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OYSTER–SPONGE INTERACTIONS AND BIOEROSION OF REEF-BUILDING SUBSTRATE MATERIALS: IMPLICATIONS FOR OYSTER RESTORATION

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ABSTRACT Subtidal oyster (Crassostrea virginica) reefs constructed during the mid 1990s in Pamlico Sound, North Carolina, initially supported high densities of oysters; however, beginning around 2007, oyster density subsequently declined. Concurrent with the decline in oyster density was an increase in the prevalence of boring sponges (Cliona) on oysters and the underlying reef substrate material at these sites. The limestone marl substrate used to build these reefs became colonized by boring sponge to the degree that bioerosion by sponge potentially compromised the suitability of the reefs for oysters. Laboratory and field experiments were conducted to determine whether oyster demographic rates of settlement, recruitment, growth, and mortality were affected by the presence of Cliona on underlying shell substrate. Oyster settlement on shells with varying levels of sponge was measured in the laboratory, and oyster recruitment, growth, and mortality in the presence and absence of sponge were measured in 2 estuaries in coastal North Carolina from 2011 to 2012. Potential alternative substrates for future reef restoration were tested in the field to determine their susceptibility to bioerosion by sponges. Substrates included 2 composed of calcium carbonate (CaCO3; oyster shells and limestone marl) and 2 non-CaCO3 substrates (concrete and granite), because bioerosion by clionids target CaCO3. Surprisingly, no differences in oyster settlement, recruitment, growth, or mortality in the presence versus absence of Cliona were observed, suggesting that effects of the presence of sponge on oysters are either chronic rather than acute or indirect and must act in concert with other oyster enemies. Notable patterns of sponge growth on the alternate substrates emerged; oyster shells were most susceptible to sponge growth, followed by marl, whereas concrete and granite were not susceptible to colonization by Cliona. Results from this study demonstrate the importance of having a strong understanding of the specific restoration methodology to be implemented, because the susceptibility of a substrate to bioerosion could have serious implications for reef longevity. Evidence is presented that consideration of the broader estuarine environment, including both biotic and abiotic factors, is vital when planning restoration actions, because the presence of Cliona was less important than the estuarine salinity gradient in altering oyster demographic rates and may shift the trajectories of restored reefs.

KEY WORDS: oyster, Crassostrea virginica, restoration, clionid sponge, salinity, bioerosion, settlement, recruitment, growth, mortality

INTRODUCTION

Efforts to restore the eastern oyster, Crassostrea virginica Gmelin (hereafter, “oyster”), along much of the Atlantic and Gulf coasts of the United States have had varying levels of success (Mann & Powell 2007, Powers et al. 2009, Schulte et al. 2009, Beck et al. 2011, La Peyre et al. 2014a). For example, in North Carolina, many oyster reefs built within no-take oyster sanctuaries during the past 30 y have met or exceeded minimal criteria for success (criteria from Powers et al. [2009], additional reefs sampled by Puckett and Eggleston [2012]). Other restored reefs in North Carolina likely met these criteria initially, but have since had dramatic declines in oyster abundance (C. Hardy, North Carolina Division of Marine Fisheries [NCDMF], unpubl. data). Large-scale oyster restoration in Chesapeake Bay has been described as a failure (Mann & Powell 2007), whereas restoration activities within small tributaries of Chesapeake Bay have been characterized as a success (Schulte et al. 2009). The need to restore this important estuarine ecosystem engine stems from a tremendous decline in oyster abundance, with more than 90% of oyster reef habitat lost along the U.S. Atlantic coast compared with historic abundance levels (Newell 1988, Heral et al. 1990, Rothschild et al. 1994, Frankenberg 1995, Beck et al. 2011). These tremendous losses are the result of the combined effects of overfishing (Rothschild et al. 1994, Jackson et al. 2001, Wilberg et al. 2011), eutrophication and sedimentation (Cooper & Brush 1993, Powers et al. 2009), and disease (Andrews 1988, Ford & Tripp 1996, Lenihan et al. 1999, Powell et al. 2008), among other factors. Although originally focusing on augmentation of the oyster fishery, recent oyster restoration efforts have begun to focus on reef building within marine reserves that will provide ecosystem functions and services to estuaries in perpetuity. These include the supply of oyster larvae to fished areas, improved water quality, habitat for other estuarine species of commercial or recreational importance, wave energy attenuation, and many others (Peterson et al. 2003, Coen et al. 2007, Grabowski & Peterson 2007, Pierson & Eggleston 2014).

The calcareous, vertical structure created by oyster reefs provides some of the only habitat structure within otherwise homogeneous soft-bottom estuaries. Species of boring sponge in the genus Cliona are widespread bioerosers on all types of calcareous substrates (Wells 1959, Goreau & Hartman 1963, MacGeanachy 1977), and bioerosion by sponges can be severe enough to damage the structural integrity of calcareous reefs (Rützler 1975). Bioerosion by boring sponges involves penetrating a calcium carbonate (CaCO3) substrate by processing solid carbonate into smaller fragments via cellular etching (Warburton 1958b, Cobb 1969). The cellular etching process is mediated chemically by microscale dissolution around the edges of carbonate flakes, followed by mechanical removal of these flakes by sponge pseudopodia
(Warburton 1958b). The sponge then inhabits the holes and galleries created inside the CaCO₃ substrate. For oysters, additional metabolic demands can be required to fight the sponge invasion, such as producing excess CaCO₃ to “patch” holes in the inner wall of a shell valve as the sponge penetrates through the shell of the oyster (Wells 1959). Carbonate substrates are susceptible to bioerosion because of their solubility in seawater and their relatively low level of hardiness compared with non-CaCO₃ substrates (Golubic & Schneider 1979). Noncarbonate materials (such as granite or concrete) should be less susceptible to sponges because they do not contain CaCO₃ for the sponge to dissolve via the chemically mediated boring mechanism. Dispersal of Cliona can occur through direct shell-to-shell contact of an infested and uninfested oyster (Warburton 1958a), through sexual reproduction followed by larval dispersal (Fell et al. 1984, Carver et al. 2010), and through asexual reproduction by fragmentation (Hartman 1958). Sponge dispersal onto and within North Carolina oyster reserves has not been investigated and likely involves all 3 transmission mechanisms.

Direct and indirect impacts of boring sponges on oysters are varied and may interact with salinity and reef substrate to shape community dynamics on restored reefs. Guida (1977) found higher mortality of caged, adult oysters that were infested with Cliona when compared with sponge-free oysters. The increased mortality rate of Cliona-infested oysters was likely caused by a combination of sponge presence and the presence of other stressors, including parasites and oyster diseases (Guida 1977). Other investigations showed larval oyster settlement onto conspecific shells was reduced and larval oyster mortality increased in the presence of Cliona (Barnes et al. 2010). The mechanism causing lower settlement remains unknown, but Barnes et al. (2010) observed clam worms (Neanthes succinea) preying directly on larval oysters, and greater abundances of worms were seen in Cliona-infested shell treatments than in control shells. The greater abundance of N. succinea in these treatments was likely a result of sponge bioerosion, as the holes found in Cliona-infested oyster shells provide refuge habitat for clam worms. Bioeroders can even lower overall larval production from oyster reefs through repeated shell damage to individuals, which can result in an abnormally high male-to-female ratio on reefs as stressed individuals remain male rather than transitioning to female (Bahr & Hillman 1967, Davis & Hillman 1971). Bioerosion of the shells of molluscs can increase predation risk from crushing and drilling predators as shells lose structural integrity and individuals lose their size refuge from predation (Thomas 1979, Pomponi & Meritt 1990, Steffaniak et al. 2005, Buschbaum et al. 2007). Similarly, shell brittleness caused by sponges lowers the market value for harvested oysters (Wells 1959, Carver et al. 2010). The distribution of clionid sponges in estuaries in the southeastern United States is highly dependent on salinity. Oyster reefs in moderate- to low-salinity areas (<20) contain lower abundances of boring sponges as a result of the inability of the sponges to cope with long-term freshwater pulses (Wells 1961, Hopkins 1962), whereas intertidal oyster beds are relatively sponge-free as a result of desiccation and exposure to fresh rainwater during low tides (Hopkins 1962, Guida 1977). Salinity also plays a key role in the distribution of oyster reefs within estuaries (Grave 1904, Wells 1959, Wells 1961), with increasing risk of oyster disease and predation with increasing salinity (Wells 1961, Chu et al. 1993). Thus, bioerosion by clionid sponges may affect oysters directly or by altering biotic interactions with other estuarine species with potentially important consequences for oyster reef community dynamics.

In North Carolina, subtidal oyster reef restoration has created a network of no-take oyster reserves in Pamlico Sound since the mid 1990s. Reserves consist of multiple, individual, high-reliability, artificial mounds (height, ~2 m; diameter, ~10 m) constructed of limestone marl boulders across areas of 1–5 ha. At reserves targeted for monitoring from 2006 to 2008, most sites exhibited evidence of increases in oyster density, some with increases of up to 400% on recently constructed reefs (Puckett & Eggleston 2012). At some of these reserves, as well as others distributed spatially throughout Pamlico Sound (Clam Shoal, Ocracoke, Gibbs Shoal, among other reserves; see map of reserves in Puckett and Eggleston [2012]), oyster densities began to decline in late 2007. There was no evidence of oyster disease, burial by shoaling, or hypoxic conditions at these locations (C. Hardy, NCDMF, unpubl. data), which are often the typical causes of such rapid declines (Ford & Tripp 1996, Lenihan 1999, Taylor & Bushek 2008). Coincident with the decline in oyster density at these reserves was an increase in the prevalence of Atlantic oyster drills (Urosalpinx cinerea) and clionid boring sponges. Sponges were ubiquitous within oyster shells and the marl reef foundation (NCDMF and N. Lindaquist, unpubl. data). The current study focused on potential negative effects of the presence of clionid sponges on reefs by measuring oyster demographic rates, including settlement, recruitment, growth, and mortality, in the presence and absence of boring sponge. Concurrently, the susceptibility of 4 potential reef-building substrate materials to bioerosion by Cliona was investigated. These substrates included 2 composed mainly of CaCO₃ (oyster shells and marl) and 2 noncarbonate materials (crushed concrete and granite rock). The hypotheses tested were (1) presence of Cliona would lower oyster settlement, recruitment, and growth, and increase oyster mortality; and (2) CaCO₃ substrates would be more susceptible to infestation by sponges than non-CaCO₃ substrates.

MATERIALS AND METHODS

Laboratory Experiment

Larval Oyster Settlement on Shells with Varying Degrees of Boring Sponge

The influence of the level of boring sponge infestation on oyster larval settlement was investigated in the laboratory at North Carolina State University in Raleigh, North Carolina, during August 2012. Oyster shells with 3 levels of sponge infestation were used as settlement substrates: (1) clean, weathered shells with no evidence of previous bioerosion by boring sponges (hereafter, “bare”); (2) clean, weathered shells with obvious evidence of previous bioerosion by boring sponges, but no live sponge (characterized by regularly spaced holes throughout the shell; hereafter, “shells with holes”); and (3) oyster shells infested throughout the inside and outside of the valve with live boring sponge (hereafter, “live sponge”). Shells with live sponge were collected from estuaries around Morehead City, North Carolina, just prior to experimental initiation, and all shells used were similar in size (7-cm shell height [SH] × 4-cm shell width × 1-cm valve depth). Bare shells and shells with holes were allowed to soak in sand-filtered seawater from Bogue Sound, North Carolina, for 4 days for biofilm development (Fitt et al. 1990), whereas shells containing live sponge taken
from the field were left with their natural microbial biofilm. After soaking, a single shell of each type was placed into a 5-L plastic tub (n = 16), with the inner side of each valve facing upward and even spacing between each shell. Tubs were filled with 3.5 L brackish water (salinity 12) that was created by mixing sand-filtered seawater (salinity 30+) and deionized laboratory water so that the salinity in experimental tanks matched the salinity in which larvae were reared at the hatchery. No negative effects of the relatively low salinity on the live sponge were seen during the experiment. The water in tubs was held at room temperature (23°C) for the duration of the experiment, and a standard aquarium air bubbler was installed in 1 corner of each of the tanks and ran continuously throughout the experiment. The location of shell types within tanks relative to the air bubbler was randomized. The algal paste T. Isochrysis, commonly used in bivalve aquaculture, was added to each tank at a concentration of \( \frac{1}{10^6} \text{cells/mL} \) to allow larvae to feed during the experiment.

Approximately 5,000 pediveliger stage, "eyed" larvae (obtained from wild broodstock cultured by the Horn Point Oyster Hatchery, Cambridge, Maryland) were introduced into each of the tubs on August 24, 2012, and left for 4 days. Prior to introduction into tubs, a subsample of larvae was examined with a stereomicroscope and individual larvae were seen to be "eyed" and swimming actively. The 4-day experimental time period allowed larvae sufficient time to settle and metamorphose given their age and late developmental stage. At the conclusion of the settlement period, settled oysters, hereafter called spat, were enumerated.

To quantify spat settlement, the inner side of valves of all shells was examined under 12× magnification using a stereomicroscope. The inner side was selected for spat counts because oyster larvae prefer to settle on the smooth, inner side of shells rather than the rough outer side (Crisp 1967). All live spat within a haphazardly selected subsample (circle with diameter 2 cm) were counted, and data were scaled to spat cm\(^{-2}\) for analysis. Analysis of variance (ANOVA) with sponge infestation level as the fixed factor was performed to test the null hypothesis that oyster spat density did not differ among sponge infestation levels. Tukey's honest significant difference test was used to compare spat density among treatment levels. Data met all assumptions of ANOVA based on visual inspection of residual plots, and passed Levene's test for homoscedasticity.

Field Experiment

To test for an effect of the presence of Cliona on oyster recruitment, growth, and mortality, and to examine the invasion potential of sponges onto alternate reef-building substrates, "substrate bag" experimental units were created and deployed in the field from August 2011 to October 2012. Substrate bags were deployed at each of 5 sites in the North and Newport River estuaries in Carteret County, North Carolina (Fig. 1). Sites were chosen according to preliminary salinity monitoring (N. Lindquist, unpubl. data) to represent locations that would experience salinities that could affect sponge growth as well as affect oyster recruitment, growth, and mortality rates, because sponges do not survive in low salinities, whereas oysters experience relatively high sponge coverage and predation in high-salinity areas (Hopkins 1956, Wells 1959, Wells 1961). Deployment sites for substrate bags experienced average salinities ranging from \( \sim 20 \) at up-estuary sites to near-full oceanic salinity (\( \sim 35 \)) at down-estuary sites (Table 1). Variations in salinity at each site were driven by varying levels of riverine input and tidal mixing, as well as wind direction and strength. In general, the mean salinity increased when moving from site 1 (up-estuary, low salinity) to site 5 (down-estuary, high salinity), whereas the range of salinities measured at each site decreased moving down-estuary, such that sites 1 and 2 in each estuary had

![Figure 1](attachment:image.png)
TABLE 1.

Water quality monitoring data from substrate bag deployment sites.

<table>
<thead>
<tr>
<th>Site</th>
<th>Salinity mean</th>
<th>Range</th>
<th>Dissolved oxygen mean (mg/L)</th>
<th>Range (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newport 1</td>
<td>20.39</td>
<td>12.8–30.1</td>
<td>6.59</td>
<td>4.16–8.9</td>
</tr>
<tr>
<td>Newport 2</td>
<td>23.28</td>
<td>16.1–30.4</td>
<td>6.66</td>
<td>3.94–9.21</td>
</tr>
<tr>
<td>Newport 3</td>
<td>22.94</td>
<td>15.6–28.2</td>
<td>6.69</td>
<td>4.05–8.8</td>
</tr>
<tr>
<td>Newport 4</td>
<td>25.40</td>
<td>21.7–29.6</td>
<td>7.02</td>
<td>4.83–8.71</td>
</tr>
<tr>
<td>Newport 5</td>
<td>30.68</td>
<td>25.8–32.2</td>
<td>6.63</td>
<td>3.72–8.51</td>
</tr>
<tr>
<td>North 1</td>
<td>21.48</td>
<td>16.9–26.7</td>
<td>6.65</td>
<td>4.01–9.01</td>
</tr>
<tr>
<td>North 2</td>
<td>23.23</td>
<td>19.2–27.5</td>
<td>6.99</td>
<td>4.72–9.81</td>
</tr>
<tr>
<td>North 3</td>
<td>28.40</td>
<td>23.6–34.4</td>
<td>6.51</td>
<td>5.4–8.94</td>
</tr>
<tr>
<td>North 4</td>
<td>28.68</td>
<td>20.4–35.5</td>
<td>6.61</td>
<td>5.27–8.99</td>
</tr>
<tr>
<td>North 5</td>
<td>33.54</td>
<td>29.9–35.8</td>
<td>6.97</td>
<td>5.98–8.33</td>
</tr>
</tbody>
</table>

Data from opportunistic synoptic sampling between August 2011 and May 2013.

wider variations in salinity than sites 4 and 5 (Table 1; also see Fig. 2 in Dunn et al. [2014]). Substrate bags were deployed subtidally, with sites averaging 0.5–1.0 m in depth at mean low water, with mean astronomical tides of ~1 m. Each site initially had 30 bags ordered randomly at 0.5-m increments on an anchored line. The anchored line initially sat directly on the benthos, but was later raised 0.25 m above the bottom using cinderblocks and PVC tubing to avoid burial by sediment.

All experimental substrates were gathered from locally available sources, including marl and weathered oyster shells from the NCDMF as well as concrete and granite from local construction supply companies. All previous fouling organisms were removed from oyster shells prior to use. All substrate pieces were as uniform in size as possible (7 × 4 ×3-cm dimensions each for marl, granite, and concrete [length × width × height]; and for oyster shells, 7-cm SH × 4-cm shell width × 1-cm depth). Individual substrate bags were made from 15-mm plastic mesh, chosen because it was small enough to hold all materials together, but also allowed for sufficient water flow and delivery of oyster larvae to experimental substrates. Each bag contained 2 pieces of a single substrate type (oyster shell, marl, granite, or concrete) surrounded by ~100 mL extra oyster shell material (used to impose the Cliona presence/absence treatment as described later). Within each bag, 1 substrate piece had hatchery-reared oysters settled on it in the laboratory (larvae from Bear Creek Shellfish, wild broodstock from mesohaline oyster reef in Bear Creek estuary, Swansboro, North Carolina) prior to deployment in the field (hereafter, “substrate with spat”), whereas the second substrate piece in each bag was clean (hereafter, “substrate without spat”). The substrate pieces with and without spat in each bag were labeled individually using a small dot of different-color marine epoxy for identification during sampling. To expose both substrate pieces to Cliona sponge, the ~100 mL (measured by volume displacement) of extra shell material in bags was highly infested (>80% surface sponge coverage) by live Cliona (n = 6 for each substrate type at each site). All sponge-infested shell material was collected from oyster beds surrounding Morehead City, North Carolina, and was held in tanks with flow-through, unfiltered seawater until experimental deployment in the field. In addition, “control” substrate bags in which the 2 pieces of experimental oyster shell substrate were surrounded by 100 mL of clean shells without live sponge were also deployed (n = 6 at each site). The sponge-free control was paired only with the experimental oyster shell substrate. Within bags, the additional shell material surrounded the 2 experimental substrate pieces, and the plastic mesh was cinched tight enough that substrate pieces were brought into direct contact with the extra shell material. Sampling occurred 7 times throughout the course of the experiment and involved retrieving the anchored line from each site at or close to high tide, opening each bag and examining both substrate pieces. During examination of the substrate with spat, all oysters present were counted and measured from the umbo region of the shell to the anterior shell margin (SH measured in millimeters). During examination of the substrate without spat, a visual estimate of percent coverage of Cliona was made based on the surface area of the piece that was covered in yellow-orange sponge papillae protruding from or encrusting the substrate surface. To avoid sampler bias, 1 researcher (R. D.) made all estimates of sponge percent coverage. After the onsite sampling, substrate bags were immediately redeployed at each site. The water quality parameters of salinity, temperature, and dissolved oxygen were recorded with a YSI-85 at a depth of 1 m at each of the study sites while sampling.

Substrate bags were designed to investigate susceptibility of the 4 alternate substrates to bioerosion by boring sponges while simultaneously testing the effect of the presence of Cliona on...
growth and mortality of presettled oysters in the presence/absence of without spat). These were used to test separate sets of hypotheses. The substrate with spat was used to examine natural oyster recruitment, as well as

Bags contained 2 pieces of a particular substrate: 1 with oysters preattached (substrate with spat) and 1 without oysters preattached (substrate without spat). These were used to test separate sets of hypotheses. The substrate with spat was used to examine natural oyster recruitment, as well as growth and mortality of presettled oysters in the presence/absence of Cliona, whereas the substrate without spat was used to test susceptibility of each substrate to Cliona sponge growth.

<table>
<thead>
<tr>
<th>Experimental substrate in bag (sample size/site)</th>
<th>Cliona present/absent in bag</th>
<th>Hypotheses tested using substrate with spat (duration of data collection)</th>
<th>Hypothesis tested using substrate without spat (duration of data collection)</th>
</tr>
</thead>
</table>

Bags contained 2 pieces of a particular substrate: 1 with oysters preattached (substrate with spat) and 1 without oysters preattached (substrate without spat). These were used to test separate sets of hypotheses. The substrate with spat was used to examine natural oyster recruitment, as well as growth and mortality of presettled oysters in the presence/absence of Cliona, whereas the substrate without spat was used to test susceptibility of each substrate to Cliona sponge growth.

Oyster recruitment, growth, and mortality by mimicking the situation of a recently settled oyster either on a reef heavily infested by Cliona or on a sponge-free reef. The substrate without spat was used to test the null hypothesis that each of the 4 substrates would be equally susceptible to growth of Cliona (Table 2). The substrate with spat in oyster shell bags with sponge-infested material was compared with the spat experimental shell from sponge-free bags to test the null hypotheses that oyster recruitment, growth, and mortality would not be affected by the presence of Cliona (Table 2). Individual methods for analyses of oyster recruitment, growth, and mortality, and sponge growth are described later.

**Recruitment of Oysters in the Presence and Absence of Sponge**

During summer 2012, natural oyster recruitment was monitored in substrate bags containing the oyster shell experimental substrate. Natural recruitment was not monitored in 2011 because substrate bags were deployed after the major peak in oyster spawning in this area of North Carolina (Mroch et al. 2012, Puckett & Eggleston 2012). Oyster recruit density (defined as individuals younger than 4 mo) on experimental oyster shells in bags with and without sponge present was compared to test for an effect of the presence of Cliona on oyster recruitment. At the initiation of recruitment monitoring, many experimental shells had oysters attached that had survived from the predeployment laboratory setting, as well as some natural settlers from fall 2011. Thus, a census of all oysters on each experimental shell was done in April 2012 to produce a baseline oyster abundance measure for use as a covariate in analyses of recruitment density, because the presence of conspecifics can influence larval oyster settlement (Hidu 1969, Keck et al. 1971, Hidu et al. 1978). Oyster abundances were standardized for comparison by using approximately equal-size oyster shells (dimensions as noted earlier). Counts of all newly recruited oysters on the substrate with spat in each bag were made during 3 sampling events (July, September, and October 2012). Year 2012 recruits were clearly distinguishable from individuals settled prior to summer 2012 based on their size (e.g., mean SH at the July 2012 sampling event in the Newport River estuary: 2012 recruits, 6.68 ± 0.8 mm; pre-2012 settlers, 42.8 ± 2.1 mm). Some bags were lost or destroyed such that oyster recruit density data were available from 114 of the original 120 oyster shell substrate bags (n = 56 bags with sponge-infested shells and n = 58 bags with no-sponge shells), so only these 114 were included in statistical analyses. Repeated-measures analysis of covariance (ANCOVA) was used to test for differences in recruit density between oyster shells in bags with and without Cliona, among sampling events (i.e., the within-subject effect), among sites, and with varying abundance of preattached conspecifics (i.e., the covariate). The number of newly recruited oysters on a replicate shell made up the response variable during each of the 3 sampling periods (repeated measure), and estuary, site, and sponge presence/absence were fixed factors. The baseline oyster abundance from April 2012 was the covariate. Oyster recruit density data met assumptions of ANOVA on visual inspection of residuals and quantile plots, and passed Levene’s test for homoscedasticity. The sphericity assumption of repeated-measures ANCOVA designs was addressed by calculating adjusted P values for all within-subject effects tests (Greenhouse & Geisser 1959). Greenhouse-Geisser (G-G)-adjusted P values did not differ greatly from unadjusted P values in any case, but are included in Results as a reference. Full statistical models initially included all interactions, with subsequent removal of nonsignificant interaction terms.

**Growth of Oysters in the Presence and Absence of Sponge**

All surviving laboratory-set oysters on the substrate with spat in bags originally out-planted to the field in August 2011 were measured (SH) in September, October, and December 2011, and in April 2012. The mean SH of all oysters on the shell with spat in each bag at each sampling event was calculated and served as the response variable. The substrate with spat in oyster shell bags with sponge-infested material (13.75 ± 1.03 spat per
shell when out-planted to the field, mean ± 1 SE) was compared with the with-spat experimental shell from sponge-free bags (10.33 ± 0.77 spat per shell). All oysters measured for growth analysis were settled at the same time, so SH was used as a proxy for oyster growth. Mean SH data were used to test for differences in oyster growth between bags with and without Cliona, between estuaries, and among sites using ANOVA, with sampling time, estuary, site, and sponge presence/absence as fixed factors. Assumptions of ANOVA were met based on visual inspection of residual and quantile plots, and data passed Levene’s test for homoscedasticity. Interaction terms were treated as described earlier.

Mortality of Oysters in the Presence and Absence of Sponge

Mortality of oysters in bags with and without Cliona was calculated for August 2011 through April 2012. For each sampling event, the cumulative mortality of the original total number of laboratory-set individuals on each with-spat piece of oyster shell substrate was calculated according to the equation

\[
\text{Mortality} = \frac{\text{No. live}(t_0) - \text{No. live}(t)}{\text{No. live}(t_0)},
\]

where No. live \((t_0)\) represents the count of live oysters on each oyster shell substrate with spat prior to being out-planted in the field, and No. live \((t)\) represents the count of live oysters from that cohort still alive at sampling event \(t\). Individuals from the original cohort were distinguished from natural recruits by size difference, which was apparent through spring 2012. Cumulative mortality values were used to test for differences between substrate bags with and without Cliona, as well as among estuaries and sites. Mortality data were analyzed with a repeated-measures ANOVA model with time (repeated measure), estuary, site, and sponge presence/absence as fixed factors. Assumptions of repeated-measures ANOVA were met based on visual inspection of residual and quantile plots, and data passed Levene’s test for homoscedasticity. The sphericity assumption of repeated-measures ANOVA were met based on visual inspection of residual and quantile plots, and data passed Levene’s test for homoscedasticity. Interaction terms were treated as described earlier.

Growth of Boring Sponge on Reef-Building Substrates

Differences in growth of boring sponge onto the 4 alternate substrates was tested by estimating percent coverage of sponge on substrate pieces without spat. Sponge tissue samples were taken from a subset of bags of each substrate type and were confirmed as Cliona spp. using the methods of Old (1941). Some bags were lost or destroyed such that sponge growth data were available from 258 of the 300 bags initially deployed, so only these 258 were included in statistical analyses. Sponge growth was analyzed with a repeated-measures ANOVA; the percent coverage of Cliona on the substrate without spat in each bag was the response variable at each sampling period (7 sampling events), and estuary, site, and substrate were treated as fixed effects. Assumptions of repeated-measures ANOVA were met based on visual inspection of residual and quantile plots, and data passed Levene’s test for homoscedasticity. The sphericity assumption and nonsignificant interaction terms were addressed as described earlier.

RESULTS

Larval Oyster Settlement on Shells with Varying Degrees of Boring Sponge

Level of infestation by clionid sponges had no statistically significant effect on mean settlement of oyster larvae onto oyster shells (1-way ANOVA, \(F_{1,45} = 0.52, P = 0.5956\)). There was nearly equal spat settlement on bare shells and shells with holes from previous sponge infestation (mean ± 1 SE: bare, 22.79 ± 5.27 spat/cm²; with holes, 21.27 ± 4.81 spat/cm²), whereas shells with live sponge attracted ~25% fewer spat (16.43 ± 3.48 spat/cm²) compared with both shell types without live sponge (1-way ANOVA comparing shells with and without live sponge; bare and with-holes data pooled, nonsignificant \([P = 0.3193]\)).

Recruitment of Oysters in the Presence and Absence of Sponge

A significant interaction between estuary and site (unpubl. data) required oyster recruitment data from each estuary to be analyzed separately. The baseline abundance of oysters on experimental shells was not a significant covariate for either estuary (repeated-measures ANCOVA, both \(P > 0.1\) for covariate) and was not included as a factor in final statistical models for either estuary. The presence of boring sponge had no effect on oyster recruitment to oyster shells in either estuary (2-way repeated-measures ANOVA, \(F_{1,50} = 0.44, P = 0.5114\) and \(F_{1,52} = 1.10, P = 0.30\) for sponge presence factor for Newport and North, respectively; Fig. 2). There was a significant effect of site on oyster recruit density in both estuaries (2-way repeated-measures ANOVA, \(F_{4,50} = 46.14, P < 0.0001\) and \(F_{4,52} = 5.40, P = 0.001\) for site factor for Newport and North, respectively), with recruitment generally increasing moving down-estuary over most sampling events.

Growth of Oysters in the Presence and Absence of Sponge

A significant interaction between sampling time and other main effects required oyster growth data to be analyzed separately for each sampling event. The presence of boring sponge in substrate bags did not have a significant effect on oyster growth (Fig. 3) for any of the 4 sampling events, nor were there significant differences in mean SH between oysters from the 2 estuaries (Table 3). There were variable effects of site, with a significant site effect on oyster growth for 3 of the sampling events (Table 3), although for 2 of these sampling events (October 2011 and April 2012), there were significant estuary × site interaction effects, which prevented pairwise means comparisons among sites. In general, oysters at the highest salinity sites had larger SH than up-estuary sites 1 and 2 (Fig. 3). No oysters at site 3 in either estuary survived through December 2011, likely a result of burial by mud, so no comparisons of SH were made for that site beyond October 2011.

Mortality of Oysters in the Presence and Absence of Sponge

The presence of boring sponge in substrate bags did not have a significant effect on oyster mortality (Table 4, Fig. 4), nor was there a significant difference in mortality between estuaries (Table 4). Oyster mortality was affected significantly by site (Table 4), with oysters at both up-estuary sites 1 and 2 having lower mortality than oysters at the more down-estuary sites during 3 of the 4 sampling events. Oyster mortality was generally high (Fig. 4) and increased through time at most sites.
A significant 3-way interaction among estuary, site, and substrate required sponge coverage data to be analyzed separately for each estuary. The reduced model for the Newport River estuary contained the main effects of site and substrate, whereas the reduced model for the North River estuary contained a significant interaction between site and substrate, such that each site within this estuary was analyzed separately. In general, percent coverage of boring sponge was greater on CaCO₃ substrates than non-CaCO₃ alternatives, although only oyster shell was statistically different from the non-CaCO₃ substrates (Figs. 5 and 6). Concrete and granite were rarely seen with any substrates (Figs. 5 and 6). During early summer 2012, there was evidence of sponge infestation, and when sponge was seen on these substrates it was always encrusting the surface rather than boring into the interior. For the North River estuary, no statistical analysis was possible for data from site 1 because no sponge growth was seen on any experimental unit, likely a result of the inability of the sponge to survive at the low salinities experienced at this site. At North River site 2, where conditions were similar, statistical analyses revealed no significant between- or within-subject effects (1-way ANOVA for between-subject test of substrate, $F_{4,24} = 0.95$, $P = 0.4516$; G-G adjusted within-subject effects tests for time and time × substrate, both $P > 0.35$). Survival of Cliona on the extra shell material within substrate bags was also depressed at up-estuary sites compared with higher salinity locations in both estuaries (R. Dunn, unpubl. data). At North River sites 3–5, where Cliona was able to grow, there was a significant effect of substrate as well as significant within-subject effects of time (1-way repeated measures ANOVAs, all between- and within-subject effects, $P < 0.013$; Fig. 5). Sponge growth in the Newport River estuary was significantly affected by site and substrate, and all within-subject effects were also significant (2-way repeated-measures ANOVA, all between- and within-subject effects, $P < 0.0001$). In both estuaries, sponge percent coverage increased moving down-estuary (Figs. 5 and 6). Very little boring sponge colonized any of the experimental substrate pieces at sites 1 and 2 in either estuary, and ideal environmental conditions for sponge growth appeared to be at mid-estuary sites, where salinity was slightly depressed from full oceanic conditions (Fig. 5). Sponge growth ceased during winter 2011 when sponge coverage on experimental substrates remained fixed or even retracted slightly (Fig. 5), whereas there was tremendous growth during summer and early fall 2012 (Figs. 5 and 6). During early summer 2012, there was evidence of sponge colonization via either sexual reproduction or asexual fragmentation, as oyster shell substrates in control bags that did not have direct contact with sponge-infested shells began showing boring sponge growth in July 2012 (Figs. 5 and 6). After Cliona colonized control shell bags, these shells began to exhibit higher levels of sponge coverage than the marl, granite, or concrete substrates that had been exposed to Cliona throughout (Figs. 5 and 6).
TABLE 4.
Results of repeated-measures analysis of variance of oyster mortality rates in the Newport and North River estuaries.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Type III SS</th>
<th>F value</th>
<th>P value</th>
<th>G-G P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estuary</td>
<td>1</td>
<td>0.086</td>
<td>0.48</td>
<td>0.4896</td>
<td></td>
</tr>
<tr>
<td>Site</td>
<td>4</td>
<td>13.68</td>
<td>19.06</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Sponge treatment</td>
<td>1</td>
<td>0.077</td>
<td>0.43</td>
<td>0.5135</td>
<td></td>
</tr>
<tr>
<td>Residual</td>
<td>110</td>
<td>19.74</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>3</td>
<td>2.36</td>
<td>52.18</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Time × estuary</td>
<td>3</td>
<td>0.075</td>
<td>1.66</td>
<td>0.1745</td>
<td>0.1911</td>
</tr>
<tr>
<td>Time × site</td>
<td>12</td>
<td>0.435</td>
<td>2.40</td>
<td>0.0055</td>
<td>0.0160</td>
</tr>
<tr>
<td>Time × sponge treatment</td>
<td>3</td>
<td>0.083</td>
<td>1.84</td>
<td>0.1395</td>
<td>0.1602</td>
</tr>
<tr>
<td>Residual (time)</td>
<td>330</td>
<td>4.98</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Between-adjusted and within-subject effects are presented. Standard unadjusted and Greenhouse-Geisser (G-G) adjusted probability values are given for within-subject effects.

This study demonstrates that the presence of a common bioeroding sponge (*Cliona*) does not affect oyster settlement, growth, or mortality negatively during early life, whereas the estuarine salinity gradient has a strong effect on oyster demographic rates, as growth, and mortality all increased moving down-estuary (Figs. 2–4). The type of substrate used and location chosen (Figs. 5 and 6) for oyster reef restoration within an estuary can have direct and potentially interactive effects on the viability of restored reefs. The lack of a negative effect of the presence of *Cliona* on the oyster demographic rates measured here could be a result of the relatively short duration of the study (<6 mo), if the effects of the presence of boring sponges are chronic rather than acute. Alternatively, the lack of a negative effect of sponge presence could be because other oyster enemies, such as disease, parasites, or predators, are needed to interact with *Cliona* to affect oyster demographic rates negatively.

Bioerosion by boring sponges does not appear to leave a direct, lasting impact on the ability of an oyster shell to attract settling oyster larvae after the sponge dies, because shells with evidence of previous *Cliona* infestation did not have lower spat density compared with shells without sponge excavations. The tunnels and galleries left in shells from previous sponge bioerosion, however, may provide important habitat for other infaunal species, some of which are predators of oysters (e.g., clam worms [Barnes et al. 2010]). Thus, oyster larvae settling on reefs that have had high *Cliona* sponge coverage may experience an indirect predation effect resulting from the refugia provided by previous sponge infestation. Similarly, oyster shells with a history of bioerosion by sponges or polychaetes experience greater rates of dissolution than their nonbored counterparts, resulting from the increased shell surface area available for dissolution (Waldbusser et al. 2011). Thus, infestation by boring sponge allows for increased predation of settling oysters and causes greater dissolution rates of reef material, both of which could have implications for long-term reef sustainability even if oyster larvae do not actively discriminate between shells with varying levels of sponge infestation.

Although oyster mortality was not influenced by the presence of sponge on neighboring shell material in the field, previous studies have reported increased mortality of oysters and gastropods infested with *Cliona* compared with sponge-free individuals, likely in combination with other stressors such as disease, parasites, or predators (Guilda 1977, Seed 1980, Stefaniak et al. 2005). Bioerosion alone appears to be insufficient to increase mortality levels of oysters above those of noninfested individuals, but may contribute to mortality of the oyster in conjunction with other oyster enemies by reducing shell strength or causing increased physiological demands.

Patterns of bioerosion on the alternate substrates tested here, as well as results from other studies, suggest that physical factors within the estuarine environment may interact with the substrate chosen for reef building to affect restored reef development. Direct interactions between the estuarine environment and reef substrate via taphonomic processes, dissolution, burial, and movement are well known (Waldbusser et al. 2011 and references therein, and Lenihan 1999). Geochemical, biological, and sedimentary processes likely interact to affect reef substrate dissolution rates within estuaries (Waldbusser et al. 2011), and in some cases, biological components of an ecosystem can mediate these environmental–substrate interactions. For instance, physiological tolerance appears to limit the distribution of clionid sponges to high-salinity portions of estuaries (Hopkins 1956, Hopkins 1962, Nicol & Reisman 1976, Carver et al. 2010) (Fig. 5), and in areas where sponges persist and bioerosion is common, calcareous substrates are more susceptible to degradation than non-CaCO3 reef-building materials (Figs. 4 and 5). In areas with lower salinities where sponges are not abundant, however, severe bioerosion of reef-building substrates is less problematic. Although there is no evidence that the presence of *Cliona* affected oyster demographic rates in the current study, the use of CaCO3-based substrates for subtidal reefs constructed in high-salinity areas may be problematic as a result of bioerosion of the reef base itself. If sponge bioerosion of the underlying reef material is sufficient to result in the loss of vertical reef structure, other estuarine species that use reefs as...
habitat, including finfish, crustaceans, and many other taxa, will be indirectly negatively affected.

Growth of boring sponge on the oyster shell substrate in control bags (Figs. 5 and 6), suggested that *Cliona* colonization via waterborne dispersal of larvae and/or asexual fragments (i.e., gemmules) occurred during summer 2012. It was not possible to determine which of these 2 dispersal methods allowed for colonization of control shell bags, but sponge growth on these shells became evident just after the *Cliona* larval reproduction period of May to early July (Figs. 5 and 6), which has been reported in estuaries of the northwest Atlantic (Fell et al. 1984, Carver et al. 2010). To date, the spawning period for clionids in North Carolina has not been documented, although sponges in North Carolina likely spawn earlier than those found at higher latitudes (Fell et al. 1984, Carver et al. 2010), because many spawning events are triggered by water temperature cues (Loosanoff & Davis 1950, Loosanoff & Davis 1952, Galtsoff 1961, Loosanoff 1969, Barber & Blake 1991). Noncarbonate materials are undesirable habitat for clionid sponges because these substrates are not susceptible to sponge colonization by either direct contact with sponge tissue or recruitment of sponge larvae, gemmules. As such, some non-CaCO₃ substrates may be more desirable than CaCO₃-based materials for reef restoration in high-salinity areas where *Cliona* is abundant, as long as oyster demographic rates are similar between CaCO₃ and non-CaCO₃ substrates (Dunn et al. 2014).

Oyster reef restoration in high-salinity areas must confront the often opposing effects of relatively high oyster recruitment with relatively high rates of postsettlement mortality compared with restoration at lower salinity sites where both recruitment and mortality rates are generally lower (Wells 1961, Soniat et al. 2004, Dunn et al. 2014). Restoration trajectories (sensu Suding 2011) of restored oyster reefs can vary widely even within a single estuary as a result of the dynamic nature of estuarine ecosystems (La Peyre et al. 2014b, Dunn et al. 2014). Because reef restoration inside marine protected areas focuses almost exclusively on provision of ecosystem services and functions, an understanding of the trajectories followed by reefs made of different substrates is vital to predict the community that will develop, as well as the timeline to expect reestablishment of ecosystem service provision.

Global climate change and its myriad associated problems are an overarching concern for restoration practitioners across ecosystems (Harris et al. 2006). In estuaries, oyster reefs may be affected by climate change in multiple ways, including shifts in intertidal habitat as sea levels rise (Vermeer & Rahmstorf 2009, but see Rodriguez et al. 2014), changes to estuarine circulation as patterns of rainfall shift (Wolock & McCabe 1999), increased temperature (Hansen et al. 2010), and lower pH (Feely et al. 2010), among others. These direct effects on oysters will interact with changes in the broader reef community, which includes clionid sponges. Previous work demonstrated that *Cliona celata* was able to excavate twice as much CaCO₃ from scallop shells when grown under predicted year 2100 pH compared with current pH levels (Duckworth and Peterson 2013), suggesting the potential for strong negative effects of sponges on bivalves as coastal waters become more acidic. Future research should examine effects of boring sponges on oyster demographic rates under increased temperature and lower pH conditions as well as the ability of clionid sponges to erode alternative reef-building substrates under future pH scenarios.

To date, efforts to restore oyster populations are numerous and have had a wide range of relative successes and failures (Coen & Luckenbach 2000, Coen et al. 2007, Mann & Powell 2007,
Powers et al. 2009, Schulte et al. 2009, Puckett & Eggleston 2012). Selecting an appropriate building material for oyster reef restoration, and considering both the biotic and abiotic components of the estuarine environment at a proposed restoration site, are vital first steps to subtidal oyster restoration. A scientific approach to restoration methodologies, as applied here, will provide a sound basis for management decisions in the face of the multitude of stressors faced by coastal ecosystems.

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