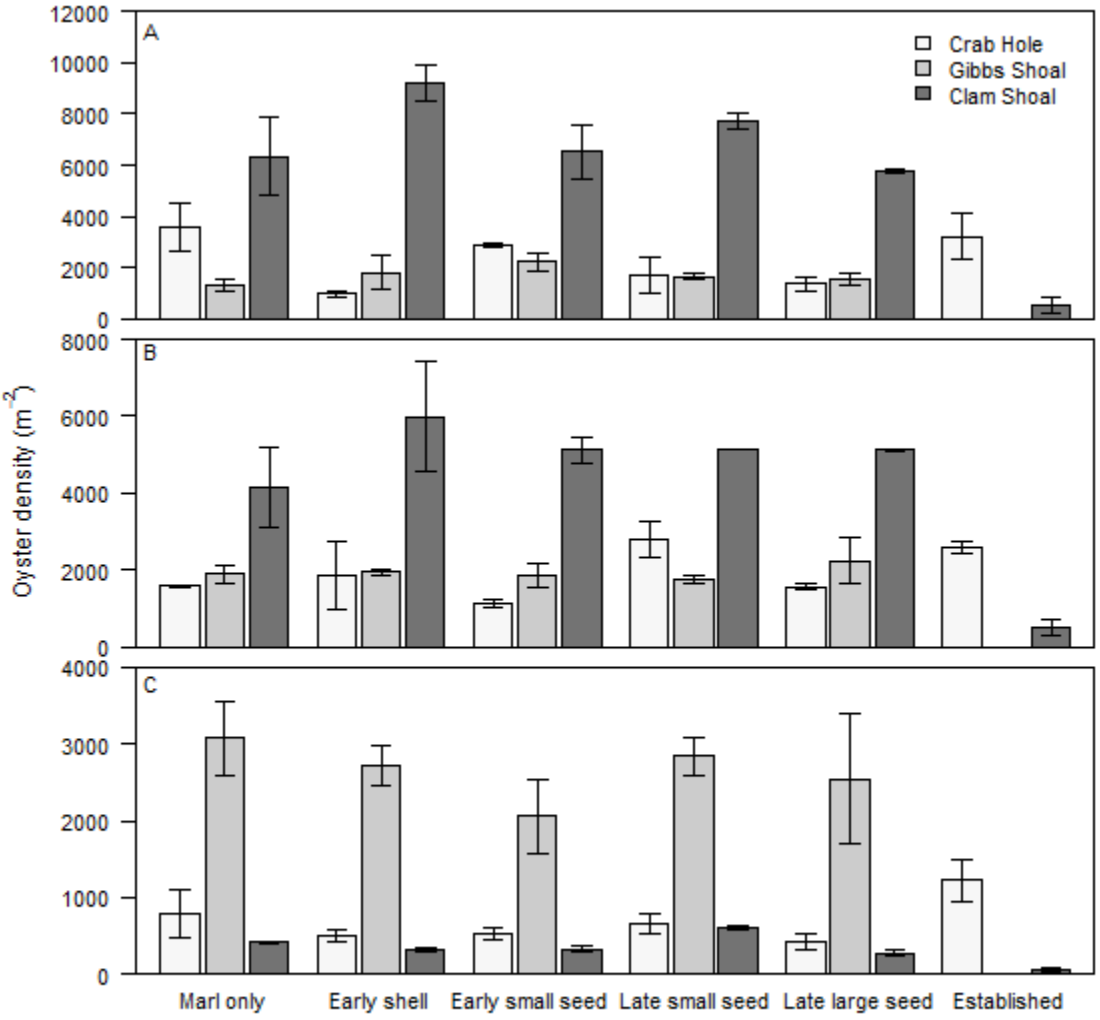


Fig. 2.6. The density of oysters (m^{-2}; mean±SE) on sampled marl from mounds seeded in 2006 in South River (A), 2008 in South River (B), 2008 in West Bluff (C) and 2006 in Ocracoke (D). Number of mounds sampled are noted above the x-axis.

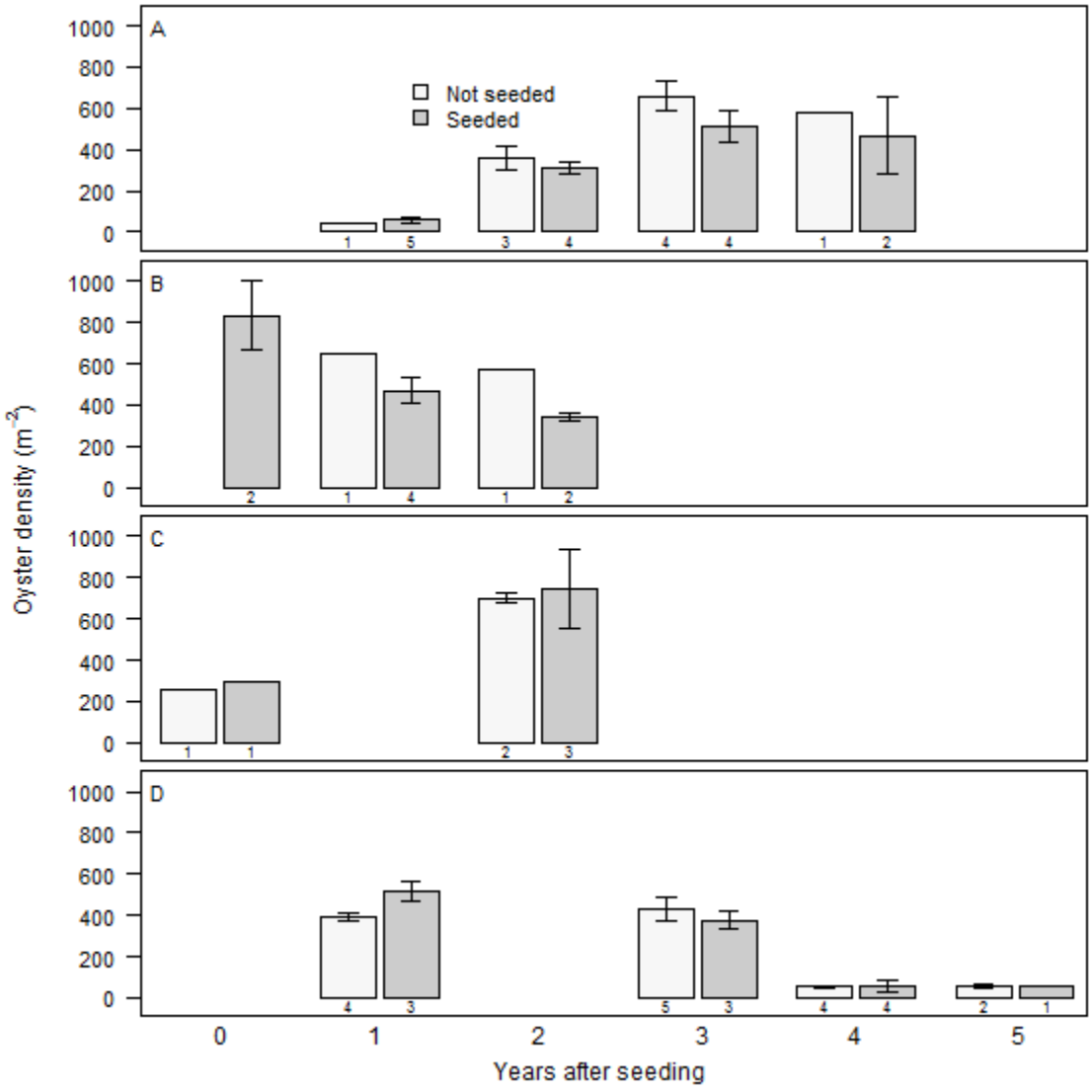


Fig. 3.1. The experimental setup of the study showing open and closed mesocosms and the initial placement of mud crabs.

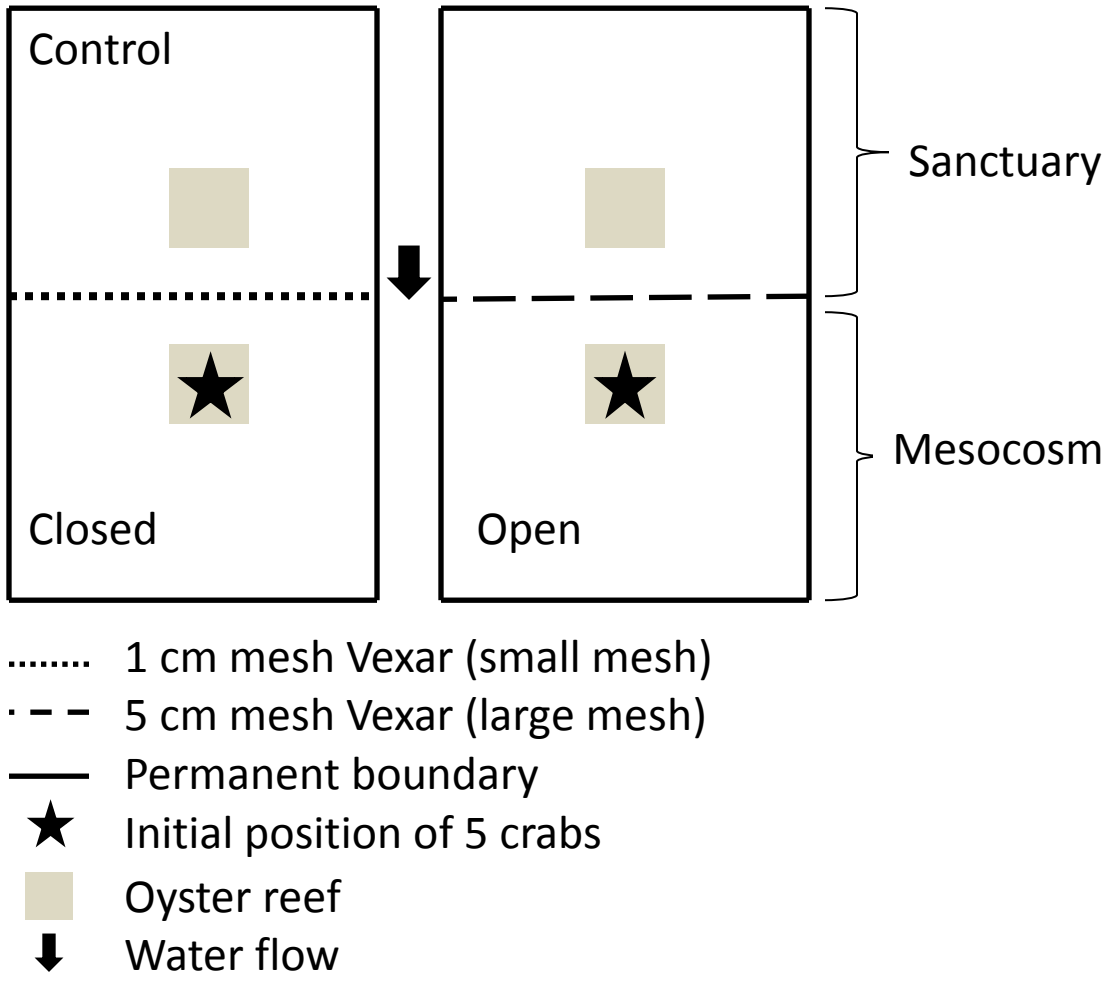


Fig. 3.2. Summary of crab (prey) data showing: (A) the mean proportion (±SE) of crabs eaten per trial by toadfish in open and closed mesocosms; (B) the mean proportion (±SE) of surviving mud crabs remaining in the mesocosms; (C) the mean number (±SE) of mud crabs that dispersed into sanctuaries; and (D) the proportion of crabs observed along the edges of mesocosms. The number of crab observations was standardized for both number of observations per trial and by the average number of crabs. Significant effects ($p < 0.05$) of mesocosm design are indicated by asterisks, and the significant effect of toadfish presence/absence is indicated by a tilde.

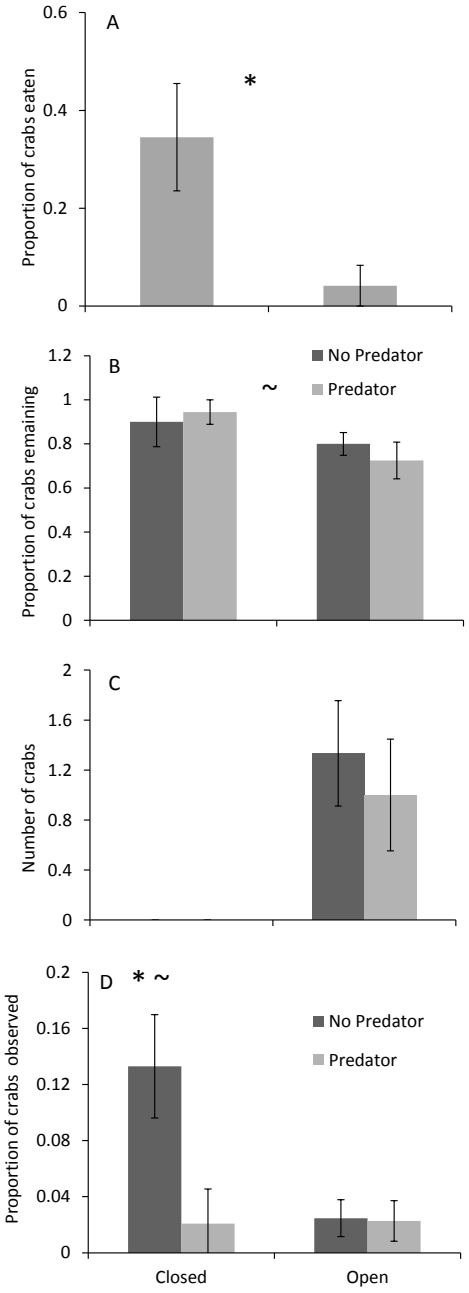


Fig. 3.3. Summary of mussel (resource) data showing: (A) the percent mortality of mussels per day in mesocosms and; (B) the percent mortality of mussels per day in sanctuaries.

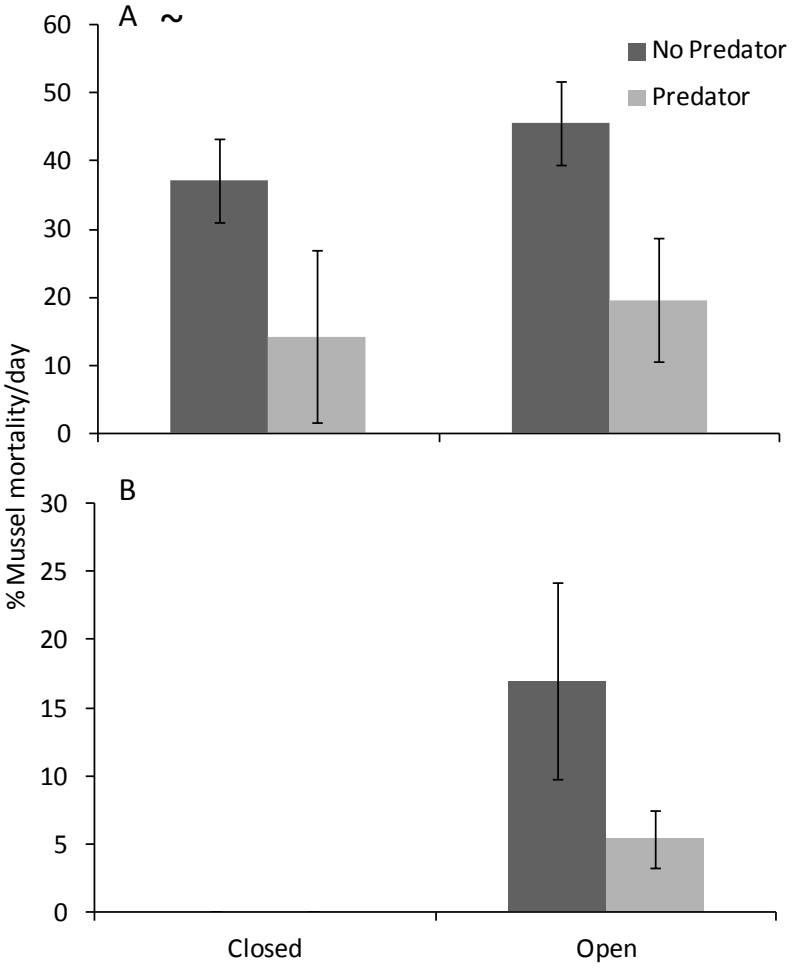


Fig. 4.1 Crab biomass consumed in treatments with 3 individual crab sizes (A), four predator treatments (B), and all crab sizes with 4 predator treatments (C) Exp 1. The biomass was calculated by multiplying the number of crabs eaten by the average biomass of individuals in the respective size class (Table 1). Lower case letters indicate significant differences between treatments. Significance was tested using 2 separate ANOVAs to keep crab biomass consistent (individual crab sizes with light gray boxplots, $p \leq 0.05$ and all crab size classes with dark gray boxplots, $p \leq 0.1$). Boxplots show inner 2 quartiles within box and whiskers extent to 1.5 times the respective inner quartile. The line through the box, cross, and dots indicate median, mean, and outliers, respectively.

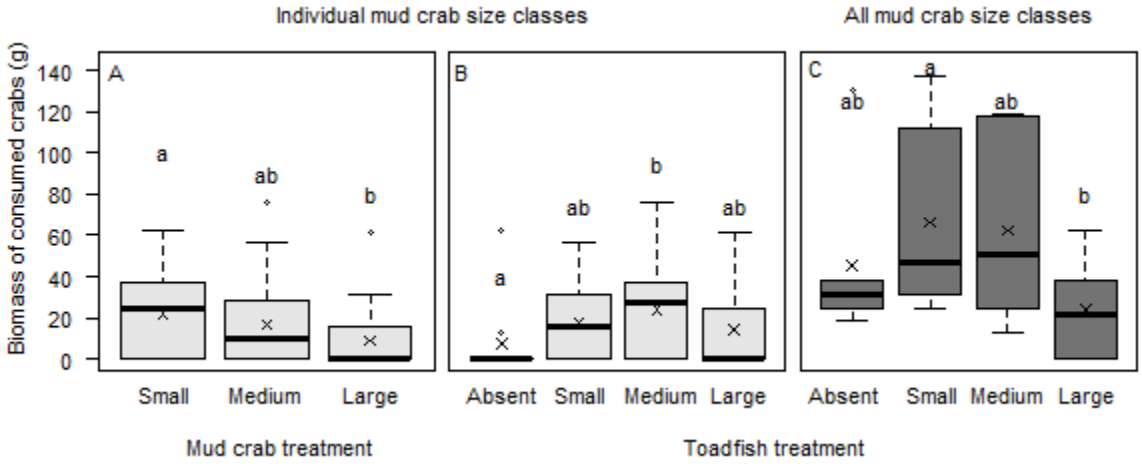


Fig. 4.2. Total number of oysters eaten (A) and the number of oysters eaten per crab (B) found in Exp 1. Lower case letters indicate significant differences between treatments ($p \leq 0.05$). Boxplots show inner 2 quartiles within box and whiskers extent to 1.5 times the respective inner quartile. The line through the box, cross, and dots indicate median, mean, and outliers, respectively.

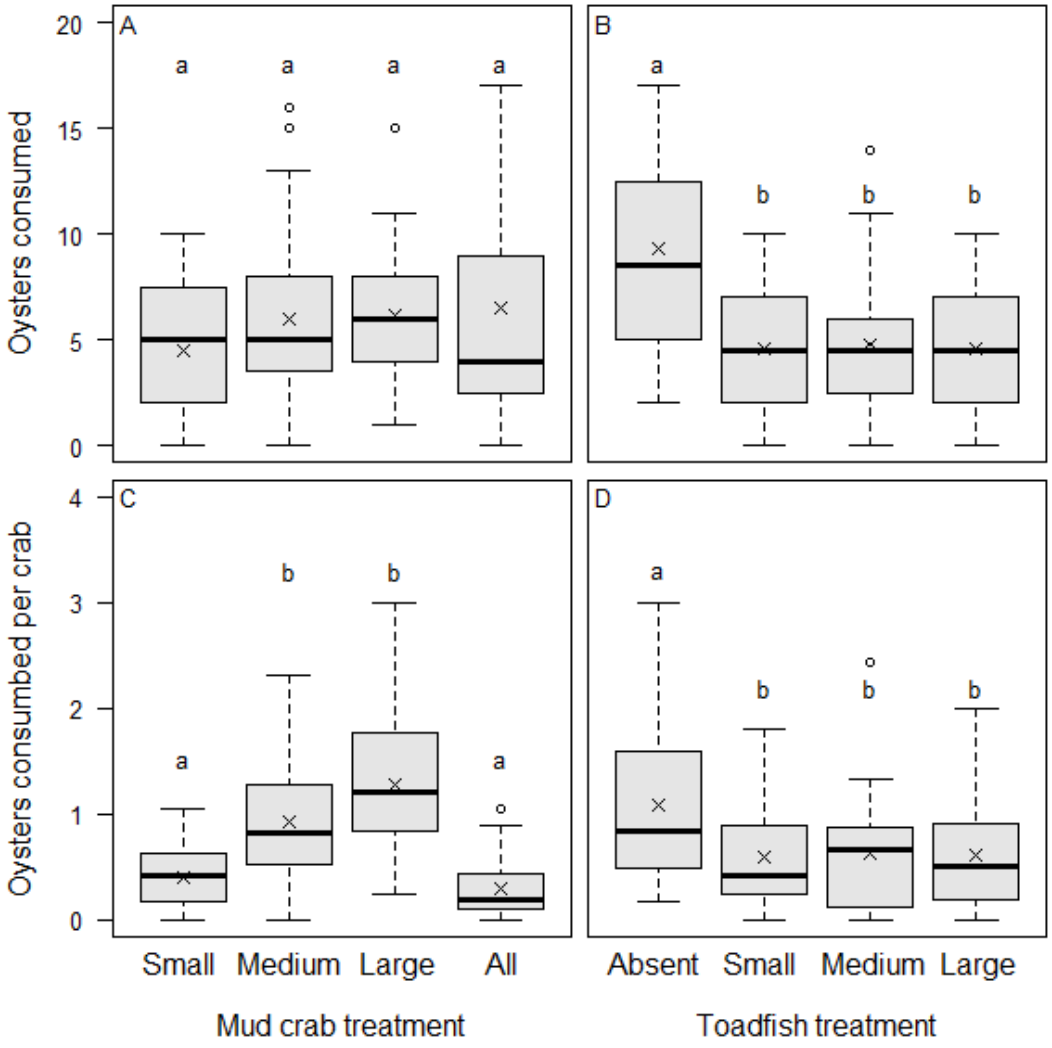


Fig. 4.3 Estimated number of oysters that survived because of prey being consumed (ER; A-C) and changes in behavior (BR; D-E). The ratio of BR to overall resource release (G-I) in Exp 1. Lower case letters indicate significant differences between treatments. Significance was tested using 2 separate ANOVAs ($p \leq 0.05$) for each BR and ER to keep crab biomass consistent (individual crab sizes in, light gray and all crab size classes, dark gray). No significance test was run on the behavioral ratio because error was propagated. Boxplots (A-E) show inner 2 quartiles within box and whiskers extent to 1.5 times the respective inner quartile. The line through the box, cross, and dots indicate median, mean, and outliers, respectively. G-I show mean (cross) and standard error.

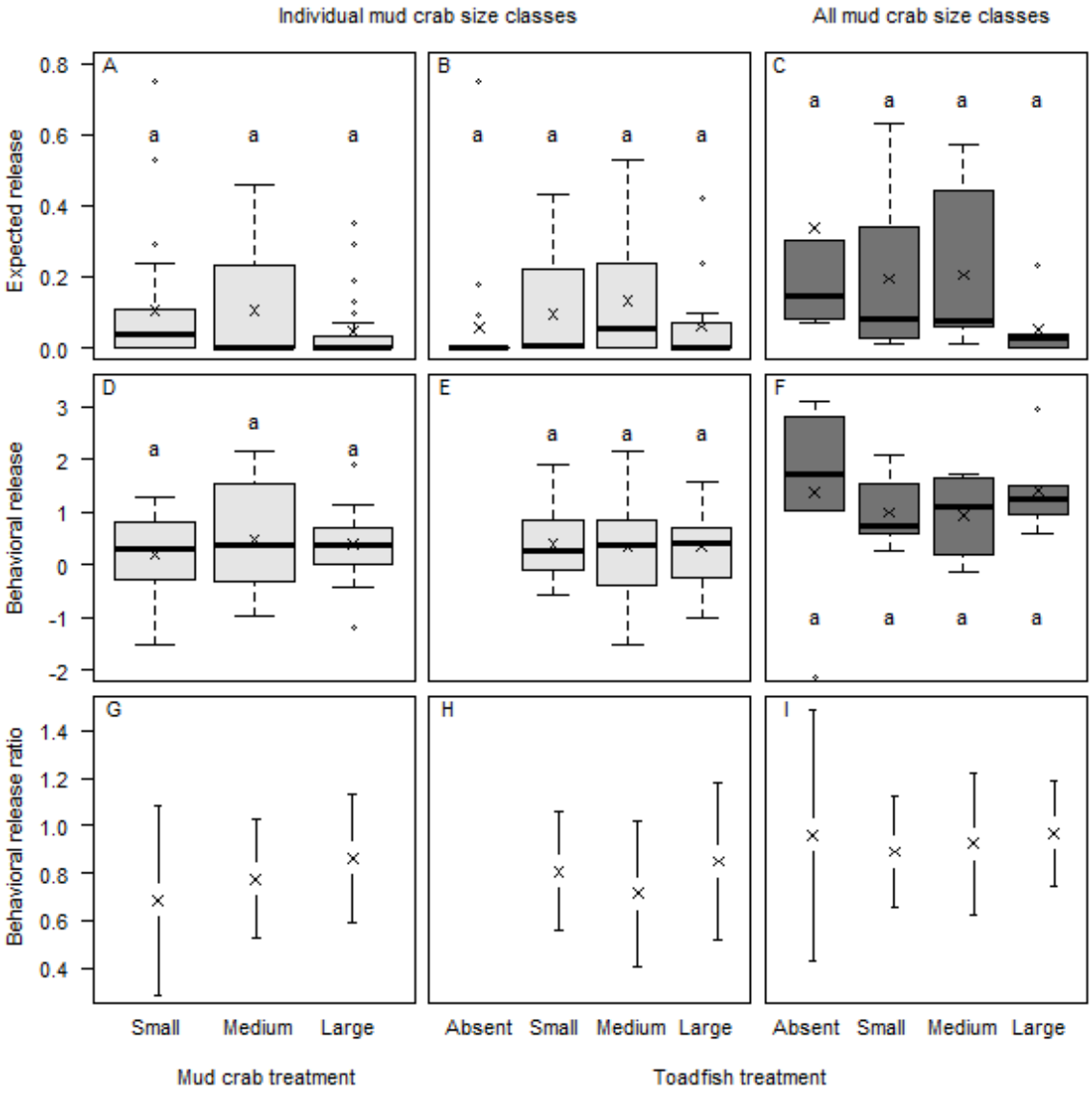


Fig. 4.4. Crab biomass consumed in Exp 2 for treatments with small crabs (A) and all crab sizes (B). The predator was either absent, present in a cage within the mesocosm, or present and free in the mesocosm. The biomass was calculated by multiplying the number of crabs eaten by the average biomass of individuals in the respective size class (Table 1). Lower case letters indicate significant differences between treatments. Significance was tested using 2 separate ANOVAs ($p \leq 0.05$) to keep crab biomass consistent. Boxplots show inner 2 quartiles within box and whiskers extent to 1.5 times the respective inner quartile. The line through the box, cross, and dots indicate median, mean, and outliers, respectively.

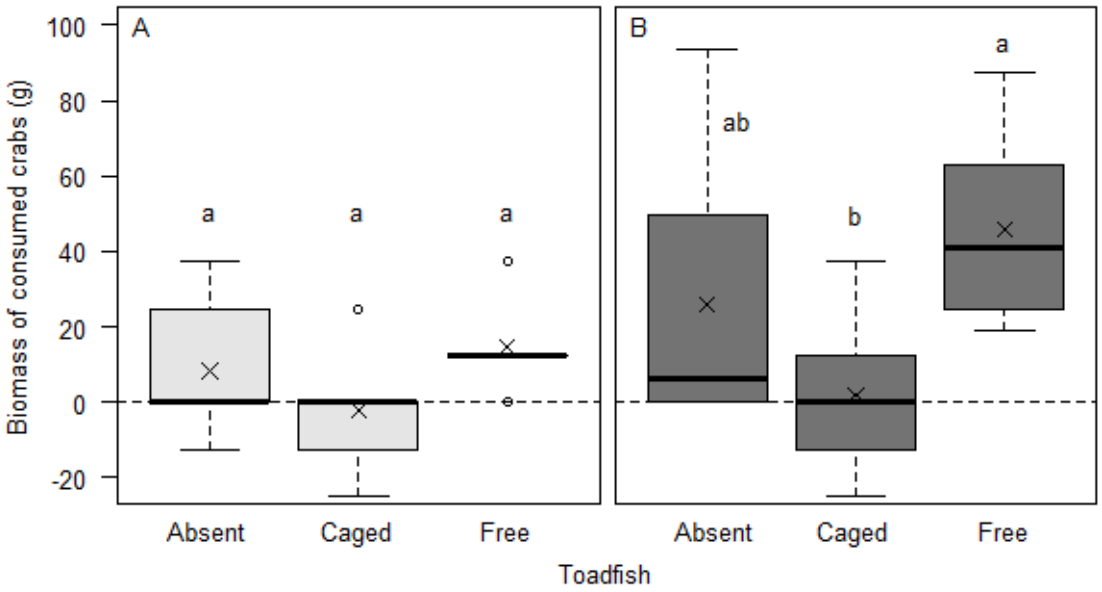


Fig. 4.5. The number of oysters eaten (A) and the number of oysters eaten per crab (B) in Exp 2. The predator was either absent, present in a cage within the mesocosm, or free in the mesocosm. Boxplots show inner 2 quartiles within box and whiskers extent to 1.5 times the respective inner quartile. The line through the box, cross, and dots indicate median, mean, and outliers, respectively.

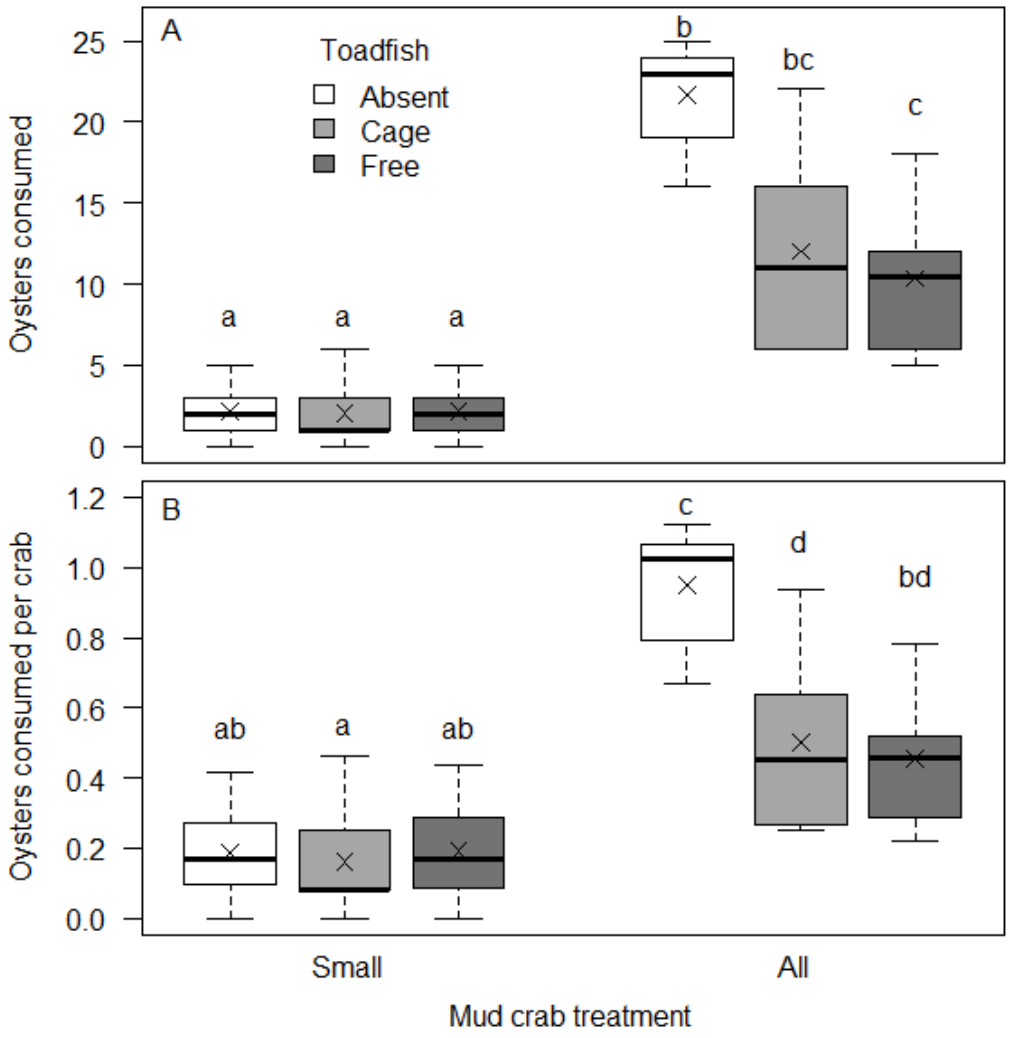


Fig. 5.1. Locations of sampling sites. Solid circles mark artificial structures: rock jetties (A and E), rock revetment (B and D) and plastic bulkheads (left circle in D). Dashed circles mark natural hard substrates (C and E).

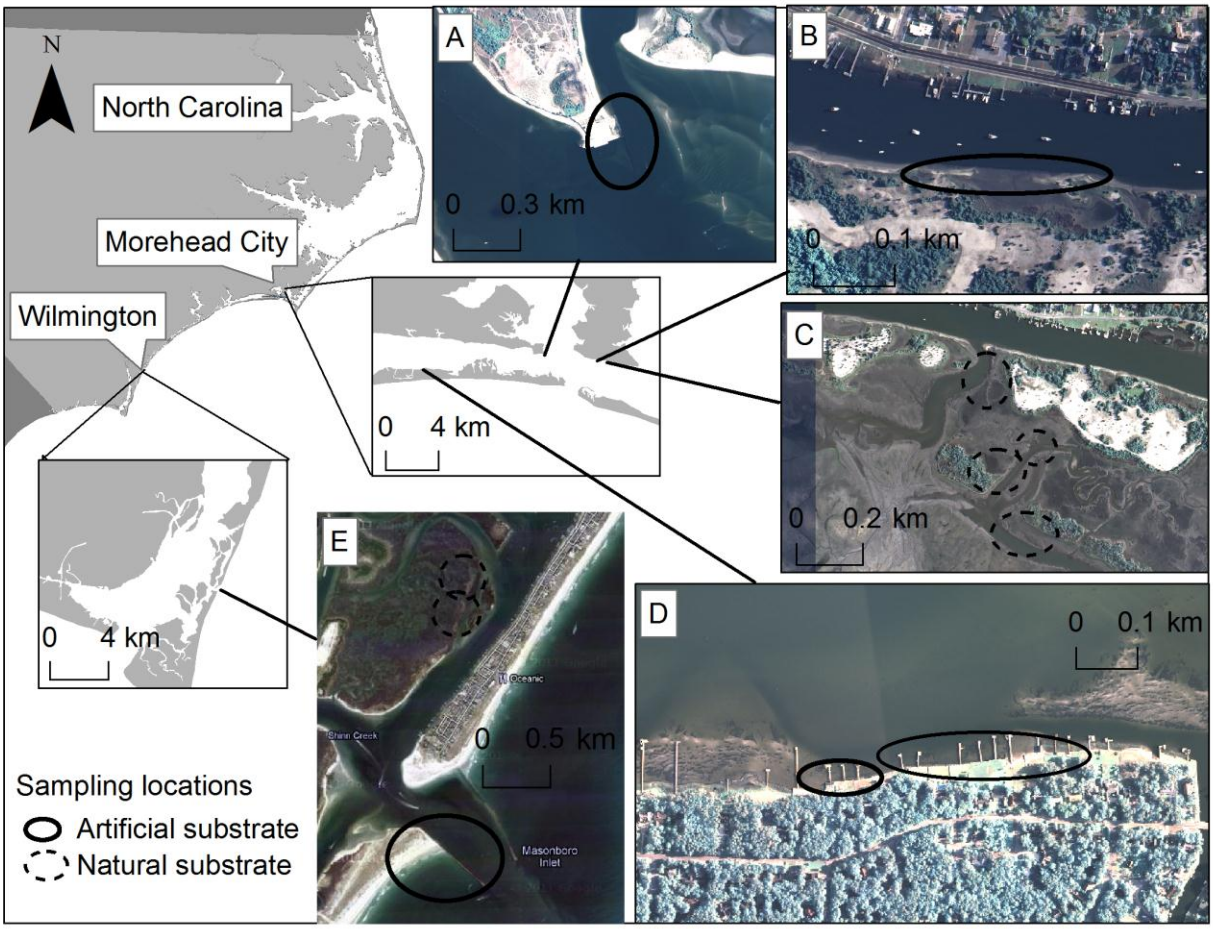


Fig 5.2. Percent cover of *Codium* (*C. fragile and C. decorticatum*), *Ectocarpus* sp, *Sargassum* sp. and the remaining macroalgae on natural (A) vs. artificial (B) substrates during 4 seasons. Boxplots indicate the inner 2 quartiles (box), distribution of points outside of the box up to 1.5 times the respective inner quartile (whisker), median (horizontal bar), and mean (open circle).

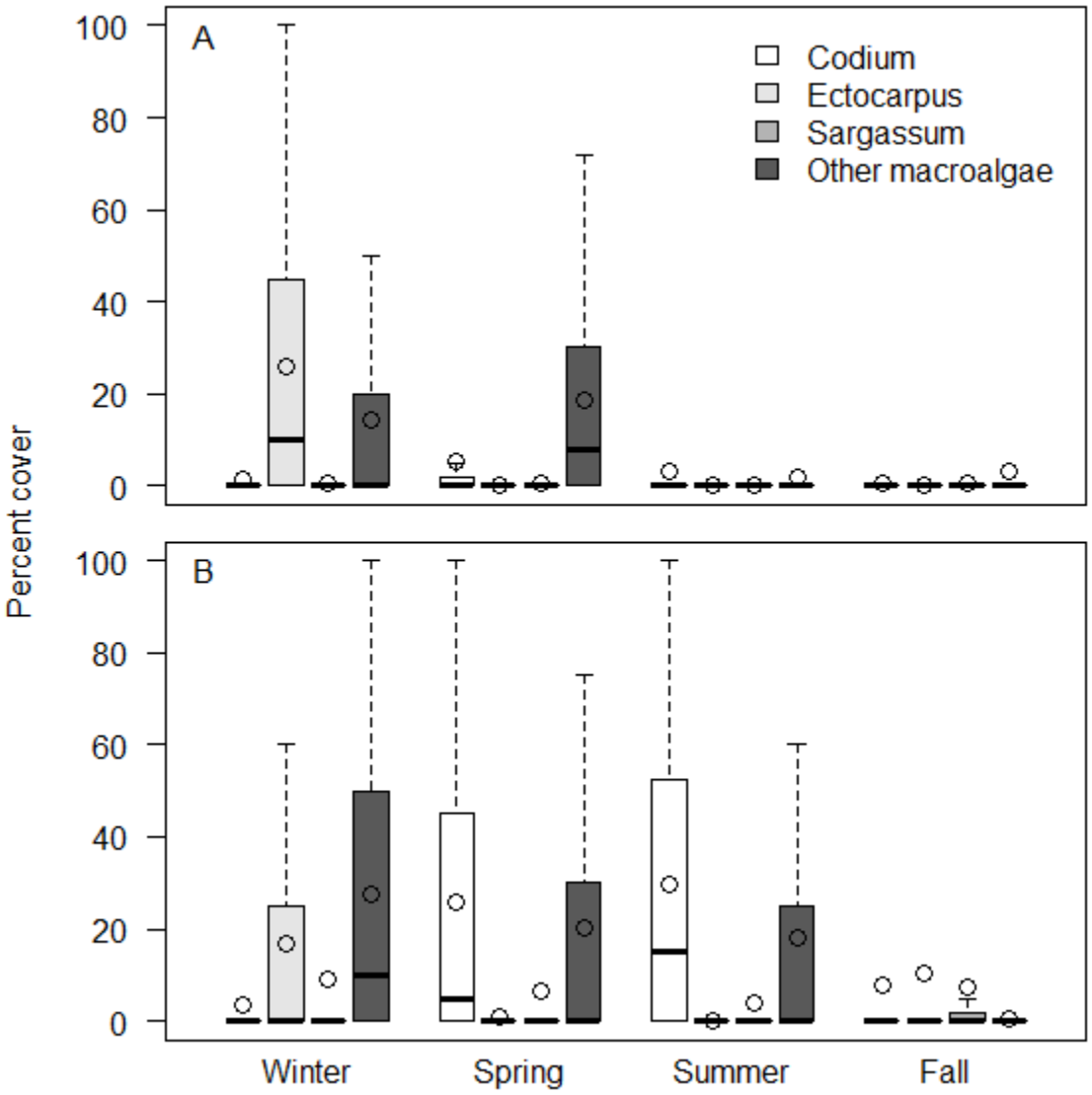


Fig 5.3. The proportion of individuals of native *(C. decorticatum) vs.* non-native (*C. fragile*) *Codium.* Proportions are displayed in separate bars for natural and artificial substrates, which are grouped by season. The number of individual plants sampled are shown above the x-axis.

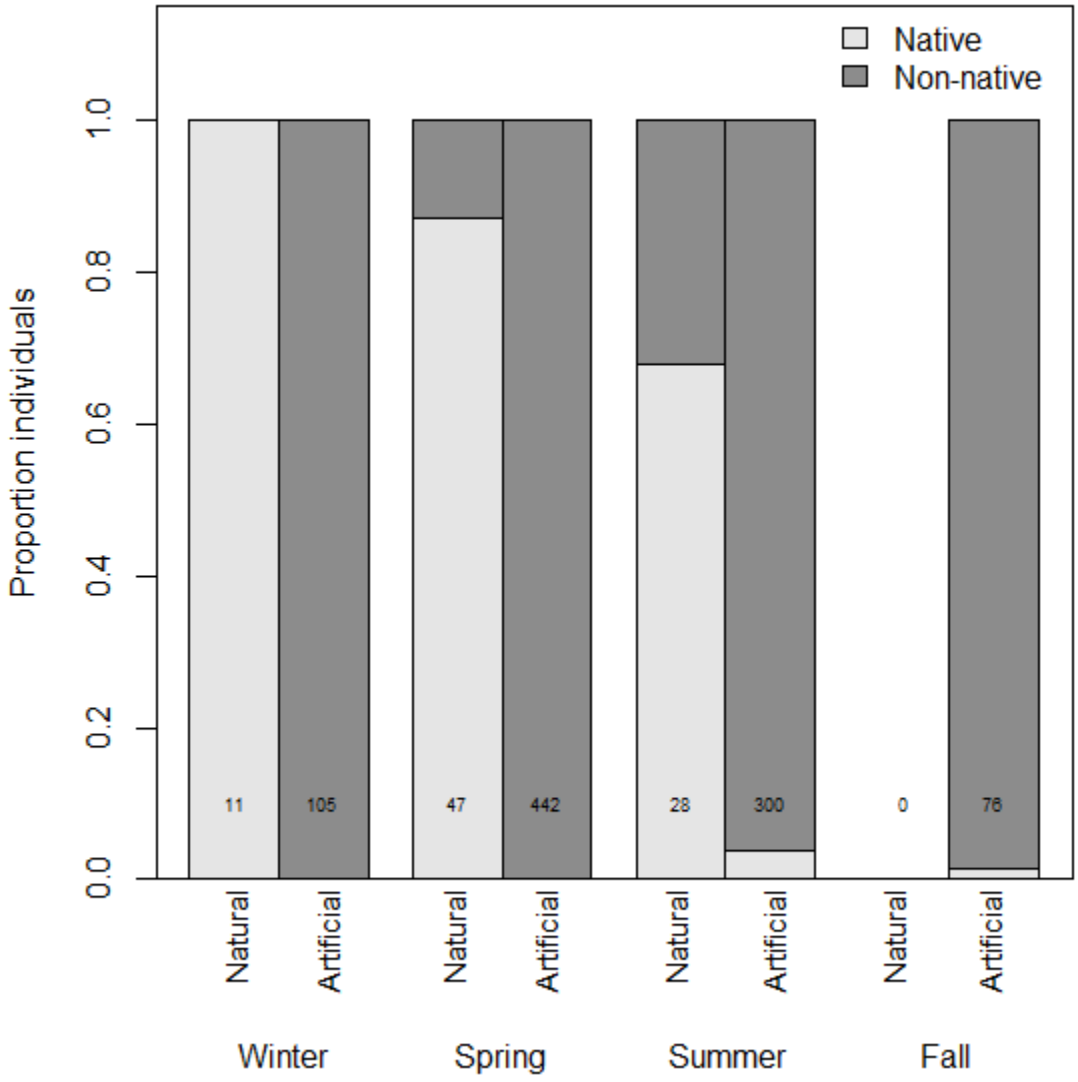
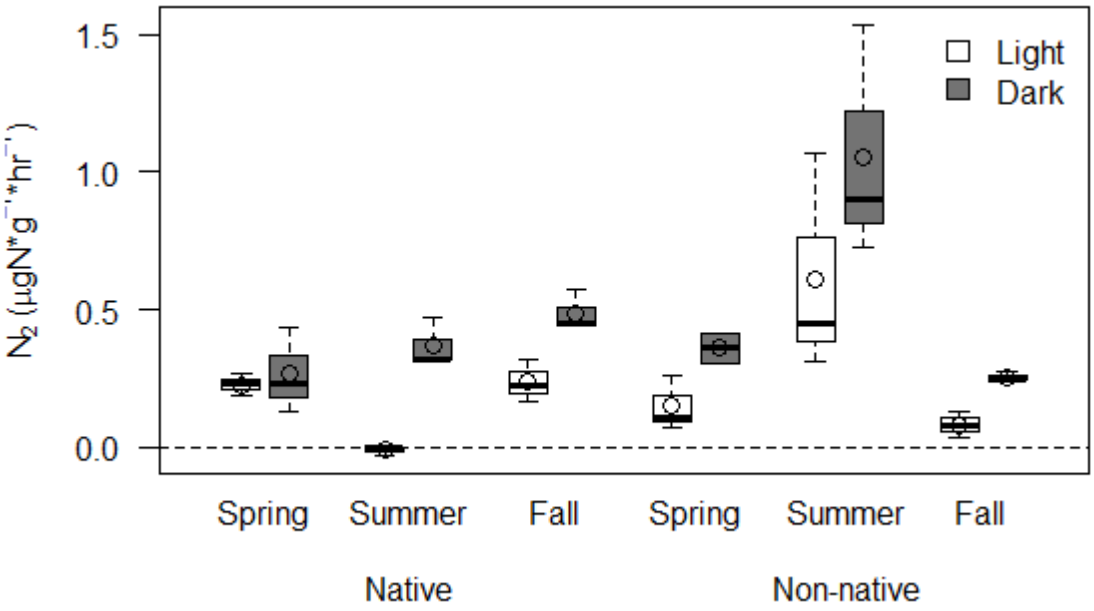


Fig 5.4. Denitrification rate from microcosms for native (*C. decorticatum*) and non-native (*C. fragile*) *Codium* during 3 seasons and light and dark sampling periods. Boxplots indicate the inner 2 quartiles (box), distribution of points outside of the box up to 1.5 times the respective inner quartile (whisker), median (horizontal bar), and mean (open circle).



Appendix 2.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 2.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 2.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 2.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=treatement, random=mound	6	10462.84
oyster size=treatement+site, random = mound	8	10438.37
oyster size=treatement+year, random = mound	7	10421.75
oyster size=treatement*site, random = mound	**14**	**10419.52**
oyster size=treatement*year, random = mound	NA	NA
oyster size=treatement*year*site, random = mound	NA	NA

Appendix 2.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 2.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+samplin, 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=treatmen, 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 2.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
Samping-spring 2011	-0.1558	0.1485	-1.05	0.29
Samping-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 2.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 2.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 2.J. 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

Appendix 3.A. Summary of studies using 3 tropic levels in a meta-analysis by Preiser et al. (2005) followed by references cited. We estimated the time scale required for prey to traverse the mesocosm based on the natural history of the prey.

Reference no.	Reference	Primary predator	Prey	Resource	Ecosystem	Mesocosm dimension	Estimated time for prey to traverse mesocosm	Predator-avoidance behavior measured
1	Brodin and Johansson (2002)	*Perca fluviatilis* ,perch Lestes sponsa,	damselfly *Daphnia magna* ,	zooplankton	freshwater stream	27 cm diameter	< 1 minute	reduced activity
2	Grabowski (2004)	*Opsanus tau* , oyster toadfish	*Panopeus herbstii* , mud crab	*Crassostrea virginica* , oyster	marine	120 cm diameter	< 1 minute	reduced activity
3	McIntosh and Townsend (1996)	*Galaxias vulgaris* , river galaxia	Stream invertebrates	algae	freshwater stream	260x35 cm	< 1 minute	reduced activity
3	McIntosh and Townsend (1996)	*Galaxias vulgaris* , river galaxia	Stream invertebrates	algae	freshwater stream	200x35 cm	< 1 minute	reduced activity
4	Rudgers et al. (2003)	*Forelius pruinosus* , ant	*Bucculatrix thurberiella* , cotton leaf perforator moth	*Gossypium thurberi* , wild cotton	terrestrial	branch	< 1 minute	reduced activity
5	Schmitz (1998)	Pisurina mira, nursery web hunting spider	*Melanoplus femurrubrum* , red-legged grasshopper	grasses	terrestrial	25x100 cm	seconds	reduced activity
5	Schmitz (1998)	Pisurina mira, nursery web hunting spider	*Melanoplus femurrubrum* , red-legged grasshopper	herbs	terrestrial	25x100 cm	seconds	reduced activity
6	Schmitz et al. (1997)	Pisurina mira, nursery web hunting spider	*Melanoplus femurrubrum* , red-legged grasshopper	grasses	terrestrial	60x60 cm	seconds	reduced activity
6	Schmitz et al. (1997)	*Pisurina mira* ,	*Melanoplus femurrubrum* , red-legged grasshopper	forbs	terrestrial	60x60 cm	seconds	reduced activity
7	Stav et al. (2000)	*Anax imperator* , dragonfly	*Culiseta longiareolata* , mosquito	periphyton	freshwater pond	34x59cm	< minute	reduced activity
7	Stav et al. (2000)	*Anax imperator* , dragonfly	*Culiseta longiareolata* , mosquito	phytoplankton	freshwater pond	34x59 cm	< minute	reduced activity
8	Stelzer and Lamberti (1999)	*Etheostoma caeruleum* , darter	*Orconectes propinquus* , crayfish	periphyton	freshwater stream	53x38 cm	seconds	reduced activity

9	Turner and Mittelbach (1990)	Micropterus salmoides, largemouth bass	*Lepomis macrochirus*, bluegill	*Daphnia pulex*, cladoceran	freshwater pond	quarter of 1500 cm diameter	seconds	altered habitat
9	Turner and Mittelbach (1990)	*Micropterus salmoides*, largemouth bass	*Lepomis macrochirus*, bluegill	*Diaphanosoma brachyurum*, cladoceran	freshwater pond	quarter of 1500 cm diameter	seconds	altered habitat
9	Turner and Mittelbach (1990)	*Micropterus salmoides*, largemouth bass	*Lepomis macrochirus*, bluegill	*Ceriodaphnia reticulata*, cladoceran	freshwater pond	quarter of 1500 cm diameter	seconds	altered habitat
9	Turner and Mittelbach (1990)	*Micropterus salmoides*, largemouth bass	*Lepomis macrochirus*, bluegill	*Chaoborus americanus*, Chaoborus flavicans, phantom midges	freshwater pond	quarter of 1500 cm diameter	seconds	altered habitat
10	Winkelman and Aho (1993)	*Esox niger*, pickerel	*Gambusia holbrooki*, adult mosquitofish	*Gambusia holbrook* i, juvenile mosquitofish,	freshwater pond	100 cm diameter	seconds	altered habitat

Appendix 3.B. Oyster reef habitat at the Rachel Carson Research Reserve, NC (76º38.5' Lon, 34º42.5' Lat); (1) dispersed, (2) intermediate sized patches, and (3) continuous habitat.

Appendix 3.C. Two-way ANOVA with mesocosm (open/closed) and trial as independent variables and percent crabs consumed per trial as the dependent variable.

Source of Variation	**df**	**MS**	***F***	***P***
Mesocosm	1	0.276	6.636	0.030
Trial	5	0.038	0.924	0.362
Residual	5	0.42		

Appendix 3.D. Three-way ANOVA with toadfish (presence/absence), mesocosm (open/closed), and trial as independent variables and percent crabs remaining in arena as the dependent variable.

Source of Variation	**df**	**MS**	***F***	***P***
Predator	1	14	0.03	0.857
Mesocosm	1	1530.7	3.66	0.075
Trial	5	267.3	0.64	0.674
Predator x Mesocosm	1	214	0.51	0.486
Residual	15	418.4		

Appendix 3.E. Two-way ANOVA with toadfish (presence/absence) and trial as independent variables and number of crabs that moved into adjacent arena as the dependent variable.

Source of Variation	**df**	**MS**	***F***	***P***
Predator	1	0.333	0.19	0.679
Trial	5	0.533	0.31	0.889
Residual	5	1.733		

Appendix 3.F. Three-way ANOVA with toadfish (presence/absence), mesocosm (open/closed), and trial as independent variables and percent mortality of mussels per day as the dependent variable

Source of Variation	**df**	**MS**	***F***	***P***
Predator	1	0.061	11.38	0.004
Mesocosm	1	0.003	0.5	0.49
Trial	5	0.014	2.64	0.066
Predator x Mesocosm	1	0.000	0.01	0.941
Residual	15	0.005		

Appendix 3.G. Two-way ANOVA with toadfish (presence/absence) and trial as independent variables and percent mussel mortality per day in adjacent arena as the dependent variable.

Source of Variation	**df**	**MS**	***F***	***P***
Predator	1	0.040	4.32	0.092
Trial	5	0.025	2.64	0.156
Residual	5	0.009		

Appendix 3.H. Two-way MANOVA with toadfish (presence/absence) and mesocosm (open/closed) as independent variables and number of crabs observed in corners, along edges, and in oyster habitat as dependent variables

Source of Variation	**Df**	**Pillai**	***approx. F***	***P***
Predator	1	0.2890	2.445	0.097
Mesocosm	1	0.2700	2.218	0.121
Predator x Mesocosm	1	0.4040	4.059	0.023
Residual	20			

Appendix 3.I. Three-way ANOVA with toadfish (presence/absence), mesocosm (open/closed), and trial (blocked) as independent variables and number of crabs observed in corners as the dependent variable

Source of Variation	**df**	**MS**	***F***	***P***
Predator	1	0.0000	0.01	0.928
Mesocosm	1	0.0003	0.14	0.714
Trial	5	0.0033	1.41	0.276
Predator x Mesocosm	1	0.0011	0.48	0.501
Residual	15	0.0023		

Appendix 3.J. Three-way ANOVA with toadfish (presence/absence), mesocosm (open/closed), and trial (blocked) as independent variables and number of crabs observed along edges as the dependent variable

Source of Variation	**df**	**MS**	***F***	***P***
Predator	1	0.020	6.37	0.023
Mesocosm	1	0.017	5.54	0.033
Trial	5	0.005	1.6	0.219
Predator x Mesocosm	1	0.018	5.95	0.028
Residual	15	0.003		

Appendix 3.K. Three-way ANOVA with toadfish (presence/absence), mesocosm (open/closed), and trial (blocked) as independent variables and number of crabs observed in oyster habitat as the dependent variable

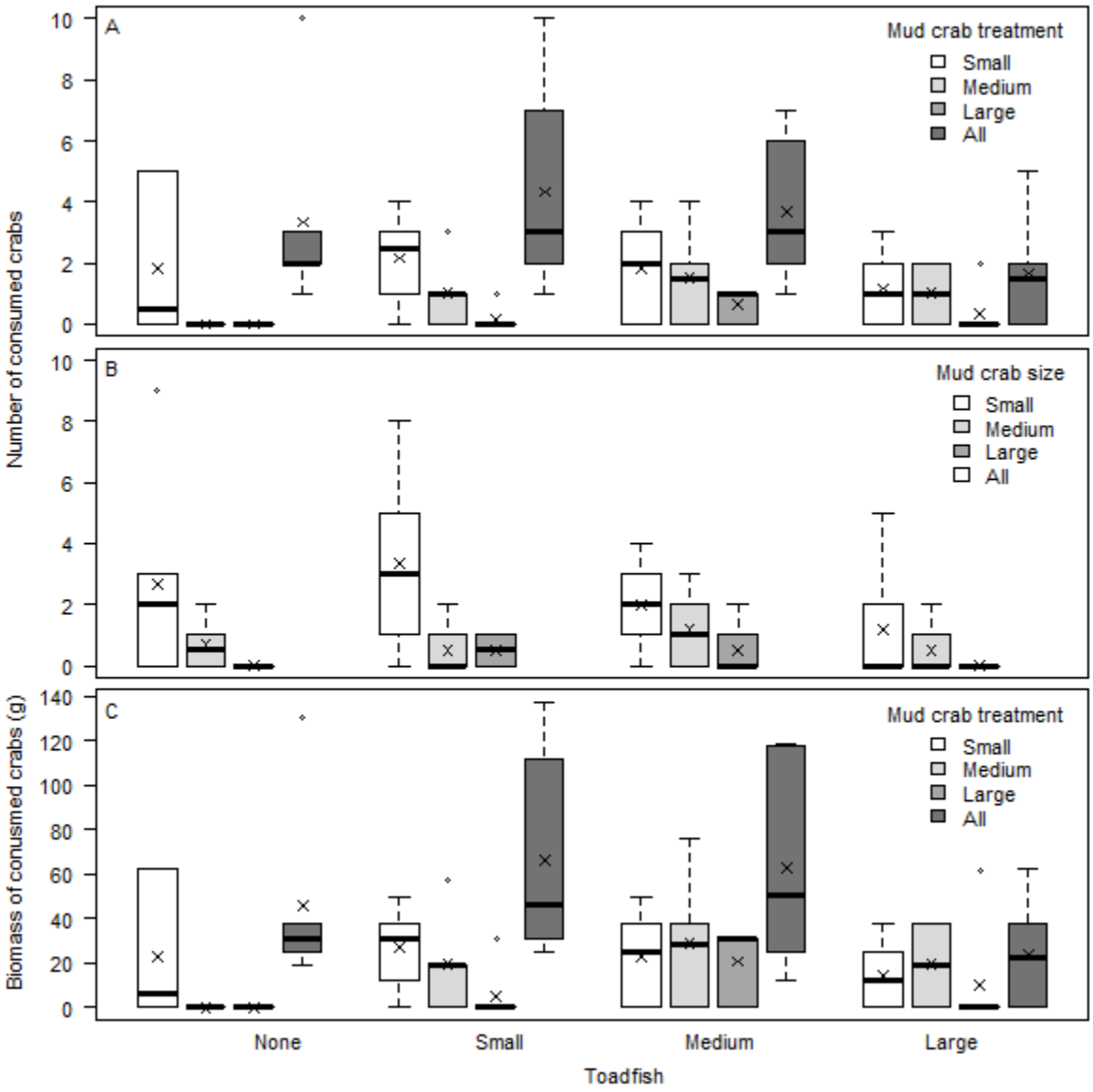
Source of Variation	**df**	**MS**	***F***	***P***
Predator	1	<0.001	0.562	0.463
Mesocosm	1	<0.001	0.562	0.463
Trial	5	<0.001	0.123	0.729
Predator x Mesocosm	1	0.002	3.240	0.088
Residual	15	<0.001		

Appendix 4.A. Toadfish and mud crabs in each of the respective size categories.

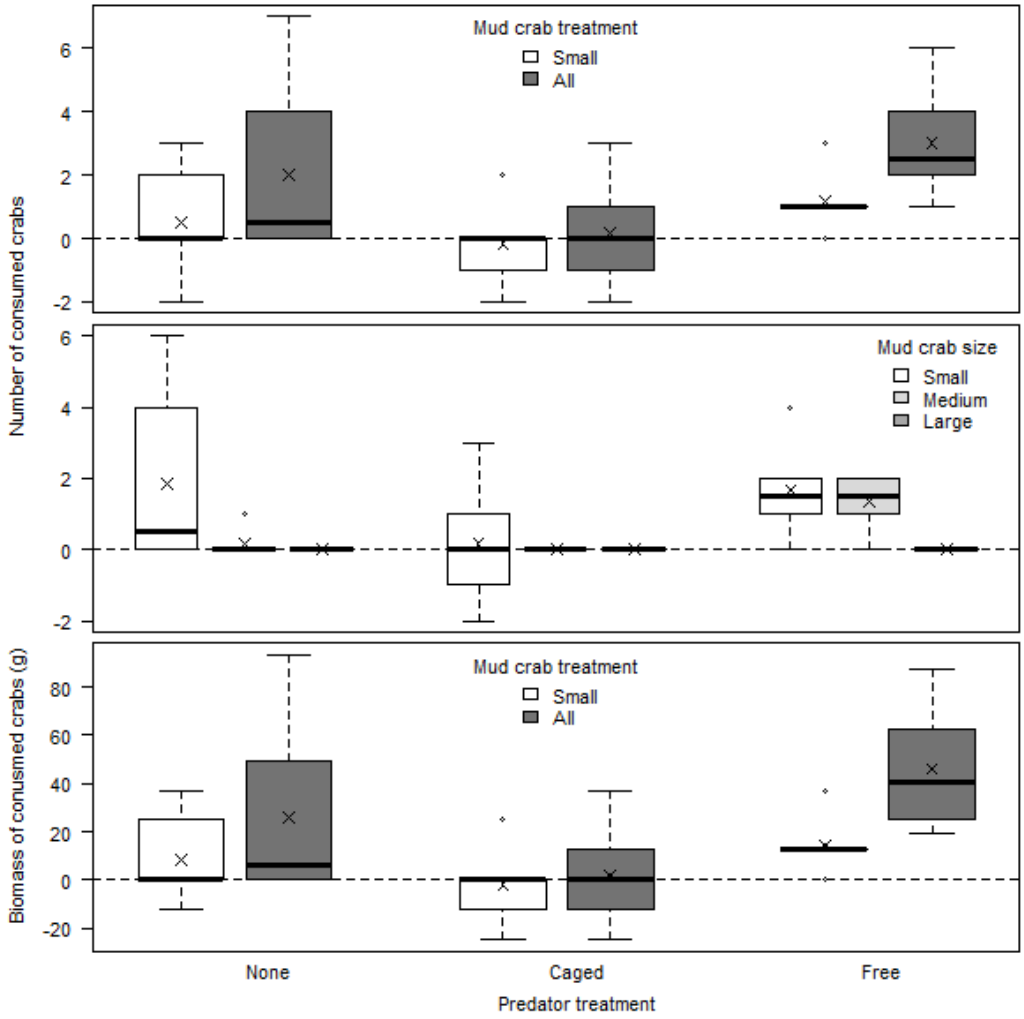
Appendix 4.B. The variables and formulas used to calculate indirect effects (upper panel) and the experimental results (lower panel). Lower case letters denote presence of predator and upper case denotes absence of predator. Estimation of the resource release resulting from consumption of prey (ER) and the reduction in prey foraging (BE). Only calculations for treatments with all crab size classes are shown.

Predator Treatment	Crabs eaten (crab)	Oyster consumption (oysters·d-^{1})	Standardized Oyster consumption (oysters·crab^{-1} ·d^{-1})	Expected resource release (ER, oysters·d^{-1})	Actual resource release (AR, oysters·d^{-1})	Behavioral resource release (BR, oysters·d^{-1})
No	P	M	C			
Yes	p	m	c	ER=c·p	AR=M-m	TMIE=AR-DMIE
No	3.33±1.36	1.91±0.39	0.09±0.02	0.34±0.19		
Small	4.33±1.43	0.73±0.18	0.04±0.01	0.20±0.10	1.18±0.31	0.98±0.28
Medium	4.50±0.85	0.78±0.29	0.04±0.01	0.21±0.10	1.13±0.29	0.93±0.31
Large	1.67±0.76	0.45±0.13	0.02±0.01	0.05±0.04	1.46±0.34	1.41±0.34

Appendix 4.C. The total number of crabs eaten in treatments (A), in the 'all prey' treatment (B), and the biomass of crabs eaten in each treatment (C). The biomass was calculated by multiplying each crab in each size category by the average biomass found for that size (Table 1). Boxplots show inner 2 quartiles within box and whiskers extent to 1.5 times the respective inner quartile. The line through the box, cross, and dots indicate median, mean, and outliers, respectively.



Appendix 4.D. The number of crabs eaten in all treatments (A), in the 'all prey' treatment separated by sizes (all crab treatments; B), and the biomass of crabs eaten in each treatment (C) in the second experiment. The biomass was calculated by multiplying each crab in each size category by the average biomass found for that size (Table 1). Boxplots show inner 2 quartiles within box and whiskers extent to 1.5 times the respective inner quartile. The line through the box, cross, and dots indicate median, mean, and outliers, respectively.



Appendix 5.A. The effect of substrate (artificial vs. natural) and season on the percent cover of *Codium*. The generalized linear model had binomial error and logit canonical link. The best model combined seasons into winter/fall and spring/summer and did not include the interaction between substrate and season.

	Estimate	Std. Error	z value	Pr(>|z|)
Intercept	-2.878	0.208	-13.847	< 0.001
Artificial (substrate)	-2.185	0.231	-9.450	< 0.001
Spring-summer (season)	1.930	0.224	8.602	< 0.001

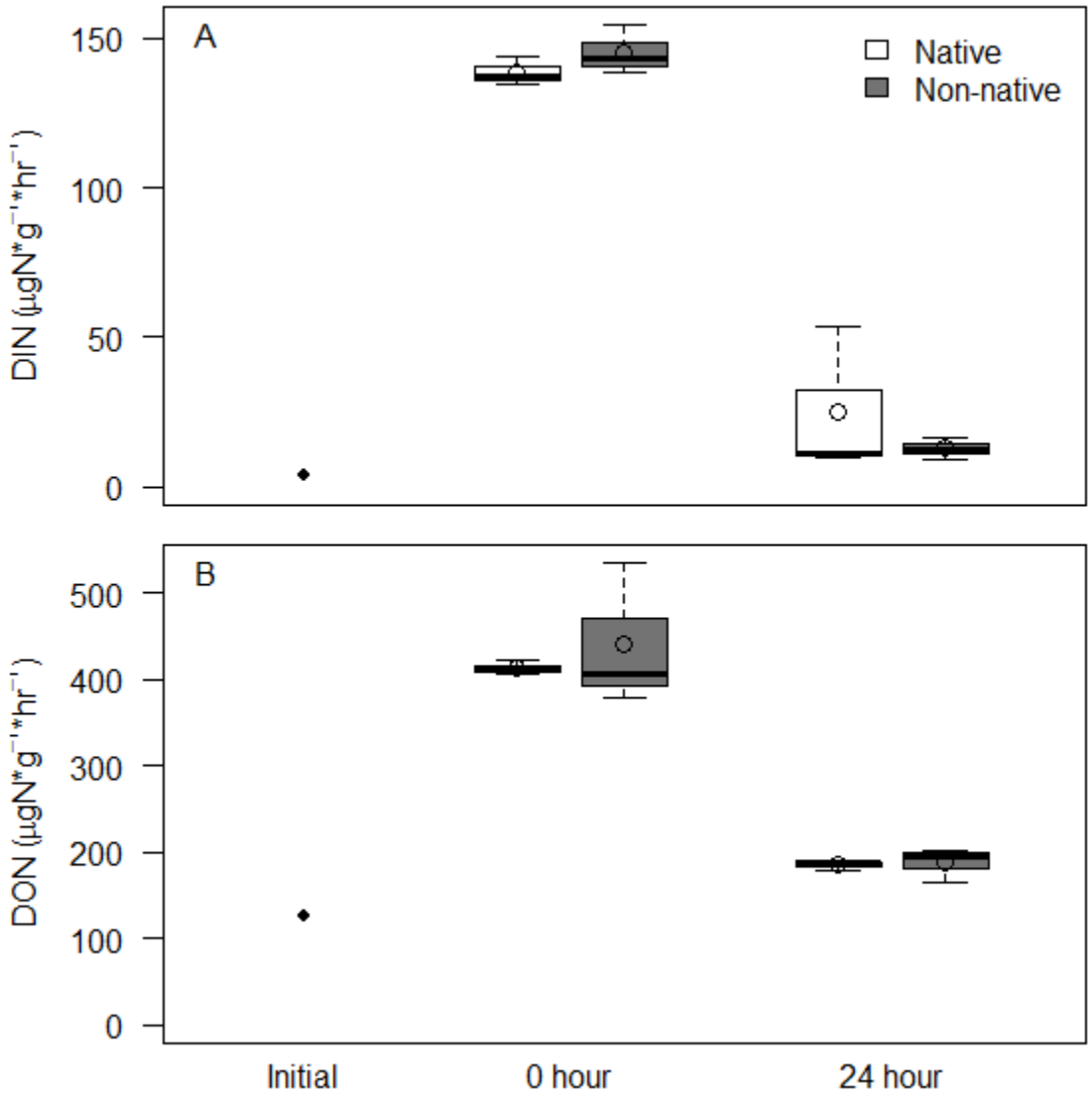
Appendix 5.B. Summary of the results of denitrification rates, which were analyzed using a mixed effect ANOVA with season, species, and light/dark as fixed factors and microcosm number as a random factor. Microcosm was included as a random variable because light/dark measurements were taken within each microcosm.

Response	Error	Predictor	Df	Sum Sq	Mean Sq	F value	Pr(>F)
N_2	Microcosm	Season	2	0.003	0.002	1.867	0.197
		Species	1	0.005	0.005	5.186	0.042
		Season x Species	2	0.021	0.010	10.984	0.002
		Residuals	12	0.011	0.001		
	Within	Light/dark	1	0.018	0.018	38.088	0.000
		Season x Light/dark	2	0.001	0.000	0.862	0.447
		Light/dark x Species	1	0.000	0.000	0.603	0.453
		Season x Light/Dark x Species	2	0.004	0.002	4.396	0.037
		Residuals	12	0.006	0.000		

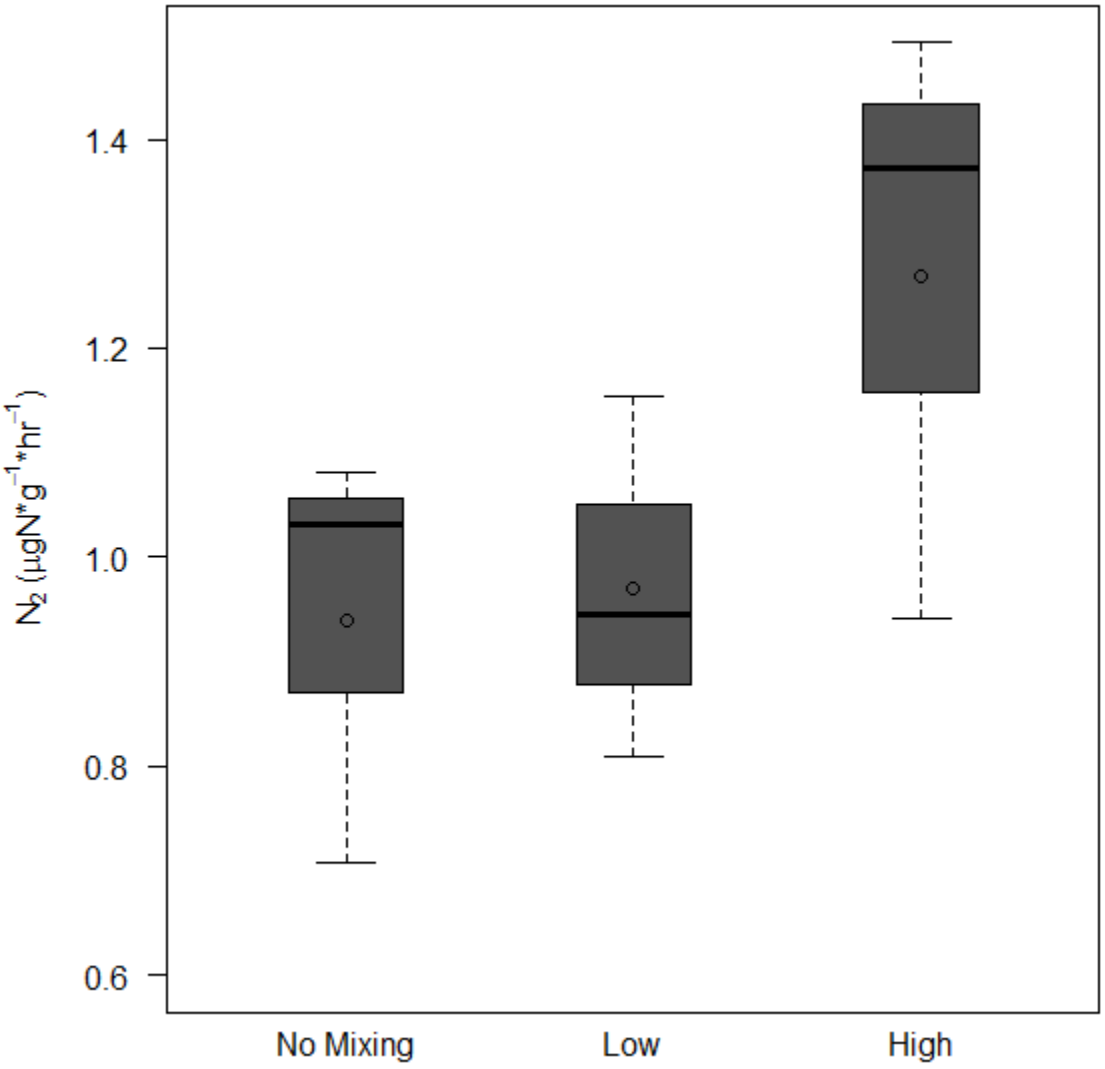
Appendix 5.C. Summary of a 2-way fixed-factor ANOVA conducted to determine the effects of Codium species and light on nitrogen fixation.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Light	1	0.470	0.470	8.482	0.012
Species	1	0.060	0.060	1.080	0.318
Residuals	13	0.720	0.055		

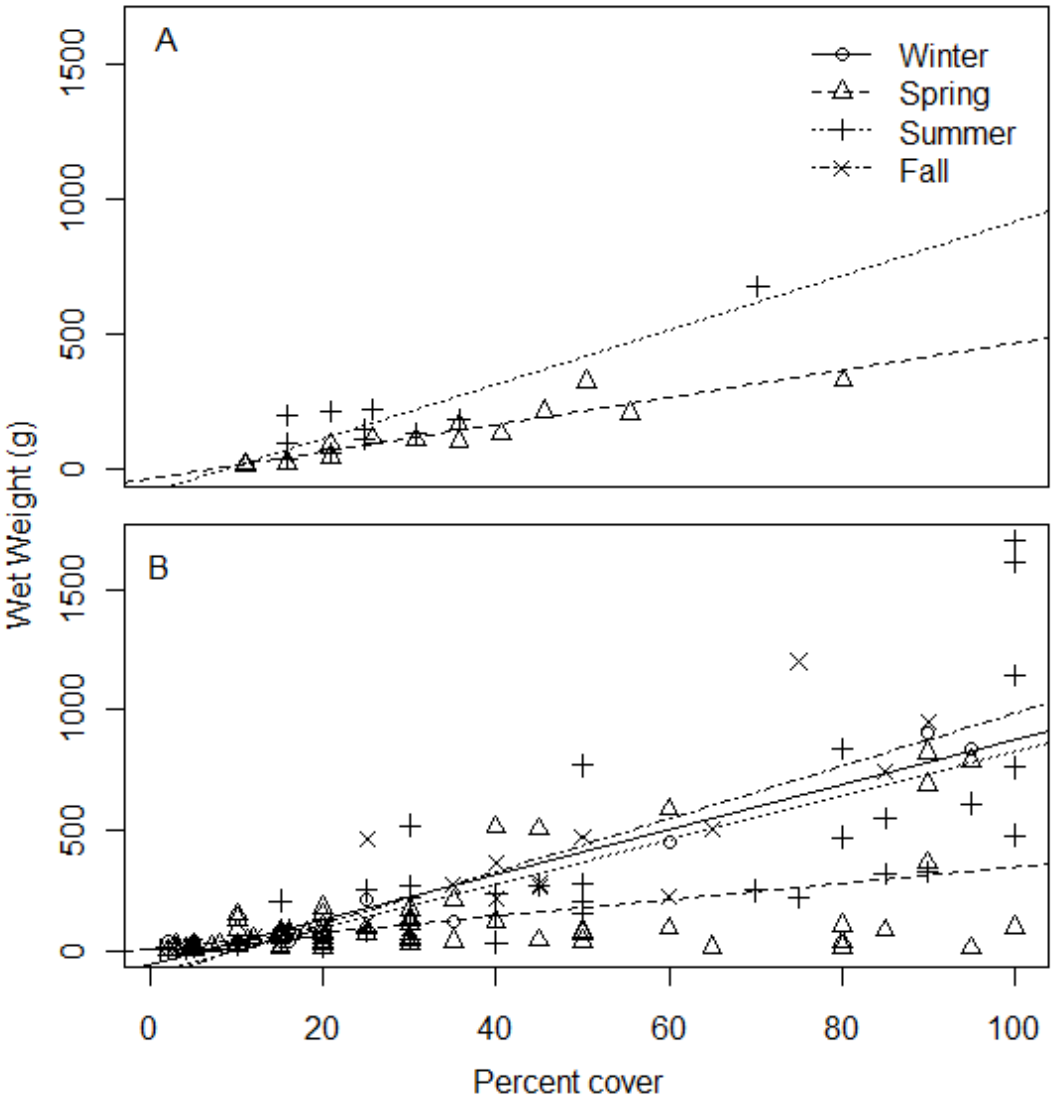
Appendix 5.D. Concentration of DIN (A) and DON (B) immediately before (solid dot), immediately after, and 24 hours after *Codium* was cut for experiments. Boxplots indicate the inner 2 quartiles (box), distribution of points outside of the box up to 1.5 times the respective inner quartile (whisker), median (horizontal bar), and mean (open circle).



Appendix 5.E. The effect of mixing on denitrification rates associated with *C. fragile* in microcosms. No significant differences between mixing intensity was detected (p=0.227). Boxplots indicate the inner 2 quartiles (box), distribution of points outside of the box up to 1.5 times the respective inner quartile (whisker), median (horizontal bar), and mean (open circle).



Appendix 5.F. The multiple regression of wet weight of Codium vs. percent cover of *Codium* on natural (A) and artificial (B) substrates parsed out by season ($F_{5,178}$ =50.16, p<0.001, R^2adj=0.57). No biomass samples were taken in fall and winter on natural substrates because little *Codium* was present.



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