

ARTICLE

Special Feature: Honoring Charles H. Peterson, Ecologist

Oyster abundance on subtidal reefs depends on predation, location, and experimental duration

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Abstract

Predation affects community structure and functioning within marine habitats. Predator–prey interactions can change through space and time. Documenting how these interactions change is essential to improve our understanding of food web dynamics and to enhance our ability to manage preferred species. In this study, our goals were to determine whether the density of subtidal oysters (*Crassostrea virginica*) differed spatially by looking at three separate restored oyster sanctuaries within Pamlico Sound (North Carolina, USA), whether oyster density changed over an interval of 16 months, and whether oyster density was related to the presence of different-sized predators by using an experimental approach. Multiple exclusion treatments were used in situ to exclude, selectively, different predator guilds from consuming oysters. Predator densities were also measured both within experimental treatments and on the restored oyster reefs by using multiple survey techniques. We found that oyster abundance differed among the four sample dates over the 16-month study and differed among the three sites. Mud crabs—one of the smallest predators measured—had the greatest predator biomass per unit of area, but the presence of other predators was largely site-dependent. Oyster abundance was affected by the exclusion of all predators, but this was dependent on sample date and location, which may suggest that mud crabs were the only predator to reduce oyster abundance in this study. In addition, large predators may have affected small predators, such as mud crabs and oyster drills, which were more abundant in treatments where large predators were excluded. The strongest evidence for top-down effects on oyster reefs occurred at one of the three field sites at the first and final sampling time, suggesting that predator effects are complex, as well as spatially and temporally variable. Field experiments that assess variables through time and at multiple locations are needed as this information could improve the success of oyster reef conservation and restoration efforts.

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KEYWORDS

biogenic habitat, body size, environmental gradients, food web, in situ experiment, intraguild predation, oyster reef, Special Feature: Honoring Charles H. Peterson, Ecologist

INTRODUCTION

Predators can influence community structure (Carpenter & Kitchell, 1988; Hairston et al., 1960; Pace et al., 1999), directly by reducing prey abundance through consumption or indirectly by altering species interactions that occur within the ecosystem (Denno et al., 2003; Paine, 1969). However, predation does not occur in isolation and other drivers, such as competition and recruitment, may also alter communities with all these drivers varying in relative importance with location (Boyce et al., 2015; Navarrete & Manzur, 2008; Peterson, 1979), time (Menge & Olson, 1990), and environmental conditions (Menge & Olson, 1990; Navarrete et al., 2000).

The many factors that affect predator–prey interactions make it inherently difficult to extrapolate results from controlled experiments to natural systems, mainly because of the complexity of natural food webs (Holt & Huxel, 2007). Community structure results from an interplay of many abiotic and biotic factors. For example, the sizes of organisms within a community influence the strength and number of interactions (Berlow et al., 2009; Emmerson & Raffaelli, 2004), as well as community structure (Petchey et al., 2008) and stability (Emmerson & Raffaelli, 2004). In addition, interaction strength of predation may be altered if smaller organisms are more abundant than larger individuals (Kalinkat et al., 2013). Therefore, thoughtfully designed in situ experiments coupled with surveys of natural abundances are needed to improve our understanding of how community structure changes through time and space.

Oyster reefs can serve as a model system to study the effects of food web interactions and environmental context on a biogenic system. Focusing on biotic factors, oyster reefs are exposed to multiple ecological attributes that vary within estuaries including: (1) oyster recruitment and survival (Geraldini et al., 2013); (2) the type and abundance of oyster predators (Garton & Stickle, 1980); (3) individual oyster predator biomass, which can vary by multiple orders of magnitude (~ 0.4 –1800 g); and (4) intraguild predation (Geraldini, 2015; Johnson et al., 2014; O'Connor et al., 2008; Figure 1a). In addition, understanding ecosystem functioning and structure, and how these can vary spatially, is important for oyster reefs because they provide multiple essential ecosystem services (Grabowski & Peterson, 2007).

Oyster reefs exist at only a fraction of their historic population (Ermgassen et al., 2012; Kirby, 2004) and, as such, are a focus of conservation and restoration efforts

around the world (Beck et al., 2011). They can form both intertidal and subtidal reefs, and in recent years, there has been growing interest in creating oyster sanctuaries

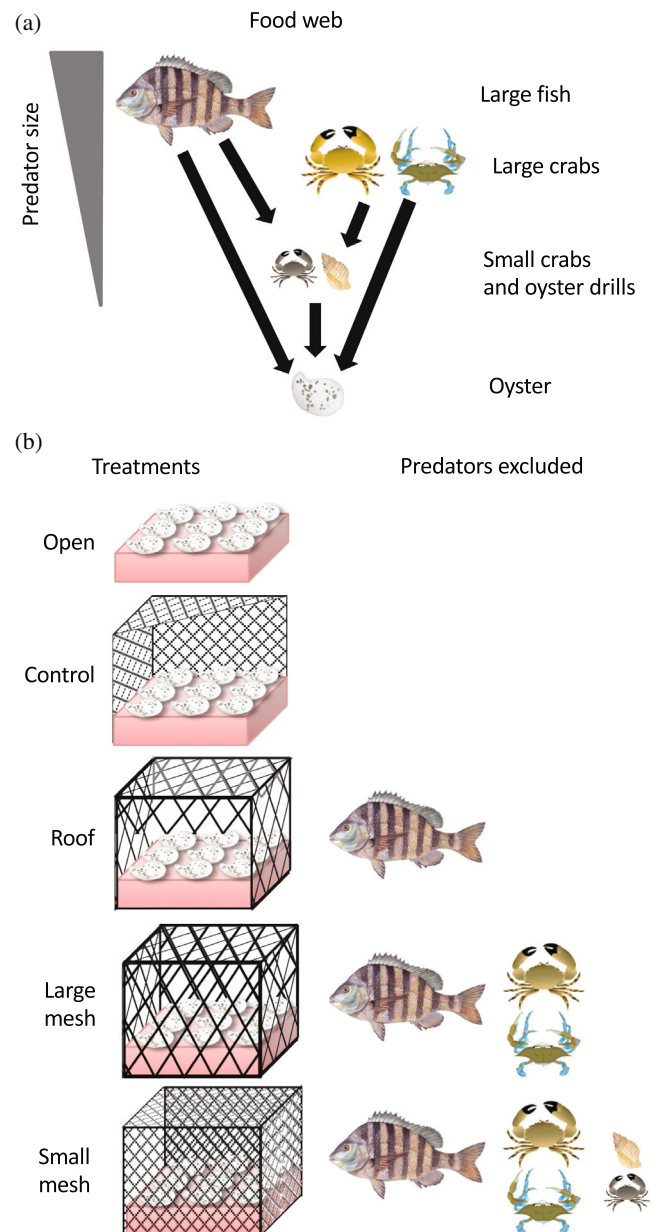


FIGURE 1 Representation of an oyster reef food web with the interactions among the predators of oysters (arrows point in direction of consumption), (a) and the design of the five different cage treatments and the respective excluded predators on the right (b). Animal illustrations courtesy of the Integration and Application Network, University of Maryland Center for Environmental Science (UMCES, Cambridge, MD, USA)

for the purpose of habitat and oyster larval provision for adjacent habitats (e.g., Powers et al., 2009). In particular, subtidal oyster sanctuaries can create recreational fishing opportunities (Peterson et al., 2003). The success of subtidal sanctuaries may be influenced by a variety of environmental or anthropogenic factors such as water flow or illegal harvesting (Powers et al., 2009). As such, there is a growing need to understand whether the mechanisms that shape intertidal oyster communities, similarly influence those of subtidal reefs.

We utilized a combination of mensurative surveys and field experiments to assess whether the abundance of eastern oysters (*Crassostrea virginica*) was affected by manipulating access of different predators within a natural subtidal marine food web at three different locations within an estuary. Although numerous studies have quantified the effect of multiple predators on intertidal oyster reefs across a range of environmental conditions (e.g., Grabowski et al., 2008, 2020; Johnson et al., 2014; Kimbro et al., 2020), predation of oysters can vary significantly between intertidal and subtidal locations (Johnson et al., 2014). Specifically, we monitored oyster abundance over 16 months within different cage designs to exclude predators based on their size and morphology, and surveyed predator abundance at all locations (Figure 1b). Specifically, the following null hypotheses were examined, whether: (1) oyster abundance would be similar through time; (2) oyster abundance would be similar at different locations; (3) oyster predator abundances would be similar at different locations; and (4) access to oysters by different predator guilds would have no effect on oyster abundance. This study builds on an extensive amount of research on oyster reefs and predator–prey interactions, but was novel in that it documents the effects of many predators in situ, including the assessment of abundance of both crab and fish predators, at multiple sites over multiple years.

METHODS

Study system

This study was conducted at three oyster sanctuaries in Pamlico Sound, North Carolina, the largest sound on the east coast of the United States (Clam Shoal, Crab Hole, and Gibbs Shoal; Figure 2). We did not have prior knowledge of abiotic conditions or the assemblage of oyster predators at these three sanctuaries. Oyster harvest is illegal within the sanctuaries, although recreational take is allowed. These oyster sanctuaries were created from 2006 to 2010, and each consisted of 50–300 constructed mounds of riprap marl rock. Each mound was approximately 3-m high, in a mean water depth of approximately 4 m, with a footprint diameter of 15 m. The mounds were

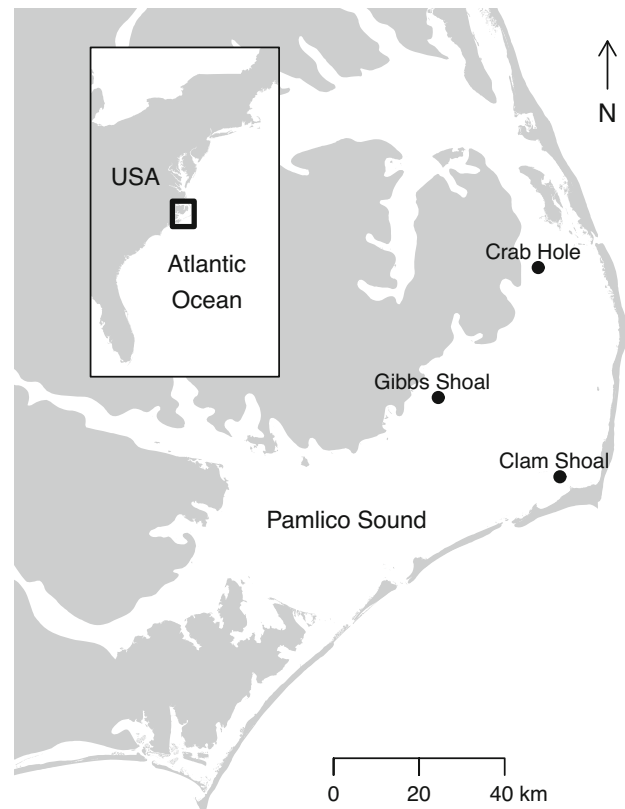


FIGURE 2 The location of the study sites within Pamlico Sound, NC

placed in a uniform grid with individual mounds separated by approximately 25 m (Geraldi et al., 2013; Mroch et al., 2012).

Experimental design

To separate the effect of predator type (e.g., predator species, abundance, and size) on oyster abundance in different estuarine settings, we used five types of cage treatments (Figure 1b). All cage treatments contained juvenile oysters (*C. virginica*) that settled onto nine cleaned adult shells. Juvenile oysters were produced from hatchery-raised larvae and naturally settled onto the cleaned adult shells (dead harvested shell) in flow through tanks (see Geraldi et al., 2013, for details). Juvenile oysters on each shell were counted, and the shell height of five juvenile oysters per shell was measured for size frequencies (greatest width of shell). Adult shells of similar size were chosen to keep surface area available to recruitment consistent. To mimic natural variation in recruitment, juvenile oysters per shell were not standardized. The adult shells were attached to plastic mesh surrounding a cement block (34 × 20 × 8 cm) by a cable tie through a hole drilled in the adult shell prior to oyster settlement.

Individual cement blocks were labeled with an identification number, and the shells (with juvenile oysters) on each cement block were organized so that each shell could be uniquely identified. Each cement block was then haphazardly assigned to a cage treatment type (henceforth referred to as treatments).

Five different treatments consisted of: open (allowed all predators); roof (roof made with a mesh of a hole size of 2.5 cm² to allow large crabs to feed on the oysters, but excluded large fish that feed by approaching the oysters from above, primarily sheepshead—*Archosargus probatocephalus*); large mesh (hole size of 2.5 cm² that excluded large fish and crabs, blue crab—*Callinectes sapidus* and stone crab—*Menippe* spp.); small mesh (hole size of 1 cm² that excluded most predators, including adult mud crabs—family Xanthidae—and oyster drills—*Urosalpinx cinerea*); and cage control (similar to small mesh but with only two walls and half the top covered in mesh). The cage control treatment allowed access to all predators but controlled for confounding effects of the cage, such as changes in water flow and/or animal recruitment (Figure 1b). The roof treatment was like the large mesh treatment, but a section of the side of the enclosure was removed so that there was a 5-cm gap in the mesh between the block and the roof (Figure 1b). Preliminary mesocosm experiments were conducted to ensure the effectiveness of predator exclusion and found no difference in oyster survival between open and roof treatments in the presence of blue crabs (large crabs fed on oysters in the roof treatment) and between roof and large mesh treatment in the presence of sheepshead (sheepshead were excluded from the roof treatment). It was not feasible to include controls for each cage type and the cage control with small mesh was chosen as this likely represents the mesh size to have the greatest effects on relevant parameters such as flow.

One replicate of each cage treatment with oysters was deployed on top of 10–12 oyster mounds at each sanctuary (a total of 10 or 12 replicates per sanctuary), which were deployed at 2 separate times, during a single summer season, to mimic natural oyster recruitment. At each sanctuary, cage treatments were deployed on six or eight mounds on 30 June to 2 July 2010 and four additional mounds on 18–24 August 2010. This resulted in a total of 170 cage treatments that included 1530 adult oyster shells containing >12,000 juvenile oysters. Divers ensured that all cage treatments were on top of each oyster mound and positioned the cage treatment so that the oysters in the treatment were at approximately the same height as the surrounding substrate of the mound. Cage treatments were sampled four times over the 16-month study. Sample dates were 18–24 August 2010, 7–14 October 2010, 18 May to 3 June 2011, and 8–13 September 2011.

Sampling consisted of divers placing the cage treatments in plastic crates lined with 1-cm plastic mesh that were then hauled onto a boat. Five out of nine adult shells within each cage treatment were haphazardly chosen, and all oysters on these adult shells were counted and the shell height (greatest length from umbo to other side of shell) was measured for five haphazardly selected oysters on each adult shell (25 oysters measured per cage treatment). To mimic natural settings, oysters were not standardized after each sampling and natural recruitment did occur. Mud crabs and oyster drills within each cage treatment were counted after thoroughly searching the cement block, oysters, and the crate used to bring the cage treatment to the boat during the fall 2010 and spring 2011 samplings. To determine the abundance of small predators within each of the treatments, the size of all mud crabs (carapace width) and oyster drills (shell height as the longest distance from lip to apex) were measured during the spring 2011 sampling. The treatment, with predators removed, was then returned to each respective oyster mound.

Predator surveys

Natural predator abundance at each of the three oyster sanctuaries was quantified using four different sampling techniques. Collection bins, traps, and visual surveys were needed because of the wide range in predator sizes and life history. Mud crab and oyster drill abundance were measured by deploying plastic bins (31.5 × 16.5 × 10 cm) onto oyster mounds. The bins contained 3 cm of cement at the base and were filled with the surrounding oyster reef material (i.e., marl rock and oyster shells). A bin was deployed at the crest and bottom edge of each mound on which we had deployed cage treatments. Bins were deployed twice for 6–8 weeks in early (May–July) and late summer (July–September) of 2010 to quantify the abundance of mud crabs and oyster drills. Bins were retrieved by divers and placed directly into 5-mm mesh cloth bags underwater to reduce the chance for fauna to escape. Contents of the bins were thoroughly searched, and all fauna >5 mm were retained. Samples were brought back to the laboratory, and all individuals were counted and massed (wet mass). The carapace width of up to 20 haphazardly selected mud crabs was measured from each bin to obtain size frequencies of this oyster predator.

The relative abundance of large crabs (blue and stone crabs) was quantified using two techniques. First, wire traps (71 × 71 × 43 cm) covered in plastic mesh (1-cm square openings) and baited with frozen fish were used to catch large crabs. Six traps were deployed on different oyster mounds at each oyster sanctuary for 4 h during

the day on three dates from May to October in 2010. The abundance, size (as carapace width), and mass of trapped individuals were recorded. Second, divers conducted visual surveys of large crabs and fish. Large crabs were counted within 50×50 cm quadrats haphazardly placed by divers six times on each reef mound with a treatment. The number and species of crabs (*C. sapidus* or *Menippe* spp.) were recorded within each quadrat. Quadrat sampling was conducted on five different occasions from May to October in 2010 and 2011 when visibility was >0.5 m.

The abundance of large fish (primarily *A. probatocephalus*) was visually quantified by divers on the same day crabs were counted in quadrats, but only when visibility was >2 m. A diver swam 10 m along a transect tape from the base of the mound to over the top of the mound. All sheephead that occurred within 1 m on both sides and 2 m ahead of the diver (20-m² transects) were counted. The total length of each fish was visually estimated within 5-cm increments. Three transects were conducted consecutively on a mound to attain a robust average number of sheephead per 20 m². Visual surveys were conducted on 1–4 mounds (2–4 times throughout the experiment) at each oyster sanctuary during cage sampling and at least once per season.

Predator biomass

The biomass of each predator was determined differently depending on the predator. The biomass of individual mud crabs ≥ 10 mm was determined from a well-fitted regression ($R^2 = 0.98$) between biomass and carapace width of mud crabs that was calculated from 22 crabs ($B = 0.00091 L^{2.7}$). The mean weight of blue crabs caught by traps was used to estimate the biomass for each individual detected visually in quadrats. The biomass of individual stone crabs was calculated by multiplying the abundance by the mean individual biomass (28 g) of similar-sized crabs collected on oyster reefs in North Carolina (O'Connor et al., 2008). The biomass of sheephead was calculated from diver-based size estimates and a length to biomass conversion for sheephead ($W = 0.0296 L^{3.05}$; Schwartz, 1990).

Statistical analysis

Given that many of our dependent variables (e.g., counts of oyster abundance) were skewed toward 0, we tested multiple generalized linear mixed-effects models (family = Poisson, *nbinom1*, or *nbinom2*; which all had log links), both with and without zero inflation (*glmmTMB* function within the *glmmTMB* package; Brooks et al., 2017). The most appropriate model was chosen based on the lowest Akaike information criterion, and the fit of the model was checked by

comparing figures of fitted values versus residuals and fitted values versus actual data. For the continuous dependent variable (oyster length), we used a similar process but with Gaussian family models with either log, identity, or inverse links. Independent fixed categorical factors were sanctuary, treatment (open, cage control, roof, large mesh, and small mesh cages), and sample date (spring 2010, fall 2010, spring 2011, and fall 2011). Changes in oyster abundance on shells deployed on mounds primarily resulted from natural recruitment and location differences, and not deployment date (Gerald et al., 2013), so we pooled all data regardless of deployment date. All interactions were included except for oyster drill abundance for which the model would not converge and only main effects were modeled. A random variable was also included in the model to account for the nested design of blocks on mounds within each sanctuary (1|sanctuary/mound/treatment). Although there was a potential for nonindependence among sample dates to affect the statistical results, part of our goal was to determine the difference among sample dates, and nonindependence was minimized by randomly measuring five of nine adult oyster shells during each sampling and removing predators within cages. Five separate statistical analyses were performed with the following dependent variables: total number of oysters per shell, number of juvenile oysters per shell (<2.5 -cm shell height), oyster length, number of mud crabs per treatment, and number of oyster drills per treatment. Only one oyster sanctuary, Clam Shoal, had oyster drills on the treatments, and the model only tested for the difference among treatments (open, cage control, roof, large mesh, and small mesh) at that location. Results of statistical models were presented using ANOVA summaries using the *Anova* function within the *car* package (Fox & Weisberg, 2011), which is based on the Type II Wald chi-squared tests, to make findings clear and concise. Differences among factor-level combinations were determined by estimated marginal means adjusted for multiple comparisons using the *emmeans* function (*adjust* = “tukey”) within the *emmeans* package (Lenth, 2020). All statistics were conducted using R software version 3.5.1 (R Core Team, 2017).

Difference in predator abundance and biomass among sanctuaries was tested for all mud crabs, large mud crabs (crabs with carapace width greater than 10-mm readily consume juvenile oysters; personal observation, Nathan R. Gerald), oyster drills, blue crabs, stone crabs, and sheephead. The abundance and biomass of oyster predators, other than mud crabs, had a high frequency of zeros (previously described linear models did not converge for multiple predators), and a nonparametric Kruskal–Wallis test was used to determine significance among sanctuaries and between seasons for these predators. This test cannot include random factors, and

the mean predator densities were calculated to obtain one value per mound at each sanctuary. The mean density measure of predators taken at each mound was used as a replicate to minimize the potential for pseudo-replication (nonindependence of samples not accounted for with a random variable) and to reduce differences in the number of samplings among sanctuaries and sampling techniques. Abundance data from underwater quadrats and traps for large crabs had the same order when ranked by abundance; consequently, quadrat data were used for the analysis to estimate density.

RESULTS

Effects of predator exclusion on oyster and predator abundance

The number of oysters on shells within treatments was influenced by the three-way interaction among sanctuary, treatment, and sample date (Table 1, Figure 3). The small mesh exclusion treatments were the only cages to have more oysters than the open treatments, but this was only significant based on the multiple comparison test among treatments at the same sanctuary and sample date at the first and last sample date at Crab Hole (August 2010 [Figure 3b] and September 2011 [Figure 3e]; Appendix S1: Figure S1). However, there was evidence of this effect with open treatments having, on average, approximately half of the oysters compared to the small mesh treatments at Gibbs Shoal at the first and final sampling dates (Figure 3b,e) and at Crab Hole at the fall (October) 2010 sample date (Figure 3c; Appendix S1: Figure S1). There were four other instances of significant differences among treatments at the same site and sample date, including more oysters within large mesh than open treatments at the first sampling at Crab Hole (Figure 3b); fewer oysters within both the large mesh and roof compared to the small mesh treatment at the final sampling at Crab Hole (Figure 3e); and fewer oysters within roof treatments compared to small mesh treatments at the final sampling at Gibbs Shoal (Figure 3e; Appendix S1: Figure S1). One sanctuary (Clam Shoal) had a high abundance of oysters during initial samplings but few oysters during the later samplings, which likely caused a significant interaction between sanctuary and sampling date (Figure 3a–e). The spring sampling period (May 2011; Figure 3d) had fewer oysters than the other sample dates that occurred after oyster recruitment periods during the summer.

The number of juvenile oysters on shells in treatments displayed similar patterns to the total number of oysters on shells and depended on the interacting effects

of sanctuary, treatment, and sample date (Table 1, Figure 3). The lowest number of juvenile oysters per shell occurred in spring 2011 (Figure 3i). The difference in juvenile oysters was significant among treatments within the same sanctuary and sample date at the final sample date with small mesh treatments having more juvenile oysters than the open, roof, and large mesh treatments at Crab Hole and more juvenile oysters in the small mesh treatments compared to the roof treatments at Gibbs Shoal (Figure 3j; Appendix S1: Figure S2).

The number of mud crabs within treatments was influenced by the main effects of sanctuary, treatment, and sample date (Table 1, Figure 4a,b). Overall, the small mesh treatments had more crabs than the roof, control, or open treatments, but this was dependent on sanctuary and sample date (Figure 4a,b; Appendix S1: Table S1). Additionally, the large mesh often had an intermediate number of crabs compared to the small mesh, and the other three treatments (Figure 4a,b; Appendix S1: Figure S3).

In contrast to mud crabs in treatments, oyster drills were only observed in the treatments at Clam Shoal and were influenced by treatment type and sample date (Table 1). In general, oyster drill abundance was higher in small, large, and roof treatments compared to open treatments, and there were more oyster drills present in roof, small, and large treatments in spring 2011 than fall 2010 (Figure 3c,d; Appendix S1: Table S1 and Figure S4).

Oyster length

There was no measured effect of treatment on oyster length (Table 1). However, there was an effect of the interaction between sanctuary and sample date with oysters generally increasing in size through time, except for the final sampling when oyster length decreased at Crab Hole and Gibbs Shoal (Appendix S1: Figure S5). The decrease in average size likely resulted from summer recruitment at these two sanctuaries, which resulted in an increase of smaller, juvenile oysters on our experimental blocks, but recruitment was almost absent at Clam Shoal where oyster length continuously increased through time.

Oyster predators across sanctuaries

The density of predators on the oyster reefs differed among sanctuaries for some of the taxa. The densities of all mud crabs ($\chi^2 = 22.2$, $df = 2$, $p < 0.001$) and large mud crabs ($\chi^2 = 10.5$, $df = 2$, $p = 0.005$) were lower at Clam Shoal sanctuary than the other two sanctuaries

TABLE 1 Summary of statistical results of five separate generalized linear mixed models

Dependent variable	Independent variable	χ^2	df	p
All oysters	Sanctuary	0.9	2	0.635
nbinom1, zi (log)	Treatment	25.9	4	<0.001
	Sample date	353.4	3	<0.001
	Sanctuary:Treatment	41.2	8	<0.001
	Sanctuary:Sample date	834.7	6	<0.001
	Treatment:Sample date	13.6	12	0.330
	Sanctuary:Treatment:Sample date	50.1	24	0.001
Juvenile oysters	Sanctuary	0.1	2	0.959
nbinom, zi (log)	Treatment	18.2	4	0.001
	Sample date	1037.1	3	<0.001
	Sanctuary:Treatment	13.9	8	0.085
	Sanctuary:Sample date	467.3	6	<0.001
	Treatment:Sample date	31.2	12	0.002
	Sanctuary:Treatment:Sample date	37.1	24	0.043
Oyster length	Sanctuary	1.5	2	0.468
gaussian (log)	Treatment	8.1	4	0.088
	Sample date	1115.1	2	<0.001
	Sanctuary:Treatment	14.5	8	0.070
	Sanctuary:Sample date	185.6	4	<0.001
	Treatment:Sample date	4.9	8	0.772
	Sanctuary:Treatment:Sample date	23.8	16	0.094
Mud crabs	Sanctuary	19.6	2	<0.001
nbinom1, zi (log)	Treatment	158.4	4	<0.001
	Sample date	29.0	1	<0.001
	Sanctuary:Treatment	5.3	8	0.729
	Sanctuary:Sample date	5.8	2	0.056
	Treatment:Sample date	6.6	4	0.160
	Sanctuary:Treatment:Sample date	8.6	8	0.381
Oyster drills	Treatment	17.2	4	0.002
nbinom1, zi (log)	Sample date	29.8	1	<0.001

Note: Each model included a random variable of oyster mound nested within each sanctuary. The dependent variable family and link used in the model are listed below the dependent variable.

(Figure 5a,b). Oyster drills were only observed at the Clam Shoal sanctuary, where there was significantly greater abundance and density than the other two sanctuaries (abundance: $\chi^2 = 9.48$, $df = 2$, $p = 0.009$; Figure 5c; density: $\chi^2 = 9.48$, $df = 2$, $p = 0.009$; Figure 5i). Of the 384 quadrats sampled during the entire study for large crabs, only 1 quadrat had a blue crab (at Gibbs Shoal sanctuary) and 5 quadrats had stone crabs (4 of 41 quadrats at Clam Shoal). There was no statistical difference among sanctuaries for the density of blue crabs ($\chi^2 = 22.2$, $df = 2$, $p = 0.223$; Figure 5d) or stone

crabs ($\chi^2 = 1.92$, $df = 2$, $p = 0.383$; Figure 5e). The density of sheepshead, as measured by visual transects, did not vary among sanctuaries ($\chi^2 = 1.92$, $df = 2$, $p = 0.383$; Figure 5f). The biomass of predators across sanctuaries did not vary for any predator: all mud crabs ($\chi^2 = 3.83$, $df = 2$, $p = 0.148$), large mud crabs ($\chi^2 = 2.82$, $df = 2$, $p = 0.244$), blue crabs ($\chi^2 = 3$, $df = 2$, $p = 0.221$), stone crabs ($\chi^2 = 1.92$, $df = 2$, $p = 0.383$), or sheepshead ($\chi^2 = 3.00$, $df = 2$, $p = 0.224$; Figure 4g-1). Even though there were large differences in means, high variance obscured significant differences, particularly for stone crabs and sheepshead.

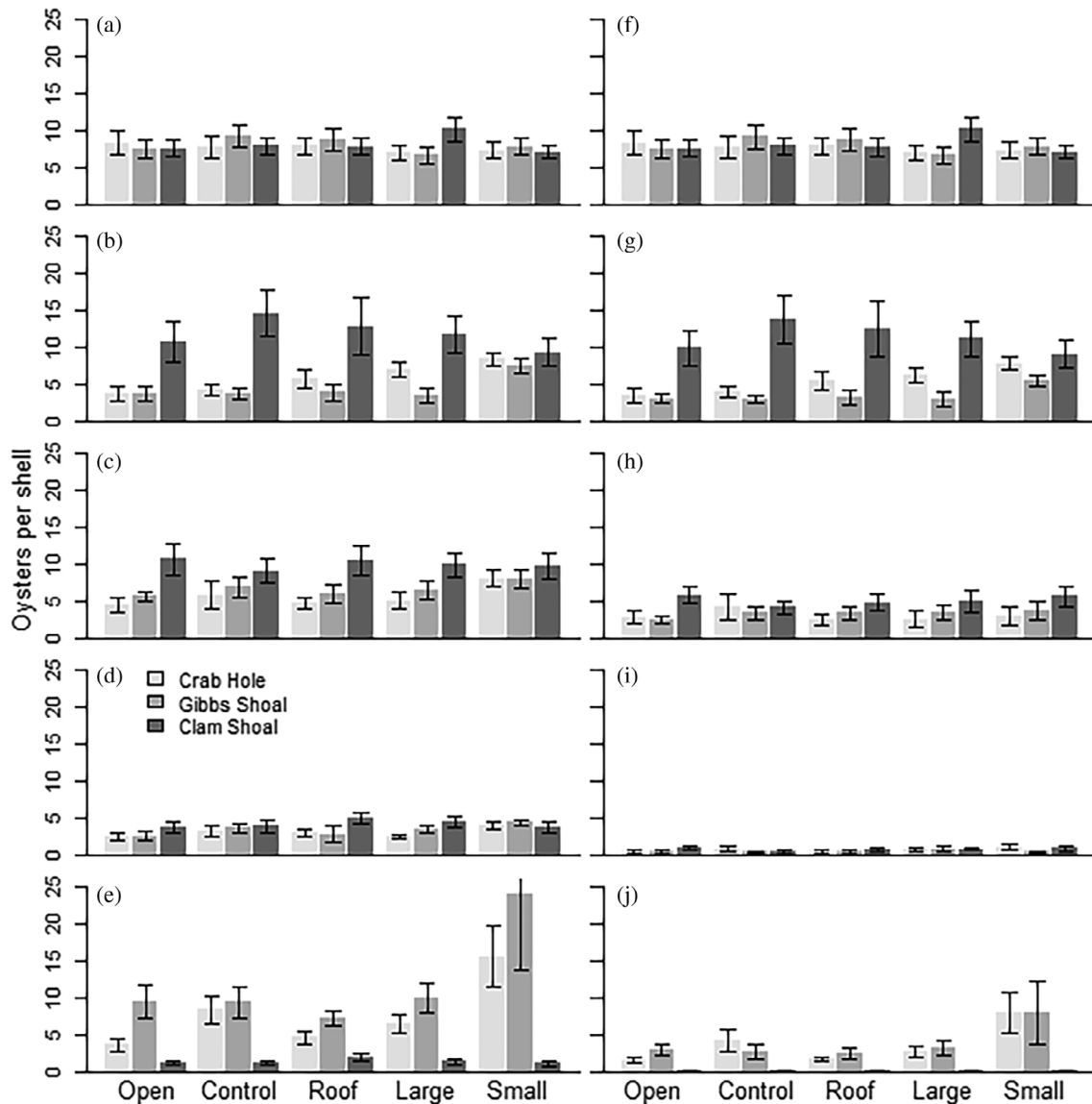


FIGURE 3 The mean (\pm SE) total number of oysters (left column) and juvenile oysters (right column) per adult shell for each predator treatment before deployment (a and f) and at the four sampling dates over 14 months, August 2010 (b and g), October 2010 (c and h), May 2011 (d and i), and September 2011 (e and j). The three different sanctuaries are indicated by bar shade.

DISCUSSION

We found that oyster abundance was dependent on predator access, location, and sample date of the experiment. Oyster abundance was sometimes enhanced when all focal predators were excluded, while allowing access by larger predators (e.g., fishes and large crabs) did not further reduce oyster abundance compared to when only small predators had access (e.g., mud crabs and oyster drills). This effect of predator exclusion was statistically significant at one of three sanctuaries at the first and final sampling event, while a similar but statistically insignificant pattern was seen at a second sanctuary. The sanctuary closest to an ocean inlet had the greatest oyster

growth but a boom-and-bust pattern in oyster abundance. Our results agree with past findings from intertidal systems that mud crabs may be the predator with the greatest effect on oyster abundance (e.g., Grabowski et al., 2008, 2020; Hill & Weissburg, 2013; Rindone & Eggleston, 2011); however, we found that this effect is spatially and temporally mediated on subtidal oyster reefs.

Oyster mean abundance decreased by nearly half during the third sampling event (May 2011, 10 months after deployment) across all predator exclusion treatments, suggesting unmeasured factors depressed abundances at all sites. The number of oysters was similar at the end of the second summer compared to the end of the first

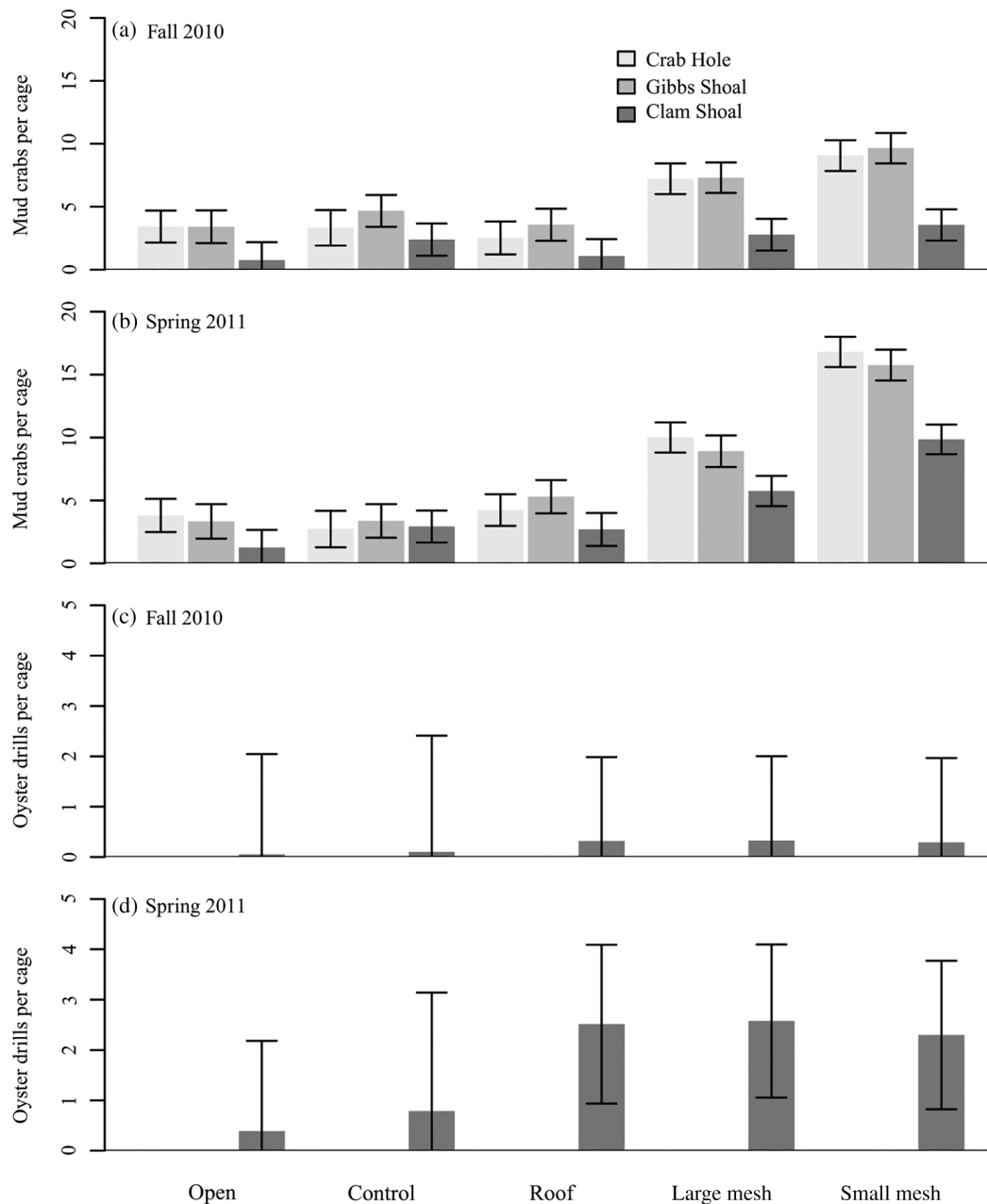


FIGURE 4 The mean number of mud crabs within a treatment (\pm SE) during the fall (October) 2010 (a) and spring (May) 2011 (b) sampling and oyster drills within a treatment during the fall 2010 (c) and spring 2011 (d) at the three sanctuaries (denoted by bar shades)

summer in all treatments except for the oysters in the small mesh treatment that excluded all measured predators. This exclusion treatment at the first and final sampling had almost twice the number of oysters (juvenile and total) than the other treatments at two of the three sites. Although this treatment difference was statistically significant at only one sanctuary, we suggest that the density of oysters was mediated by small predators, but this effect was time and location specific.

Mud crabs were more abundant inside the cage treatment with small and large mesh that excluded larger predators, suggesting that cages could have been used by

mud crabs as shelter from large predators (Gerald, 2015; Gerald & Macreadie, 2013; Grabowski, 2004; Johnson et al., 2014). This seemingly contradictory finding that oysters were enhanced in treatments that also had small predators may result from three factors. First, the majority of the mud crabs in these cages were too small to readily consume oysters (<10 mm carapace width) and instead possibly facilitated oyster recruitment by consuming sessile species that compete with settling oysters for suitable substrate (McDonald, 1982). Second, the abundance of mud crabs we measured was likely the maximum number and size present in cages because we

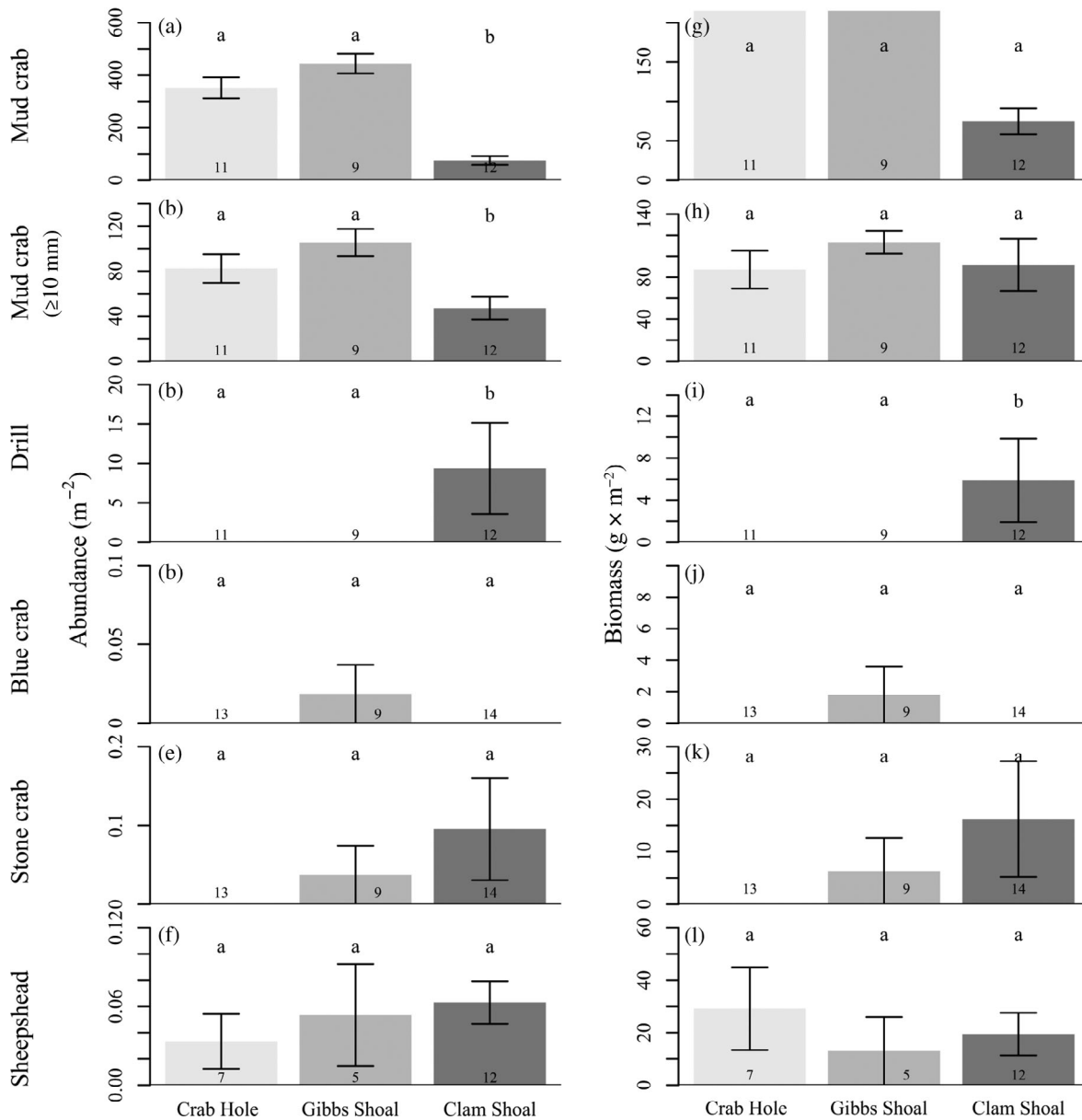


FIGURE 5 Abundance (\pm SE) and biomass (\pm SE) of predators at the three sanctuaries. The numbers above the x-axis indicate sample size. Scales of y-axes differ among groups. Statistical significance among sites within each plot is indicated by different letters.

removed them from cages after each sampling, and only very small crabs (<10 mm) that could enter through the mesh were probably present the majority of the time (Johnson et al., 2014). Finally, antagonistic interactions among mud crabs, both behavioral and consumptive (e.g., cannibalism), are as strong as predator–prey interactions (Geraldi, 2015), which may have reduced oyster predation intensity when the mud crabs were in higher abundance in the cage treatments. Overall, the effect of different predator species on a shared resource was related to species-specific biomass as the biomass of mud crabs surpassed that of any other predator, and mud crabs were likely the only species to have a measurable effect on oyster abundance.

There was no indication that oyster abundance was affected by the species of assessed predators at Clam Shoal (closest to ocean inlet), which could be explained by three plausible hypotheses. First, detection of top-down effects observed at other sanctuaries may have been prevented at this sanctuary because of the high recruitment and growth of oysters, which was most evident at the first sampling across all predator exclusion treatments. Second, although this sanctuary had the greatest density of oysters at the beginning of the study, it had the lowest number of oysters at study end. This could result from oyster diseases (Geraldi et al., 2013) or other oyster predators not measured by this study (e.g., predatory worms), which could have negatively

affected oyster density. It is worth noting that the abundance of these predators can be related to salinity (Garton & Stickle, 1980; Salewski & Proffitt, 2015), and thus, abiotic factors may have played a role in our results. The boom-and-bust pattern of oyster abundance in Clam Shoal could have reduced the measurable effect of oyster predators. In general, the oyster density at all three sanctuaries in the open treatment followed a similar spatial and temporal pattern as oyster abundance observed on the reefs (Geraldini et al., 2013). Oyster abundance and recruitment within the predator exclusion treatments displayed similar patterns through time at the two other sanctuaries, as well as similar abundances of predators, suggesting that both top-down and bottom-up pressures affect the oyster reef community at these two sanctuaries.

The effect of multiple predators on oyster reefs has been conducted in mesocosms or intertidal field experiments using cages. They report that either mud crabs (Grabowski et al., 2008; Hill & Weissburg, 2013; Johnson et al., 2014; Rindone & Eggleston, 2011) or blue crabs (Carroll et al., 2015; O'Connor et al., 2008) reduced oyster abundance. Additionally, on intertidal oyster reefs, mud crabs occur in high abundances across broad geographic ranges (Grabowski et al., 2020) and can have significant consumptive effects on juvenile oysters (Kimbrow et al., 2020). Our multisite, subtidal field experiment suggests that mud crabs were the only predator to have a measurable top-down effect on oyster abundance, although differences were only significant at one of the three oyster sanctuaries. Large crabs were relatively low in abundance, and subsequently, we found no further reduction in oyster abundance when large crabs could access the oysters. To have a true test of the effect of predator guilds, we would have needed treatments that allowed access by larger predators but excluded mud crabs. However, we do not know how this could have been accomplished *in situ*, but this should be kept in mind when interpreting the results and designing future research. Although large crabs were observed in a small percent of quadrats, results from these quadrats had the same abundance rank among sites as measured using crab traps in our study and were similar to (no blue crabs at any sanctuary and no stone crabs at Crab Shoal) or slightly greater than (~0.12 stone crabs per square meter) a previous study on stone crabs at these sanctuaries based on burrow counts (Rindone & Eggleston, 2011). Overall, we quantified the abundance and diversity of oyster reef predators on subtidal reefs at multiple locations, which is important to better understand food web structure and estimate the production of mobile fauna (zu Ermgassen et al., 2016).

Predator and prey that are closer in size have stronger interaction strengths, while greater differences in body size between predator and prey typically have weaker interactions (Heckmann et al., 2012; Kartascheff et al., 2010). Our

findings on subtidal oyster reefs agree with this statement in that mud crabs had a greater interaction strength with oysters than sheepshead fish, assuming interaction strength is measured by oyster abundance in the treatments. In addition, sheepshead, as well as larger crabs, consume both oysters and mud crabs (Abeels et al., 2009; Cutwa & Turingan, 2000; Lenihan et al., 2001; O'Connor et al., 2008), which implies intraguild predation. Intraguild predation theory predicts that the prey (e.g., the smallest predator in a theoretical food web, which would be mud crabs in this study) must be more efficient at acquiring the resource than the top predator in order to maintain the populations of both prey and predator (Polis & Holt, 1992). However, changes in environmental and biotic factors can result in more stable intraguild predator interactions, via alteration to habitat structure (Finke & Denno, 2002; Toscano & Griffen, 2013), size-structured populations (Mylius et al., 2001; Toscano & Griffen, 2013), productivity (Amarasekare, 2007), and the presence of alternative prey (Holt & Huxel, 2007). Our study supports this contention that spatial heterogeneity is important through the findings that abundance of different taxa can change through space and/or time, which may enhance the stability of species abundances within intraguild predation.

Biotic interactions are complex and can change in both space and time. Deciphering context dependency of species interactions *in situ* at different locations brings novel insights that might not be apparent at single locations or in simplified designs of traditional laboratory experiments. In this study, we confirm that predation may exert top-down effects on subtidal oyster reefs by reducing the abundance of the habitat-forming species. However, predatory top-down effects were most evident when experiments were maintained over a year, and second, it was dependent on location. Understanding the complexity of bottom-up and top-down forces on reef building species *in situ* is particularly important given that the relative strength and interactions of these drivers of oyster abundance have direct implications for the maintenance and restoration of oyster reefs.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Nathan R. Geraldi and Charles H. Peterson conceived and designed the study; Nathan R. Geraldi and Maria L. Vozzo collected the data; Nathan R. Geraldi, Andrea Anton, and Stephen R. Fegley analyzed the data; Nathan R. Geraldi wrote the initial draft of the manuscript. All authors contributed critically to editing the manuscript and gave final approval for publication.

DATA AVAILABILITY STATEMENT

Data and R code (Geraldi, 2022) are available from Zenodo: <https://doi.org/10.5281/zenodo.6349509>.

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