

INTERSPECIFIC COMPETITION AND PREDATION CONTROL
EUHALINE OYSTER REEF DEVELOPMENT

Michelle Claire Brodeur

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Approved by:

F. Joel Fodrie

Charles H. Peterson

Michael F. Piehler

Jonathan H. Grabowski

Johanna H. Rosman

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ABSTRACT

Michelle Claire Brodeur: Interspecific competition and predation control euhaline oyster reef development
(Under the direction of F. Joel Fodrie)

Species interactions, including competition and predation, can drive the development of biogenic habitats across environmental stress gradients. Using the Eastern oyster (*Crassostrea virginica*) as a model, my dissertation investigates how competition and predation limit the distribution of euhaline oyster reefs to the intertidal zone. In the field (Chapter 1) I examined how competition with epibiota, which compete for space in the subtidal and low-intertidal zones, affects juvenile oyster growth and survival. I also tested if competition between oysters and epibiota diminishes with increasing oyster body size. Removing epibiota increased oyster survival, especially in the lower-tidal-exposure treatments where epibiota cover is high. Larger oyster body size resulted in significantly higher oyster growth than smaller body sizes at lower tidal exposures. Following competition with summer epibiotic communities, oysters are covered by macroalgae in the winter and spring. In Chapter 2, I quantified baseline macroalgal dynamics and explored the effects on oyster fitness. I found that macroalgal cover is controlled mainly by abiotic forcing and does not reduce oyster growth or survival. Predation may be another mechanism that causes oyster mortality the subtidal, but the settlement and predation rates may vary across different scales. I examined how oyster settlement and predation of oysters are affected by landscape setting, aerial exposure, and structural complexity (Chapter 3). Oyster settlement increased with

inundation time and isolated reefs had greater settlement and adult densities than saltmarsh-fringing reefs. Oyster survivorship at low complexity was greater on isolated reefs than saltmarsh-fringing reefs and predation by nekton increased with increasing inundation. At high complexity, predation rates by mud crabs had highest at intermediate aerial exposures, likely due to an interaction of predator density and foraging behavior. Results from my dissertation indicate that predation and competition reduce juvenile oyster survival at low environmental stress, but net result of settlement and post-settlement processes is dependent on reef complexity and landscape setting. Restoring reefs on isolated sandflats immediately before oyster settlement in the lower-mid-intertidal will minimize competition with epibiota and predation. Maximizing reef growth by reducing species interactions will increase reef complexity and further increase survivorship from predation.

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LIST OF SYMBOLS AND ACRONYMS

%	percent
<	less than
>	greater than
±	plus or minus
°	degree
μ	average
'	minutes
”	seconds
ANOVA	Analysis of Variance
C	Celsius
K ₂ O	Potassium oxide
m	meter
mm	millimeter
MSL	mean sea level
NAVD 88	North Atlantic Vertical Datum of 1988
n	sample size
NOAA	National Oceanic Atmospheric Administration
N	Nitrogen
ppt	parts per thousand
P ₂ O ₅	Phosphorous pentoxide
RTK GPS	real time kinematic global positioning system
SE	standard error
YSI	Yellow Springs Instruments

INTRODUCTION

Biogenic habitats are an important component of landscape mosaics and the foundation for many ecological communities (Lindsey et al. 2006, Boström et al. 2011). The development habitat-building or foundation species is regulated by the integration of larval supply (Underwood and Fairweather 1989) and post-settlement processes (Menge 2000, Wright and Steinberg 2001). Species interactions, including the processes of competition (Connell 1961a, 1961b) and predation (Paine 1966), can significantly affect the growth and survival of individuals after settlement and transform how biogenic habitats are distributed and formed throughout a landscape. However, the degree to which settlement processes and species interactions influence the development of biogenic habitats may vary with different spatial scales, altering how individual habitat patches develop across landscapes.

The differences in species tolerance to aerial (tidal) exposure and interactions with neighbors can produce distinct zones of foundation species in littoral environments, limiting where some biogenic habitats can develop. Interspecific interactions, including competition for shared resources and predation, that mediate species distributions are context dependent, and have been tested across numerous environmental stress gradients (Menge 2000, Helmuth 2002, Harley 2003, Crain et al. 2004, Liancourt et al. 2005). Patterns across multiple ecosystems have been used to develop environmental stress models that describe the relative importance of physiological stress, competition, predation, and facilitation at varying levels of recruitment (Menge and Sutherland 1987a, Menge 2000, Menge and Sutherland 2001,

Bruno et al. 2003). For organisms at intermediate trophic levels, the relative importance of predation is greatest at low environmental stress, competition becomes most important at intermediate stress levels when recruitment is high, while abiotic stress tolerance is typically most important in determining the relative abundance of a species at high stress levels. In the marine environment, desiccation and thermal stress are associated with increasing aerial exposure in the intertidal zone; therefore, aerial exposure tolerance determines how high in the intertidal an organism can survive, and can dictate their ability to withstand competition or escape predation determines the lower limit.

The landscape setting, or spatial arrangement of habitats within a mosaic, has also been found to affect both the supply and survival of settlers, and ultimately the development of biogenic habitats (Turner 1989). The position of a habitat within a landscape can dramatically alter settlement rates if adjacent habitats interfere with the transport of settlers or habitats are isolated and have low connectivity from source populations (Roughgarden et al 1987). After settlement, new recruits of habitat building species are exposed to other community members that are competing for the same space and other resources (e.g., food, light, etc.). However, similar to mechanisms that drive species distributions across environmental stress gradients, higher recruitment to an area also increases the importance of interspecific and/or intraspecific competitive interactions for shared resources (Menge 1976, Menge and Sutherland 1987b, Menge et al. 2010). Varying predation pressure due to differences in the spatial arrangement and corresponding habitat connectivity can further alter predator abundance and foraging activity can also dramatically alter the pattern of individual habitat patches and abundance adults in biogenic habitats (Micheli and Peterson 1999, Lenihan et al. 2001, Rilov and Schiel 2006). It is the integration of these processes

across landscapes and within individual habitat patches that determines the resulting distribution and demographics of foundation habitats.

Eastern oyster (*Crassostrea virginica*) reefs in euhaline waters are a model system to test how settlement, interspecific competition, and predation vary across environmental stress gradients and landscape settings to affect the development of a foundation species. Within estuaries, Eastern oyster, *Crassostrea virginica*, reefs exhibit disparate development across gradients of salinities (Wells 1961), habitat settings (Grabowski et al. 2005), and aerial exposures (tidal emergence) resulting in differences in oyster demographics from the scale of an individual reef to an entire estuary, presumably due to differences in settlement, competition, and predation (Fodrie et al. 2014, Walles et al. 2016). In the euhaline portion of the estuary, oyster reefs develop predominantly in the intertidal zone, and patterns of oyster settlement and the distribution of adults can vary within individual reefs. Oyster settlement has been found to decrease with increasing aerial exposure due to lower submergence time on intertidal reefs; however, adult oyster density increased with greater aerial exposure (Fodrie et al. 2014).

The restricted subtidal distribution of oyster reefs in euhaline waters has been attributed to strong post-settlement interspecific competition with epifauna that inhibits subtidal reef growth (Chestnut and Fahy 1953, Ortega and Sutherland 1992, Osman and Whitlatch 1995, Bartol et al. 1999, Bishop and Peterson 2005, Fodrie et al. 2014). In the low-salinity region of estuaries where epibiotic communities are less diverse or absent (Ortega 1981), oysters extend into the subtidal zone. One factor that may contribute to oyster reef success in subtidal low-salinity waters is a reduction of oyster mortality from competition. Many epifaunal organisms are unable to withstand long periods of aerial exposure and can't

survive in the low to mid intertidal, reducing competition with oysters in the intertidal zone compared to the subtidal zone. Although oysters in euhaline waters have been observed to grow faster and have higher oyster abundance in the intertidal than at subtidal depths, these findings have been primarily observational and the effects of inter-specific competition and predation have been confounded by intraspecific density of oyster recruits (Bishop and Peterson 2006, Fodrie et al. 2014).

More is known concerning the role of predation on oyster reefs; however, the understanding of how landscape setting and aerial exposure interact to affect settlement and predation rates remains tenuous. Across the intertidal gradient, predation of newly recruited oysters is greater in the subtidal (Johnson and Smee 2014), but the magnitude or pattern in predation rates may not be a linear from the intertidal to subtidal zones or be the same at all landscape settings. Previous work has found that intertidal reefs restored along saltmarshes typically have lower oyster densities than isolated reefs (Grabowski et al 2005, Carroll et al 2015); however, it is unknown if settlement and/or predation drive these differences. Additionally, increasing reef structural complexity may change predator accessibility and foraging success (Turner 1989). High structural complexity increases prey survival on oyster reefs (Crowder and Cooper 1982, Grabowski 2004) because it reduces predator foraging efficiency relative to unstructured habitats (Summerson and Peterson 1984). An understanding of how settlement and predation vary across landscape settings and aerial exposure is needed to better understand the mechanisms regulating reef growth, as well as how the resulting habitat complexity further alters oyster survival.

My dissertation research focused on understanding if and how species interactions affect oyster reef development in euhaline estuaries. Building upon previous work on how

species interactions affect species distributions across environmental stress gradients and landscapes, I directly explore how these processes interact to regulate development of a crucial foundation species. I conducted a series of field manipulations to evaluate how settlement, competition and predation affect the fitness of oysters. Specifically, I examined how summer epibiotic communities (Chapter 1) and winter macroalgal fouling communities (Chapter 2) compete for space with juvenile oysters and affect oyster growth and survival across the intertidal. Additionally, I examined how landscape setting and aerial exposure affect the relative rates of new oyster settlement and predation of oyster spat, as well as how habitat complexity further modifies predation rates (Chapter 3).

A thorough understanding of oyster reef community ecology, especially the interplay between settlement and mortality from species interactions and how they vary across landscapes and environmental stress gradients, is crucial for effectively conserving and restoring oyster reefs. In North Carolina, for example, restoration projects within the last decade have been sited in the subtidal zone in euhaline waters, even though early work observed that reefs grow best in the intertidal zone (Lanier 1981). Here I build upon recent work that experimentally identified that restoration efforts should focus at elevations within the mid to low intertidal zone in euhaline systems (Fodrie et al 2014). By exploring how species interactions restrict oyster reef development in the subtidal, we can refine science-based oyster restoration methods to avoid negative ecological interactions and maximize restoration success.

Study Objectives

1. Effects of epifaunal competition on juvenile oysters
 - a. *Objective 1:* Determine if competition with epibiota restricts oysters from subtidal

zones by reducing growth and survival of juvenile oysters.

Hypothesis 1: Removal of epibiota will reduce competition, increasing growth and survival of oysters causing growth and survival to be equal across all aerial exposures.

- b. *Objective 2:* Determine the effects of juvenile oyster body size on how competition with epibiota affects oyster growth and survival across the aerial exposure gradient.

Hypothesis 2: Increasing oyster body size will reduce the negative effects of competition with epibiota, particularly at low aerial exposures where epibiota abundance is high.

2. The influence of seasonal macroalgae on intertidal oysters

- a. *Objective 1:* Characterize the temporal patterns of macroalgal cover across the intertidal gradient.

Hypothesis 1: The species composition of macroalgae will shift and the cover of macroalgae will increase with decreasing aerial exposure.

- b. *Objective 2:* Quantify the effects of eutrophication on seasonal macroalgal cover.

Hypothesis 2: Increasing the nutrient concentration in the water will enhance growth of macroalgae.

- c. *Objective 3:* Determine the effect of macroalgae on oyster growth and abundance.

Hypothesis 3: Macroalgae will filter oyster larvae and food particles, reducing the oyster abundance and growth.

3. Importance of landscape setting and structural complexity on oyster settlement and predation of oyster spat

- a. *Objective:* Determine how oyster landscape setting affects oyster settlement across

the aerial exposure gradient.

Hypothesis: Oyster settlement will increase with decreasing aerial exposure and be higher at isolated oyster reef settings due to higher transport of larvae.

- b. *Objective 2:* Elucidate how oyster reef landscape setting affects predation on oysters across the aerial exposure gradient.

Hypothesis 2: Reefs fringing salt marshes will have higher settlement rates than isolated oyster reefs and predation will increase with decreasing aerial exposure (higher submergence time for marine predators).

- c. *Objective 3:* Determine the effects of habitat structural complexity on oyster predation.

Hypothesis 3: Reducing habitat complexity will increase predation rates of oyster spat.

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CHAPTER 1: EFFECTS OF INTERSPECIFIC COMPETITION ARE MEDIATED BY BODY SIZE AND ENVIRONMENTAL STRESS IN A BIOGENIC REEF COMMUNITY¹

Introduction

An underlying goal of ecology is to understand the mechanisms controlling species distributions and abundance. The impacts of physiological stress and interspecific interactions, including competition for shared resources (Connell 1961a, 1961b) and predation (Paine 1966), have been tested across numerous environmental stress gradients (Menge 2000, Helmuth 2002, Harley 2003, Crain et al. 2004, Liancourt et al. 2005). Patterns consistently observed across these systems have been integrated into environmental stress models that describe the relative importance of physiological stress, competition, predation, and facilitation at varying levels of recruitment (Menge and Sutherland 1987, Menge 2000, Bruno et al. 2003). For organisms at intermediate trophic levels, abiotic stress tolerance is the most important in determining the relative abundance of a species at high stress levels. Communities that experience severe environmental stress are characteristically comprised of predominately sessile species, as mobile species leave habitats when conditions become too harsh (Menge and Sutherland 1976, 1987). At low environmental stress, the relative importance of predation is greatest, while competition becomes most important at intermediate stress levels when recruitment is high.

¹ Chapter 1 is under review for the journal Marine Ecology Progress Series with coauthor F.J. Fodrie.

The littoral zone is an ideal system to test environmental stress models on intermediate trophic levels. The differences in species tolerance to aerial exposure and interactions with neighbors produce distinct zones across a small area, providing an opportunity to use experimental manipulations to examine underlying mechanisms. The competitive ability of sessile species is typically inversely related with abiotic stress tolerance (Connell 1961b, 1961a, Liancourt et al. 2005), indicating a tradeoff between these two characteristics. For example, the salt marsh grass *Spartina patens* cannot withstand the physiological stress of the low intertidal zone where increased inundation results in greater salinity and anoxic stress (Bertness 1991). Conversely, *Spartina alterniflora*, can tolerate the stress of the low intertidal, but is competitively excluded from the less stressful, high intertidal zone by *S. patens* when recruitment of *S. patens* is high. For marine invertebrates, desiccation and thermal stress are associated with increasing aerial exposure in the intertidal zone (i.e., higher elevations), therefore aerial exposure tolerance determines how high in the intertidal an organism can survive (Connell 1961a, 1972, Hacker and Gaines 1997).

In areas where competitive interactions are the primary drivers of community dynamics, any changes in the competitive ability of an organism (due to changes in body size, chemical defenses, or morphology) may alter species interactions and ultimately affect zonation. Body size, in particular, is an important characteristic that can affect the outcome of interactions between species, as the ability of an organism to acquire resources, reproduce and avoid being consumed changes with ontogeny (Buss 1980, Sebens 1982). In sessile communities, where species primarily compete for space, the ability to overgrow or resist overgrowth is particularly important (Jackson 1977). For instance, competition between octocorals and colonial ascidians is determined by size, with the larger individual

overgrowing the smaller in the rocky subtidal of New England (Sebens 1982). Through faster growth rates, organisms can reach a size refuge and become less susceptible to competition with neighbors that settle simultaneously (McFadden 1986, Wethey 2002). Conversely, if an organism recruits earlier than its competitors, it may have an increased window for growth to also obtain a larger body size (Sutherland and Karlson 1977).

The advantage of increased body size to competitive ability should be the greatest at moderate stress levels, where competition is the most important in regulating populations (see Fig. 1). As the importance of competition declines at low and high environmental stress the advantage of increased body size for competitive ability should also become less important in controlling species distributions. Recent work has shown that changes in competitive ability, due to temperature-dependent growth increases, can shift distributions along intertidal gradients (Wethey 2002).

Eastern oyster (*Crassostrea virginica*) reefs in euhaline waters are an excellent model system to test how body size, interspecific competition and aerial exposure interact to affect foundation species. Oysters can withstand extended periods of aerial exposure and create reefs by growing vertically upon conspecifics in the intertidal, forming a natural environmental stress gradient of increasing aerial exposure. Many organisms in reef-associated communities grow directly attached to the reef substrate (i.e., epibiota), allowing for clear observation of competition for space between oysters and epibiota. Across the aerial exposure gradient, reef growth in euhaline waters is restricted between 10% and 55% aerial exposure (Ridge et al. 2015). The restricted distribution of oyster reefs in euhaline waters has been attributed to strong post-settlement interspecific competition with epifauna that inhibits subtidal reef growth (Chestnut and Fahy 1953, Ortega and Sutherland 1992, Osman and

Whitlatch 1995, Bartol et al. 1999, Fodrie et al. 2014). Oysters in euhaline waters have also been observed to grow better in the intertidal than at subtidal depths, but these findings have been primarily qualitative and/or confounded by intraspecific density of oyster recruits (Bishop and Peterson 2006, Trimble et al. 2009, Fodrie et al. 2014). Additionally, in the low-salinity region of estuaries where epibiotic communities are less diverse or absent (Ortega 1981), oysters extend into the subtidal zone, indicating that oyster reef success in subtidal waters may in part be due to the reduction of oyster mortality from interspecific competitors.

Although reduced oyster fitness across the aerial exposure gradient has been attributed to interactions with epibiota, there has been no empirical test to determine if interspecific competition limits the distribution of oysters in euhaline estuaries. Furthermore, oyster settlement also occurs over the summer months (Ortega and Sutherland 1992) and growth is variable, creating a range in the size of oyster recruits on reefs. However, it remains unclear whether larger oyster recruits are less susceptible to the negative effects of interspecific competition. I conducted a field experiment to evaluate: (1) how does interspecific competition with epibiota affect oyster fitness across the aerial exposure gradient; and, (2) do the effects of competition with epibiota on oyster fitness vary with oyster size? I hypothesized that interspecific competition between oyster spat and epibiota would increase with decreasing aerial exposure time, and limit the vertical distribution of oysters to the intertidal zone. I also hypothesized that increased oyster body size would reduce the negative effects of interspecific competition on oyster fitness in the subtidal.

Methods

I manipulated epibiota presence to experimentally determine if interspecific competition negatively affected oyster spat fitness, and if an increase in the body size of spat ($<5\text{mm}$ or 40%) alleviated the negative effects of epibiota on oyster fitness across an aerial exposure gradient. I manipulated epibiota presence (ambient epibiota, epibiota removed), oyster spat size class (small, 9.9 ± 0.3 mm shell height; large, 13.8 ± 0.4 mm shell height), and aerial exposure stress (from mid-intertidal to subtidal or -0.3, -0.5, -0.7, and -0.9 m (relative to the North Atlantic Vertical Datum of 1988)) in a fully crossed design ($n = 8$).

Oyster spat were collected by deploying recycled oyster cultch shells (105 mm shell height on average) onto intertidal flats in May 2012 and allowing oyster larvae to settle on the shells. Shell was deployed at Middle Marsh and Carrot Island, both of which are located within in the euhaline portion of Back Sound, North Carolina in the Rachel Carson National Estuarine Research Reserve and experience a mean monthly tidal range of 0.9 m. Habitats in both sites are representative of estuarine marsh complexes in relic flood tidal deltas and consist of a mosaic of natural oyster reefs, seagrass meadows, saltmarshes and sandflats. Once spat had naturally seeded cultch shell, the shells were collected in mid -July.

I prepared the collected shells by gently scraping off all epibiota and reducing the density of juvenile oysters through targeted removals to an abundance of 7 spat on the smooth interior of each shell. This standardized oyster density for each replicate cultch shell and spaced oysters sufficiently to limit competition between individual oysters for attachment space. Oyster spat collected from the two sites formed distinct size classes (Analysis of Variance; $F_{15,108} = 59.40$, $p < 0.001$) with the small size class shell height averaging 9.9 ± 0.3 mm (Middle Marsh) and the large size class shell height averaging $13.8 \pm$

0.4 mm (Carrot Island). Differences in body size are most likely due to small differences in settlement timing and not representative of distinct oyster stocks due to the proximity between the two sites (<1 km).

The naturally seeded cultch shells were randomly assigned a position along a vertical rack at one of four depths (-0.3, -0.5, -0.7, and -0.9 m NAVD 88), secured at the umbo, and suspended downwards. The experimental rack was deployed in Middle Marsh on July 16, 2012, adjacent to natural oyster reefs to place each treatment at the desired aerial exposures. Oysters were suspended to reduce the influences of other confounding processes that reduce oyster fitness, particularly sedimentation and predation. Although I was unable to exclude all mobile nektonic predators from accessing experimental racks, the racks reduced the abundance of predators that reside in the reef (e.g., mud crabs, stone crabs, oyster drills etc.) to practically zero based on serial observation during rack maintenance and monitoring (e.g., on average sheephead grazing scars covered >1% of experimental shells over the duration of the experiment). A Trimble[®] RTK GPS, with a vertical precision of <1.5 cm, was used to measure the elevation at the base of the experimental racks. Aerial exposures were calculated for target elevations using water level data and methods of Ridge et al. (2015). Using these data, the elevations of -0.3, -0.5, -0.7, and -0.9 m NAVD 88 had aerial exposures of 23.9%, 4.9%, 0.2%, 0% of the day, respectively.

To maintain epibiota removal treatments, all invertebrates that had settled onto the interior part of the oyster shell were gently scraped off approximately every ten days throughout the experiment. Since competition is most important when recruitment is high, I quantified recruitment (abundance) of epifaunal and mobile invertebrates to the experimental shells approximately every three weeks (on every other epibiota removal trip). Monitoring

ensured that epifauna densities were high enough to allow epifauna to interact and compete for attachment space and/or indirectly interfere with oysters (reducing food or degrading local water quality). Monitoring also confirmed that epibiota removals were effective throughout the experiment, and significantly lowered the cover of fouling invertebrates (repeated measures ANOVA, $p < 0.001$). At the end of the experiment, average cover on ambient epibiota treatments was $98.0 \pm 0.03\%$ ($\mu \pm 1\text{SE}$) and cover in the epibiota removal treatments was $18.4 \pm 0.02\%$ (Fig. 2, $F_{15,108} = 380.354$, $p < 0.001$). The size (shell height, measured as the longest vertical axis from the umbo) of visible oysters was also measured; however, high cover of fouling organisms on shells with ambient epifauna treatments prevented us from rigorously quantifying oyster survival and size until the end of the experiment when I could completely remove all epibiota.

After four months (November 2012) I collected shells from the experimental racks and enumerated species abundance of all organisms and estimated percent cover of all sessile invertebrates in the lab. When invertebrates recruited directly on top of each other I measured the total cover of each species; therefore, the percent cover of all species summed could exceed 100%. Once quantified, I removed all epifauna from the shells to enumerate oysters and measure shell height of all living oysters. Oyster survival (%) was calculated at the end of the experiment. Growth of surviving oysters was also calculated as the relative increase (%) in average shell height of each replicate ($[\text{final size} - \text{initial size}] / \text{initial size}$) from the beginning to the end of the experiment.

Statistical Analysis

To ensure that epifauna removal was effective and to determine how epifauna species composition and cover varied with aerial exposure, I tested the effects of oyster size, epibiota

presence, and aerial exposure treatments on the total percent cover of epibiota, canopy-forming epibiota (Sutherland 1974), and most common invertebrate species found on shells. The cover of canopy-forming epibiota did not meet the assumptions of normality or equal variance and therefore was Box-Cox Y transformed before I tested the effects of the independent variables with a three-way Analysis of Variance (ANOVA). The effect of independent variables on the total cover of all organisms, percent cover of barnacles, and abundance of mobile invertebrates were tested with Kruskal-Wallis tests because values did not meet assumptions of parametric statistics after transformation.

To understand how oyster fitness was affected by size, epibiota presence, and aerial exposure I also conducted a three-way ANOVA testing the independent and interactive effects of our manipulations on oyster survival and average growth. Both dependent variables failed to meet the assumptions of normality and equal variance and were Box-Cox Y transformed (growth) or arcsine square root-transformed (survival) to meet these assumptions. Post-hoc comparisons were conducted with Student's *t*-test for all significant interactions and main effects to determine whether there were significant differences between unique treatments. All analyses were performed using JMP software version 10.0 (SAS Institute 2010).

Results

Oyster Survival

Oyster survival was significantly lower in ambient epibiota treatments ($8.6 \pm 4.3\%$ survival) than in epibiota removal treatments ($66.3 \pm 4.3\%$ survival, Fig. 3A, $F_{15,108} = 171.289$, $p < 0.001$). Larger body size increased oyster survival by 1.5 times relative to the

small size class; survival of large oysters was 7.5 percentage points greater than small oysters regardless of aerial exposure (Fig. 3B, $F_{15,108} = 5.248$, $p = 0.016$). There were no differences in oyster survival across aerial exposures ($F_{15,108} = 0.719$, $p = 0.543$) or interactions between aerial exposure, epibiota removal, or body size.

Oyster growth

Growth of the surviving oysters in response to epibiota presence varied with aerial exposure (epibiota x aerial exposure, $F_{15,57} = 4.173$, $p = 0.020$). When epibiota were removed, oysters grew by an average of $180.6 \pm 13.7\%$ of their initial body size during the duration of the experiment. There were no significant differences between aerial exposures (Fig. 4A), but when epibiota were present, oyster growth diminished with decreasing aerial exposure. Oyster growth of ambient epibiota treatments at the highest two aerial exposures (23.9% and 4.9% exposed) were not different than all epibiota removal treatments. However, growth of oysters exposed to ambient epibiota cover decreased by an order of magnitude relative to epibiota removal treatments. Under ambient epibiota cover, oyster body size only increased by $45.5 \pm 78.6\%$ at 0.2% exposure and $81.0 \pm 51.2\%$ at 0% exposure, respectively.

The effect of epibiota on oyster growth across the aerial exposure gradient was also dependent on body size (Fig. 4B, epibiota x size, $Z_{15,57} = 4.402$, $p = 0.040$). There was no difference in growth between ambient and removal epibiota treatments for the large size class ($138.7 \pm 24.0\%$ and $148.9 \pm 13.6\%$, respectively). Conversely, growth of small spat exposed to ambient epibiota cover was reduced to $70.1 \pm 24.8\%$. There were no interactions between body size and aerial exposure that affected oyster growth.

Epibiotic community

Epibiota quickly recruited to shells, and barnacles were the first and most abundant species to settle. By the first monitoring period (August 8th), barnacle densities were 99.9 ± 6.1 ($\mu \pm 1\text{SE}$) barnacles/shell in ambient epibiota treatments and included *Balanus* spp. and *Chthamalus fragilis*. High barnacle densities persisted through the end of the experiment (Fig. 2A). Barnacles represented 95% of the epibiota community overall and covered $93.1 \pm 2.1\%$ of the inner side of cultch shells in ambient epibiota treatments, as compared to covering $14.3 \pm 0.1\%$ for epibiota removal treatments ($F_{15,108} = 521.929$, $p < 0.001$). Total epibiotic cover ($F_{15,108} = 1.196$, $p = 0.315$) and individual species cover did not significantly change with aerial exposure (Fig. 2A), but there was a significant reduction of canopy-forming invertebrates (bryozoans *Bugula neritina* and *Bugula avicularia*, the hydroid *Tubularia crocea*, and tunicates *Stylea plicata* and *Didemnum duplicatum*) with increasing aerial exposure (Fig. 2B, $F_{15,108} = 7.476$, $p = 0.001$). Abundances of other bivalves, gastropods, and polychaetes were present in low densities, but exhibited no patterns across treatments.

Discussion

I found that interspecific interactions between oysters and epibiota reduced the fitness of oyster recruits; however, oyster body size and aerial exposure stress mediated the magnitude of the response. In the absence of competitors, growth and survival of oysters were equal at all aerial exposures and oyster body sizes. When competitors were present, however, overgrowth of encrusting epibiota caused high mortality due to competitive exclusion of oyster spat across all aerial exposures. Additionally, interference competition

with canopy-forming fouling organisms increased with decreasing aerial exposure and reduced oyster growth at low aerial exposures. Thus, our results confirm that high recruitment of epibiota causes high mortality and reduced growth of oyster recruits on reefs. However, oysters with a larger body size may experience greater survival and growth rates relative to small spat when exposed to competitors.

The presence of epibiota significantly reduced survival of oyster spat irrespective of oyster size or aerial exposure, presumably due to the high recruitment and overgrowth of encrusting barnacles across all aerial exposures. This result is consistent with previous work that has shown that barnacles reduce oyster spat survival via overgrowth (Boudreaux et al 2009). Since barnacles are tolerant to aerial exposure and settlement is continuous throughout summer (Sutherland and Karlson 1977), oyster spat were almost completely covered by barnacles in the ambient epifauna treatments throughout our experiment. Barnacle recruitment is also high in areas of high flow (Ortega 1981) and have an affinity to settle on new shell material (Ortega and Sutherland 1992). These factors enhanced barnacle densities on experimental racks, and combined with the isolation from predators of barnacles found on reefs, may have caused the considerable overgrowth of oysters relative to what is found on both natural and newly restored reefs.

Based on the community patterns I observed across treatments, the mechanism most likely generating reduced growth of surviving oysters was some form of interference from canopy-forming epibiota (Svensson and Marshall 2015, Thompson et al. 2015). Canopy-forming overgrowth may have suppressed flow (Buss and Jackson 1981), thus reducing food supply (Okamura 1990, 1992) and oxygen concentrations (Lenihan et al. 1996, Ferguson et al. 2013). Additionally the presence of canopy-forming invertebrates may have reduced

oyster feeding because oysters may perceive the risk of predators taking refuge in the canopy (Sih 1980) or the allelopathic effects of competitors (Kennedy et al. 1996, Lopanik et al. 2004). Reduced oyster growth with decreasing aerial exposure correlated with the presence of canopy-forming fouling organisms (including bryozoans, hydroids and tunicates), which supports previous growth patterns of intertidal oysters. Bishop and Peterson (2006) also observed that despite a 93% reduction in feeding time, *Crassostrea ariakensis* grew 34% faster in the intertidal than the subtidal areas where oysters were covered by epibiota. Growth patterns also parallel results from restored experimental reefs along the same aerial exposure gradient, in which oysters exhibited lowest growth in the subtidal (Fodrie et al. 2014). Because I did not track the growth of individual oysters throughout the experiment, size-selective predation or mortality from physiological stress may have confounded growth measurements throughout the experiment. However, final oyster growth was greater than one standard deviation of the mean initial oyster shell height for both size classes in ambient epibiota treatments, indicating that the limited growth observed was not strictly due to mortality of the largest individuals in each replicate, but incorporates growth.

Since oyster feeding only occurs when oysters are inundated, increased aerial exposure can shorten feeding time and reduce oyster growth (Peterson and Black 1987, Roegner and Mann 1995, Bishop and Peterson 2006). However, growth was equal across aerial exposures in epibiota removal treatments indicating that in the absence of interference by epibiota, intertidal oysters were not resource limited by inundation for the aerial exposures tested in this study. Oysters may not be resource limited at greater aerial exposures because the transport or concentration of food or is sufficiently high that oysters can consume enough food during relatively short inundation times (Lenihan et al. 1996). However, since oysters

were elevated from the benthos at high aerial exposures in our design, oysters may have experienced enhanced flow and food delivery relative to that of a reef.

Considering that interference competition with canopy forming epibiota increased with decreasing aerial exposure, I expected oyster body size to become more important to oyster fitness as aerial exposure decreased. However, high cover of encrusting epibiota (barnacles) reduced survival drastically across all aerial exposures causing no differences among aerial exposures or interactions between body size and aerial exposure. Although oyster survival was low across all size classes when epibiota were present, larger individuals exhibited greater survival than smaller oysters. These results also suggest that an increase in oyster body size also prevented oysters from experiencing reduced growth when exposed to epibiota. Thus, increased body size created a partial size refuge from competition for larger oyster spat, where growth was less hindered by interactions with the canopy forming epibiota at low aerial exposures than small spat.

To reach a size refuge where stress from epibiotic competition is reduced, oysters must first progress through more vulnerable states of development. On protected reefs that have reduced barnacle recruitment due to lower barnacle larval supply and survival (Ortega 1981), I would expect oyster spat to survive to larger body sizes, as survival was higher when epibiota were absent in this study. Oysters that settle early enough before heavy summer epibiota settlement occurs may have enough time to grow to be large enough to reach the size refuge. However, canopy-forming epibiota found at lower aerial exposures will still reduce growth of all spat that settle at or below the low intertidal. Since oysters that settle in the low intertidal have reduced growth rates when surrounded by epibiota, oysters that settle in the subtidal may not be able to grow fast enough to escape mortality from competition or

predation, and contribute to the patterns observed in intertidal oyster reef development (Chestnut and Fahy 1953, Ortega and Sutherland 1992, Osman and Whitlatch 1995, Bartol et al. 1999, Fodrie et al. 2014).

The interplay of environmental stress, species interactions and body size largely determines the growth and survival, and thus, distribution of species that live along environmental stress gradients. The design I employed separated the effects of interspecific competition from other mechanisms that are thought to reduce fitness. This study provides evidence that as juveniles, interference competition and overgrowth reduces fitness of intermediate trophic level species at low environmental stress. Additionally, a small (4 mm), but proportionately significant (40%), increase in body size can alleviate the negative effects of competitors on growth and mortality of intermediate species.

Understanding how competition and aerial exposure affect the fitness of foundation species is a critical component for effectively restoring essential habitats. Interference from competitors should be considered as a major factor influencing survival and growth of foundation species when designing effective restoration projects. For oysters, restoration practices that deploy cultch shells with oyster spat that have been raised to a size > 14 mm may allow oyster spat to be more resistant to competition due to epibiota overgrowth. Restoring reefs with deliberate seasonal timing at moderate aerial exposure stress to minimize interactions with epibiota will maximize growth and allow individuals to reach a size where they are less vulnerable to the negative effects of competitors.

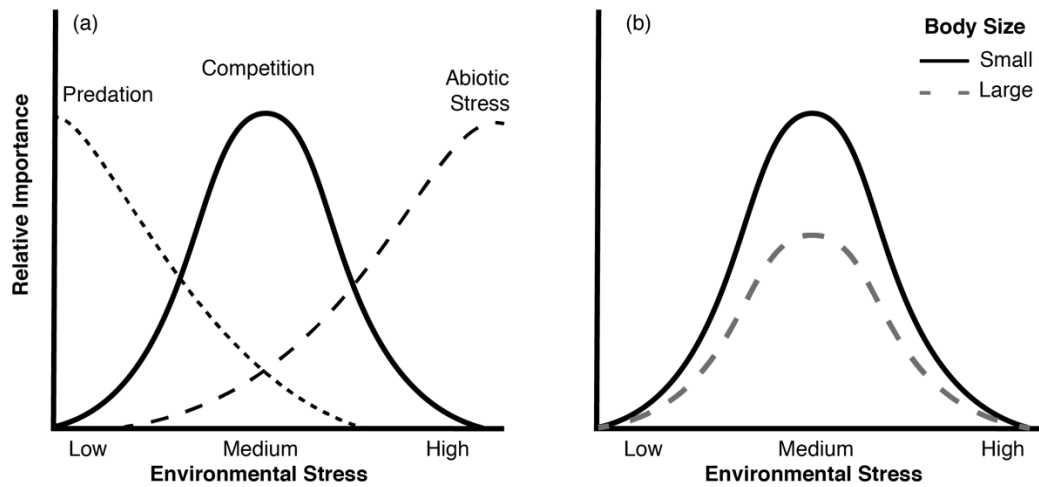


Figure 1.1 (A) Conceptual model showing the relative importance of physical stress, predation, and competition across environmental stress gradients (Adapted from Menge and Sutherland 1987) and (B) the relative importance of competition for juvenile oysters of large (grey dashed line) and small (black solid line) body sizes. Increasing oyster body size is predicted to reduce the relative importance of competition at intermediate environmental stress.

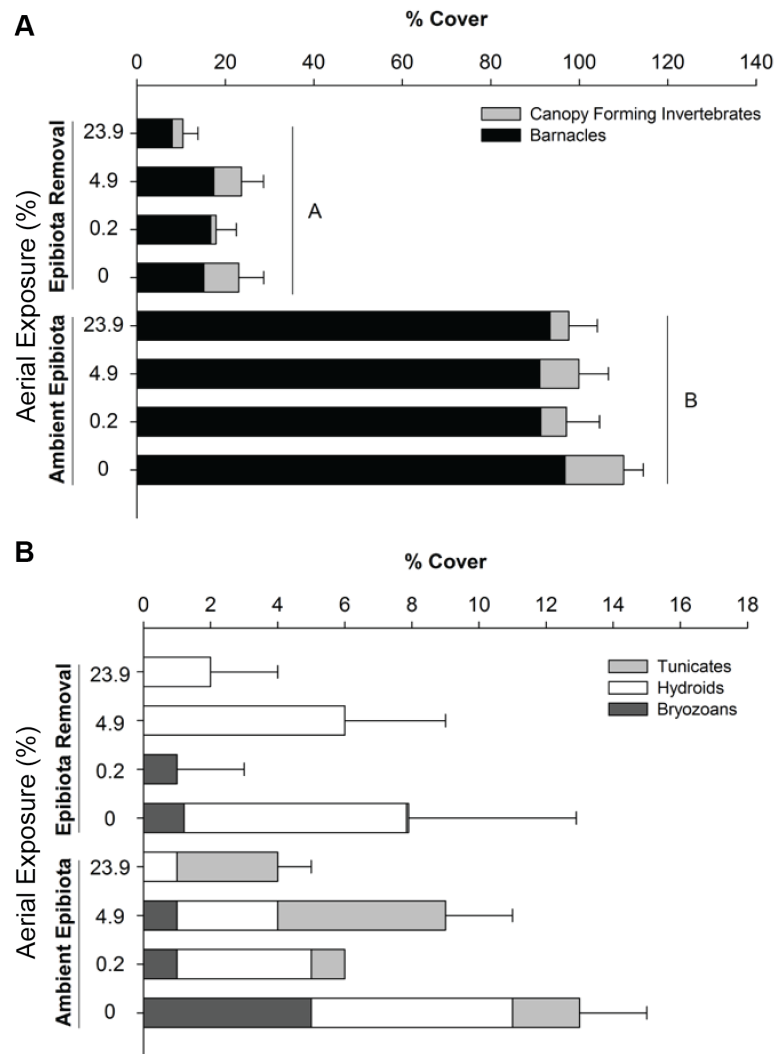


Figure 1.2 Total percent cover ($\% \pm 1\text{SE}$) of (A) all invertebrates (B) and total cover of canopy forming invertebrates after four months. Epibiota presence (ambient, removed), oyster body size (small, large), and aerial exposure (23.9, 4.9, 0.2 and 0% exposure) were manipulated and crossed in a fully orthogonal design. Error bars represent standard error of total cover or abundance of organisms and letters represent significant differences from post hoc comparisons.

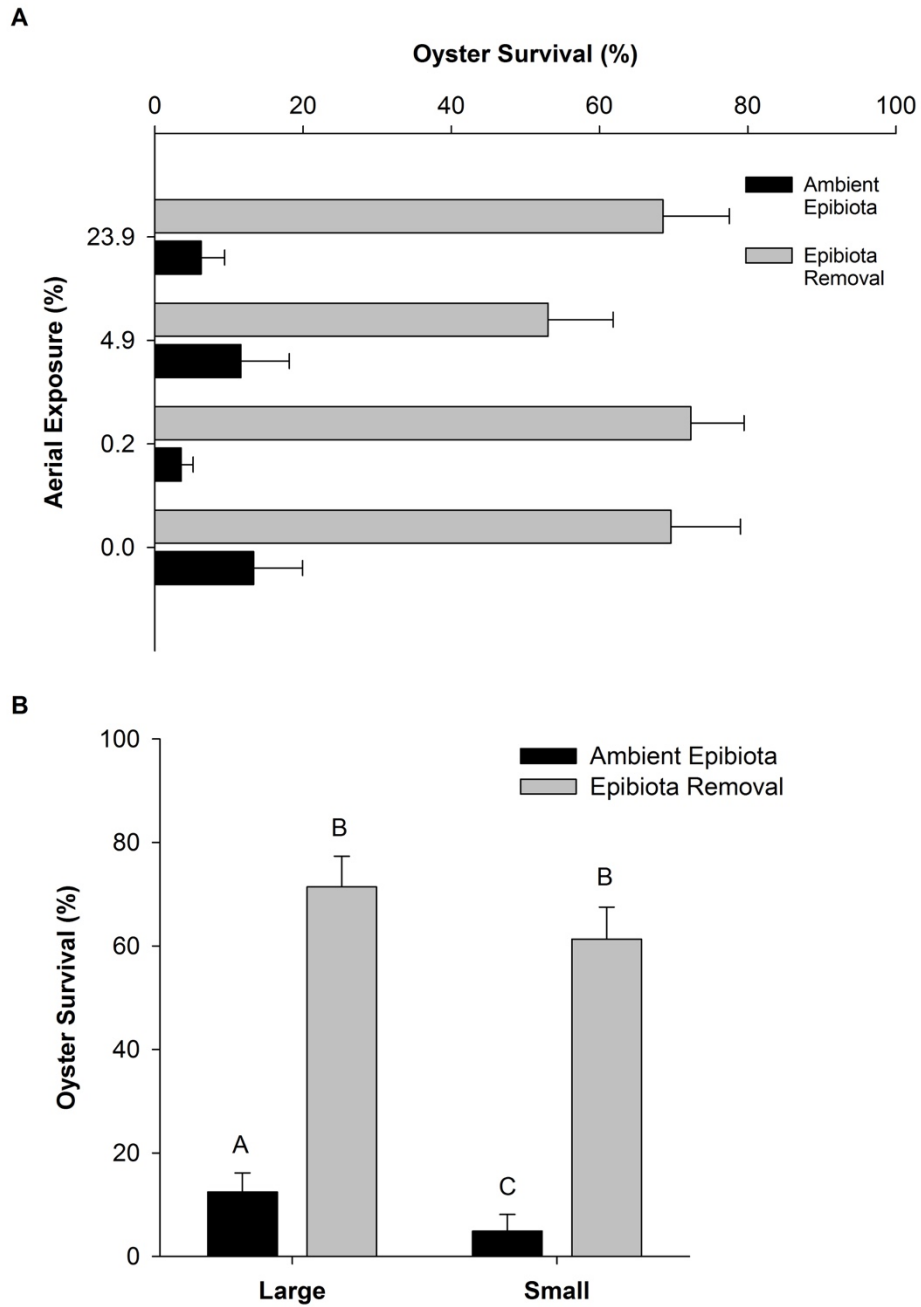


Figure 1.3 Oyster survival ($\% \pm 1\text{SE}$) for two epibiota treatments at: (A) four aerial exposures (23.9, 4.9, 0.2 and 0% exposure); and, (B) two size classes (small, 9.9 ± 0.3 mm; large, 13.8 ± 0.4 mm). Letters represent significant differences from post hoc comparisons.

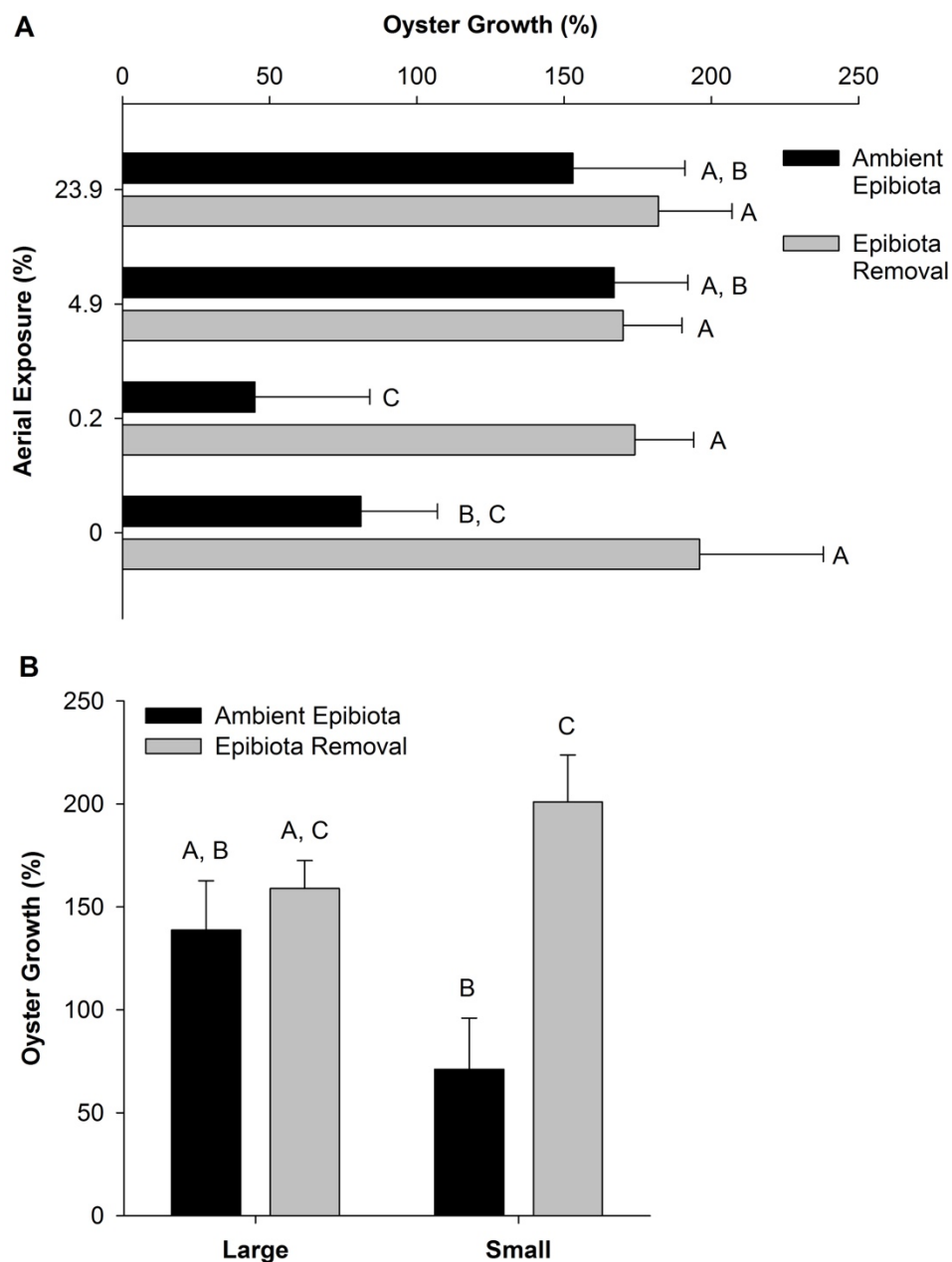


Figure 1.4 Growth of the surviving oysters at the end of the experiment ($\% \pm 1\text{SE}$), measured as the relative increase (%) in size during the duration of the experiment, for two epibiota treatments at: (A) four aerial exposures (23.9, 4.9, 0.2 and 0% exposure); and, (B) two size classes (small, 9.9 ± 0.3 mm; large, 13.8 ± 0.4 mm). Letters represent significant differences from post hoc comparisons.

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CHAPTER 2: DYNAMICS AND DRIVERS OF SEASONAL MACROALGAE ON BIOGENIC REEFS AND THE EFFECTS ON OYSTER FITNESS

Introduction

Foundation species facilitate epifaunal communities by providing suitable habitat for settlement (Bertness and Callaway 1994, Bruno et al. 2003). Eastern oyster (*Crassostrea virginica*) reefs are some of the only naturally occurring hard substrates in soft-bottom estuaries and provide essential habitat for diverse epifaunal assemblages of barnacles, bryozoans, tunicates, and macroalgae (Wells 1961, Sutherland and Karlson 1977, Bahr and Lanier 1981, Meyer and Townsend 2000). Although oyster reefs serve as an essential settlement substrate for epifauna (Grabowski and Peterson 2007), high rates of epifaunal recruitment can be harmful to oyster fitness and the growth of oyster reefs (Bishop and Peterson 2005, Barnes et al. 2010, Barnes and Dick 2014). Therefore, a thorough understanding of the mechanisms that control the development of epifaunal communities is critical for addressing how eutrophication and climate change may affect oyster reefs.

The presence of macroalgal mats are one mechanism that has the potential to affect oyster fitness and control overall reef health (Williams 1948, Rhodes 1970). Macroalgae is part of epifaunal communities throughout the year; however, beginning in January, larger intertidal mats, consisting primarily of *Ulva* spp. and *Ectocarpus* spp., become established, and persist through May (Williams 1948, O'Connor et al. 2011). Currently, there is no baseline for the seasonal dynamics of macroalgal assemblages and little quantitative data on mechanisms regulating the onset and eventual decline in algal cover on intertidal oyster reefs.

Numerous abiotic factors can co-regulate the development and persistence of macroalgal mats on oyster reefs. Nutrients limit macroalgal primary productivity and seasonal nutrient patterns may control macroalgal dynamics (Valiela et al. 1997). Fast growing, ephemeral macroalgae respond quickly to increases in nutrients, but also have a high nutrient demand; therefore, productivity is more often limited by nitrogen (Taylor et al. 1995). Photosynthesis, and ultimately primary production, is also limited by water temperature (Beer and Eshel 1983). If summer water temperatures rise above the thermal limit for the dominant macroalgal species (Rivers and Peckol 1995), it will reduce in macroalgal cover in the summer months. Additionally, intertidal macroalgal communities are also limited by desiccation tolerance, which is a function of the interaction between the air temperature and the duration epifauna are aerially exposed at low tide (Underwood 1981, Henley et al. 1992). Macroalgae on intertidal oyster reefs may be able to withstand exposure at extreme winter low tides, but not once the air temperature exceeds thermal tolerance.

Biotic mechanisms may also control macroalgal cover, as the decline of macroalgae coincides with seasonal increases in invertebrate and fish communities in the spring (Nelson 1979). Mesograzers - small herbivores including amphipods - play important roles in controlling benthic macroalgal communities (Duffy and Hay 2000). However, when fishes recruit to the estuary in the summer (Baillie et al. 2014), they exhibit strong top-down control on mesograzers and reduce grazer abundance (Duffy and Hay 1994, 2000), suppressing herbivory on macroalgae by late May. Additionally, one of the most dominant fish species in mid-Atlantic estuaries (pinfish, *Lagodon rhomboides*) is omnivorous and may consume algae and directly reduce macroalgal cover (Stoner 1979, 1980), or indirectly by inadvertently breaking up macroalgae as it feeds on mesograzers (Duffy and Hay 1994).

The seasonal dynamics of macroalgal mats are likely impacted by human-driven eutrophication and warming. Use of the coastal zone has resulted in nutrient enrichment, which promotes algal blooms and has cascading negative effects on coastal ecosystems (Valiela et al. 1997). Increases in local nutrient concentrations can augment macroalgal biomass or even extend the duration of seasonal algae if it is nutrient limited. Additionally, human-induced ocean warming can shift the timing of macroalgal development to earlier in the year if water temperature restricts when macroalgae can grow (Gao et al. 2012). Warming also alters the timing of reproduction of mesograzers and fishes, causing temporal mismatch and changes in top-down forcing on macroalgae (Platt et al. 2003, Kordas et al. 2011). Increasing water temperature also strengthens herbivore-algal interactions (O'Connor 2009, Carr and Bruno 2013) as metabolic rates of consumers increase at a greater rate than the metabolic rates of primary producers. This may result in a trophic skew, causing a faster decline in seasonal macroalgae by enhanced grazing. In sum, the relative importance of biotic and abiotic factors will determine how macroalgal mats respond to changes in water quality within the estuary.

Changes in the quantity or timing of seasonal macroalgae may have cascading effects on fitness of the underlying oysters. Drift algae has been found to smother and filter larvae (Olafsson 1988), reduce currents (Escartin and Aubrey 1995), and/or interferes with oyster feeding (Norkko and Bonsdorff 1996), overall reducing the fitness (growth and survival) of individual oysters. Additionally, previous research has documented that accumulation of drift algae greater than 2kg wet weight m⁻² strongly inhibits oyster recruitment (Thomsen and McGlathery 2006). Currently, macroalgal mats are not present on reefs during major oyster spawning and settlement periods (Ortega and Sutherland 1992), but if continued

eutrophication or regional warming increases the biomass and/or timing of macroalgal cover, it may have subsequent negative effects on oyster reef recruitment and growth.

The main objectives of this study were to quantify the seasonal macroalgal dynamics and to understand the abiotic and biotic mechanisms controlling macroalgal cover. Further, I wanted to understand if macroalgae negatively affects the underlying oyster reef. Specifically, our questions were: (1) What are the seasonal patterns of macroalgae on intertidal oyster reefs? (2) Is the seasonal decline in macroalgal cover controlled by biotic and/or abiotic forcing? (3) How does nutrient enrichment alter the seasonal distribution of macroalgae? And, (4) does seasonal macroalgae have negative effects on oyster fitness? I answer these research questions using seasonal monitoring and manipulative field experiments. By quantifying the seasonal dynamics and understanding how abiotic and biotic mechanisms control macroalgal growth, we can better manage oyster reef communities as eutrophication and climate change continue to affect coastal ecosystems.

Methods

Study site

I quantified seasonal macroalgal dynamics and conducted a series of experiments to assess how abiotic and biotic mechanisms affect macroalgal cover and how macroalgae affects the underlying oysters. This study was conducted in Middle Marsh, North Carolina, within the Rachel Carson National Estuarine Research Reserve (34° 41' 34.98"N 76° 36' 43.44"W). Middle Marsh is representative of estuarine marsh complexes occupying a relic flood tidal delta with a tidal range of 0.9 m and consists of a mosaic of natural and restored oyster reefs, seagrass meadows, salt marshes and sandflats.

Monitoring

I selected 16 intertidal oyster reefs located within Middle Marsh at two restoration settings; half being natural reefs and the other half were restored reefs from 1997 and 2000 (Grabowski et al. 2005). On each reef, I established 0.25m²-monitoring plots at two elevations to quantify the seasonal cycle of macroalgae. All elevations are reported in reference to the North American Vertical Datum established in 1988 (NAVD88), where local mean sea level is approximately -0.03 m NAVD88. High elevation plots were marked at -0.26 ± 0.02 m and low elevation plots were at -0.40 ± 0.01 m, near mean low water (MLW). Once macroalgae began to develop in January 2012, I estimated the percent cover of each species within each plot every two weeks at low tide. Monitoring occurred until total macroalgal cover was below 10% on average in May 2012.

On each monitoring date, I also quantified nekton and mesograzer communities at each reef. Two unbaited minnow traps were deployed at the base of the reef to capture local nekton, and grazer abundance was monitored by deploying frayed ropes which grazers typically recruit to. After a 24-hour soak time, fishes were identified, enumerated, and weighted and grazer ropes were brought back to the lab to quantify mesograzer abundance.

To understand abiotic forcing on macroalgae, I examined water quality and water level at each reef. Water temperature and salinity was measured continuously within Middle Marsh using an YSI sonde (Yellow Springs Instruments) as part of the National Estuarine Research Reserve System System-wide Monitoring Program. Tidal elevation was calculated based on data collected from NOAA Tide Station on Pivers Island, Beaufort, NC (station #8656483) referenced to MSL.

Effects of nutrients on macroalgal cover

To determine if increasing nutrient concentrations in the estuary would increase the abundance or duration of macroalgal cover I conducted a manipulative field experiment where I altered nutrient concentrations at half of the reefs and monitored macroalgal cover ($n = 4$). In December 2010 before the macroalgal mats began to develop, PVC dispensers were filled with 100 g OsmocoteTM slow release fertilizer (N:P₂O₅:K₂O, 15:9:2%) and placed on the reef. Macroalgal percent cover was estimated from January through the end of May 2011 when cover was 10%. Fertilizer was replaced every two weeks to ensure that nutrients were constantly being released into the reef.

Effects of top-down control on macroalgal cover

To understand if biotic mechanisms control seasonal macroalgae, I conducted a caging experiment whereby I excluded mesograzers and nekton from the reefs in a fully orthogonal design ($n=6$). Once macroalgae had reached 100% cover on reefs in March 2011, I collected oyster clumps with attached algae from nearby reefs and placed them into a 0.10-m² vexar basket. Baskets were then placed onto plots on an intertidal sandflat and were assigned a randomized treatment.

A half-cage was used and compared to the uncaged control to determine if artifacts from excluding nekton with a cage, including reduced light attenuation and flow, significantly affected the cover of macroalgae. For nekton exclusions, I placed oyster baskets within 1-m³ cages (1-cm mesh), and for mesograzer exclusions, I suspended a plaster block above the plots, containing the degradable insecticide carbaryl. Deterrent blocks were made by incorporating Sevin[®] into a slowly dissolving plaster matrix using the methods of Poore et al. (2009). Carbaryl is effective against arthropods, but has limited effects on other

organisms (Carpenter 1986, Duffy and Hay 2000, Dumbauld et al. 2001) as it degrades quickly (Armbrust and Crosby 1991, Whalen et al 2013).

Macroalgae was monitored every two weeks until macroalgal cover began to decline, when I monitored macroalgae every week until total average cover declined to less than 10%. If macroalgae cover declined at the same rate in control treatments as the grazer and nekton exclusion (no biotic control), it suggests that abiotic controls are the primary driver of the seasonal decline of macroalgae. It would assume that macroalgae was controlled by biotic controls if macroalgae declined slower when you excluded grazers and nekton than the control. If only grazers were responsible for top-down biotic control, nekton exclusion treatments would have the fastest rates of macroalgal decline because nekton removal would allow grazers to reproduce and graze freely, increasing herbivory on macroalgae. However, if fishes also consume macroalgae directly, it would indicate that macroalgae in the grazer exclusion treatment decline faster than when both grazers and nekton were excluded.

Effects of macroalgal cover on oyster fitness

Concurrently, I conducted a macroalgal removal experiment to understand the effects of seasonal macroalgal fouling on oyster density. I created 0.25-m² plots, adjacent to all monitoring plots (1-m away from high and low elevation plots at each restored or natural reef) and manually removed all algal biomass every two weeks from December through May 2011 (the entire time seasonal macroalgae was present). After algae declined in June, all oysters within a 0.10-m² quadrat in the macroalgal-removal plots and monitoring plots were collected, enumerated, and measured for shell height. The differences in oyster density of adult (>75mm, legal harvesting size in North Carolina) and juvenile oysters (<75 mm) in monitoring and removal plots were calculated for each set of plots.

Additionally, I conducted an experiment to understand the effects of macroalgal fouling on oyster growth. I collected clumps of ten ~6-month old juvenile oysters in January 2012 that had recruited to experimental oyster reefs created the previous summer. Individual oysters were labeled and measured before deploying pairs of clumps at the two plot elevations (high, low) on intertidal reefs. One clump was placed in the monitoring plot and another clump one meter away at the same elevation in the algal removal plot. Every two weeks, I manually removed all algal biomass within a 0.25m² area around one clump per pair, being careful not to disturb the oysters, until macroalgal cover naturally declined to less than 10%. All oyster clumps were collected in May and individuals were measured to calculate growth over the 5 months.

Statistical Analyses

To assess the effects of plot elevation and restoration setting (fixed effects) on macroalgal cover over time, I fit a linear mixed effect model using the maximum likelihood framework with plot nested within sampling date (random effect). For the nutrient experiment I used the same mixed model design as with the algal monitoring nutrient treatment with plot elevation and restoration setting were fixed effects. For the cage experiment, I first examined if there was a significant difference in macroalgal cover between the control and cage-control treatments. Since there were no cage effects (cage > 0.42), cage-control treatments were dropped from the analyses. Linear mixed effects models were also fit for the caging experiment with nekton exclusion and mesograzer exclusion as fixed effects and sampling date as a random effect to determine the effects of manipulated treatments through time.

I examined the effect of macroalgae on oyster density (both spat and adults) and

growth by calculating the difference in oyster density or growth between ambient and algal-removal treatment plots at each elevation. This allowed me to standardize the effect of macroalgal cover relative to the characteristics of each individual oyster reef. The difference was calculated so a negative value represented that algal removal had a negative effect on the density or growth of oysters and a positive value meant that removing macroalgae increased oyster density or growth. Two-way ANOVAs were used to analyze the effect of landscape setting and elevation (fixed factors) on adult density, spat density and oyster growth.

Tukey's post-hoc comparisons of significant treatments and interactions were used to determine which levels of each treatment were significantly different from one another in each experiment. All analyses were run in R 3.2.3 and linear mixed model analyses were run using package lme4 (Bates et al. 2015, R Core Development Team 2016).

Results

Monitoring

Two dominant genera, *Ulva* and *Ectocarpus*, composed 95% of the macroalgal community on the intertidal oyster reefs from January to May 2012. *Ulva* was comprised of *Ulva lactuca* and *Ulva intestinalis*, while *Ectocarpus* consisted of *Ectocarpus fasciculatus* and *Ectocarpus siliculosus*. The remaining species comprised less than 5% of total cover and included *Porphyra* spp, *Striaria attenuata*, *Gelidium americanum*, *Hypnea muciformis*, *Nemacystus howei*, *Scytosiphon lomentaria*, *Chondria* spp., *Lomentaria baileyana*, *Dyasia baillouriana*, *Ceramium* spp., and *Aspercocous fistulosus*.

Total macroalgae cover exhibited a unimodal seasonal cover pattern within all plots on the intertidal oyster reefs (Fig. 1A, time, $P < 0.001$). Macroalgal mats developed in

January 2012, with mat cover peaking from February 6 – March 14, 2012, before declining through May 2012 to $3.8 \pm 2.0\%$ across all treatments. Cover of macroalgae at low elevations on restored reefs developed the quickets and reached $67.0 \pm 10.7\%$ on February 6, 2012 and was significantly greater than all other plots. Low-elevation plots on natural reefs and high-elevation restored plots did not have significantly different cover with $37.8 \pm 13.2\%$ and $38.1 \pm 13.4\%$ on February 6th, respectively (setting x elevation, $P = 0.032$) and high plots on natural reefs had the lowest cover on February 6th with $21.0 \pm 27.6\%$ cover. During peak growth, restored reefs had 1.9 times higher macroalgal cover than natural reefs (restoration setting, $P < 0.001$) and high plots had 1.8 times greater cover than low elevation plots (elevation, $P < 0.001$). Macroalgal cover in low plots on restored reefs persisted longer than all other treatments (setting x elevation, $P = 0.032$, elevation x time, $P = 0.001$).

Plot elevation had a significant effect on *Ulva* cover, with low plots peaking at 40% on February 20th and having higher coverage than high plots throughout the monitoring period (Fig. 1B, elevation, $p < 0.001$). The effect of elevation on *Ulva* cover during the end of the growing season was dependent on restoration setting (setting x elevation, $p=0.038$). On restored reefs, *Ulva* cover on low plots was nearly twice as high as the percent cover of *Ulva* in high plots, until the last sampling date (May 14, 2012) when algal cover on all reefs had declined to near 0%. However, on natural reefs, low elevation plots began to decline immediately after peaking on February 20th and were not significantly different than high plots.

Ectocarpus developed to 20% cover on reefs within the first two weeks and remained relatively consistent until March 15th, when cover began to decline (Fig. 1C). Overall restored reefs had higher cover and high plots had lower cover than low elevation plots

(setting, $P < 0.050$), until *Ectocarpus* began declining. Coverage on all natural reefs and high-restored plots had declined to less than 1% by April 29th, but *Ectocarpus* persisted at low plots on restored reefs until May 1, 2012.

Water temperature increased with time throughout the macro-algal monitoring period and ranged from 3.09°C to 28.26°C (Fig. 2A). Average water temperature when the total macroalgae cover began to decline was $18.28 \pm 2.16^\circ\text{C}$, ranging from 13.80°C to 24.96°C in the period between sampling intervals on March 20, 2012. Water salinity ranged from 23.6 to 36.2 ppt over the course of the monitoring period and was 33.0 ± 3.0 ppt on average (Fig. 2A). Overall, water level was lower during the winter and spring when macroalgae was present than the rest of the year (Fig. 2B). During the monitoring period, mean water level increased from -0.07 m in January to 0.10 m on average in May.

Natural reefs had 2.9 ± 1.0 grazers per sampling rope until April 12, 2012, as macroalgae began to decline, when mesograzers increased to 11.1 ± 4.0 mesograzers on average throughout the remainder of the monitoring period (Fig. 3A). Restored reefs had higher initial mesograzers density than natural reefs, with 10 grazers on average per sampling rope, peaking at 57 ± 21 grazers on April 12th and then declined back to 9.8 ± 4.7 mesograzers by the time the algae began to senesce.

The most common nekton collected were pinfish (*Lagodon rhomboids*), Penaeid shrimp, and mummichogs (*Fundulus heteroclitus*), which together comprised over 90% of the catch (Fig. 3B). Similar to mesograzers abundance, nekton abundance was dependent on reef type. Natural reefs had higher abundances of nekton than restored reefs. Initially, catch per unit effort (CPUE) of nekton was less than 1 individual for both restored and natural reefs in January. CPUE on natural reefs increased to reach 8.3 ± 1.5 individuals/set on April 12,

2012, while restored reef CPUE remained low and only increased to 2 ± 1.5 individuals/set on average during the last six weeks of sampling.

Effects of nutrients on macroalgal cover

The response of macroalgal cover to nutrient enrichment was dependent on restoration setting and plot elevation (Fig. 4A, nutrient x setting x elevation, $P = 0.043$). On restored reefs, nutrient enrichment increased *Ulva* cover at the end of the growing season, but the relative increase was greater for plots at low elevation than high (Fig. 4B). Additionally, the peak macroalgal cover persisted two weeks longer for nutrient enriched plots compared with non-nutrient enriched plots at low elevations on restored reefs. However, on natural reefs, macroalgal cover was lower within nutrient-enriched plots until April 26, 2011 when all treatments converged for the remainder of the monitoring period (*Ulva* cover, setting x elevation x time, $P = 0.019$). Nutrient enrichment had no effect on *Ectocarpus* cover (Fig. 4C, nutrients, $P = 0.347$) and did not cause any changes in community composition.

Effects of top-down control on macroalgal cover

When nekton were excluded (removing top-down control on mesograzers) total cover of macroalgae declined significantly earlier than all other treatments from $77.5 \pm 2.8\%$ on April 8, 2012 to $8.3 \pm 7.5\%$ on May 9, 2012 (Fig 5A, cage x pesticide, $P = 0.032$; cage x date, $P = 0.007$). Macroalgal cover persisted at an average of $41.8 \pm 4.7\%$ in treatments where nekton were not excluded, until declining on May 16, 2012. Although removing top-down control on mesograzers caused a faster rate of decline in total macroalgal cover, all treatments were not significantly different from each other only one week later on May 23rd (time, $P < 0.001$).

The rate of decrease in *Ulva* cover in the grazer exclusion treatment was greater than

the grazer and nekton exclusion treatments (Fig. 5B, cage x pesticide, $P < 0.001$), indicating that the presence of nekton was associated with a quicker decline in *Ulva*. Post hoc analyses also indicated that *Ulva* cover in the grazer exclusion was not statistically different from the control.

The nekton exclusion only had significant effects on *Ectocarpus* cover (Fig. 5C, cage, $P < 0.050$). *Ectocarpus* cover in the nekton-exclusion and nekton- and grazer-exclusions treatments did not exceed 2% cover over the course of the experiment. However, when grazers were excluded, *Ectocarpus* reached 15% and was not statistically significant from the control treatments.

Effects of macroalgal cover on oyster fitness

Macroalgal removal significantly reduced the abundance of both oyster spat (Fig 6A, $P < 0.001$) and adult oysters (Fig 6C, $P < 0.001$) on natural reefs, but the difference in oyster density between ambient cover and macroalgal removal on restored reefs was not different from zero (adults, $P = 0.593$; spat, $P = 0.767$). Macroalgal removal had no effect on oyster growth (Fig 6B), and there were no differences between elevations ($P = 0.889$) or restoration settings ($P = 0.996$).

Discussion

I quantified a baseline for oyster-associated seasonal intertidal macroalgal cover. Overall, macroalgae developed on intertidal reefs in January and fully disappeared by the end of May in 2012. Restored reefs had higher cover of macroalgae than natural reefs, and low elevations (low-intertidal or approximately mean low water) had greater cover than high elevations (mid-intertidal). The two most dominant species, *Ulva* and *Ectocarpus*, formed

two distinct zones; *Ulva* was the most dominant species in the intertidal zone and established on oyster reefs by the beginning of February and slowly declined until mid-May. *Ectocarpus* cover extended past the low elevation plots, peaking at the start of March and immediately declining one month earlier than *Ulva*, to less than 10% by the end of the May.

I found that predominantly abiotic mechanisms (a combination of water temperature, air temperature, and aerial exposure) control the decline of macroalgae in the spring. However, these results suggest that any changes in nekton abundance patterns could affect macroalgal dynamics. Nutrient enrichment increased cover and delayed the decline of *Ulva* in high elevation plots on restored reefs. This suggests that intertidal, *Ulva* in the mid-intertidal may be nutrient limited due to decreased inundation times compared to subtidal plots. Finally, I expected macroalgae to have negative effects on oyster fitness through reducing local water flow in surrounding water quality and food supply, but found that macroalgae did not reduce oyster density or growth and may have even protected oysters from exposure to winter air temperatures.

Experimental manipulations of biotic forcing indicate that nekton suppress mesograzers herbivory, but that there are limited biotic effects on the seasonal decline of macroalgal cover. When top-down control (nekton) on mesograzers was removed, the total cover of macroalgae declined faster than in the abiotic only treatment (e.g., in nekton and grazer exclusion treatment). Additionally, the rate of decline in total macroalgal and *Ulva* cover in amphipod exclusion treatments was equal to rate of decline in the control (ambient conditions). This indicates that nekton caused the decline of *Ulva* by either reducing grazer density (Duffy and Hay 2000) or suppressing mesograzers herbivory through non-consumptive effects on mesograzers (Duffy et al. 2005). Although nekton indirectly

benefited macroalgae by significantly reducing herbivory, they also reduced macroalgal biomass through direct herbivory. When only amphipods were excluded, macroalgal cover declined slightly faster than when both nekton and grazers were excluded. Pinfish are omnivorous and may have directly consumed macroalgae as they do when algae is present in high quantities (Stoner 1979) or other carnivorous fish may have inadvertently broken up macroalgae while foraging for mesograzers (Duffy et al. 2001). However, since total macroalgal and *Ulva* cover in control treatments (ambient) were not different from the grazer and nekton exclusions, it suggests that abiotic factors primarily control decline of algae in the spring.

Abiotic stress due to increasing water temperature and desiccation were most important in determining how and when macroalgal communities declined. *Ectocarpus* can grow rapidly even at sub-optimal temperatures (Bolton 1983), allowing rapid establishment in the low intertidal and subtidal zones early in the winter. *Ectocarpus* began to decline in early March, when maximum temperature (17°C) exceeded the optimum temperature of *Ectocarpus siliculosus* populations in North Carolina (Bolton 1983). *Ulva* can also withstand a wide range of temperatures (Fong and Zedler 1993), but tolerates desiccation better than *Ectocarpus* (Fong et al. 1998), allowing *Ulva* to survive in the low to mid intertidal zone. *Ulva* began to decline at both elevations on natural reefs and high plots on restored reefs once water temperature exceeded 15°C and rose to the upper range of reported optimal temperatures for *Ulva* species (10-20°C; Fong et al. 1998). Similarly, previous work has found that *Ulva intestinalis* has been found to have higher photosynthetic rates at 15°C than at 5°C or 25°C (Rivers and Peckol 1995). Additionally, desiccation stress may have interacted with rising water temperature to affect macroalgal cover. The average water level

increased between January and May, potentially reducing some desiccation stress and may have buffered macroalgae from higher water temperature (Henley et al. 1992). I also observed a quick decline in all plots when salinity dropped to 26 ppt during a storm. Since salinity was well within osmotic tolerance of *Ulva* and *Ectocarpus*, the high wind and wave action may have caused the senescence of macroalgae (Underwood 1999).

Nutrient enrichment yielded mixed results in algal community response, which is most likely tied to physiological differences in community and the complexity of intertidal hydrodynamics. *Ulva* has rapid nitrogen uptake and growth rates, as well as a large nitrogen storage capacity (Fujita 1985). This allows *Ulva* to respond rapidly to enrichment and then record the nutrient signal through time as storage in tissues (Fong et al. 1994), which is frequently used as a bioindicator of nutrient enrichment. However, I found limited effects of nutrients on macroalgal cover and only found greater cover on high elevation plots. Since I only observed increases in *Ulva* cover on high restored plots due to nutrient enrichment, *Ulva* may only be nutrient limited in high plots because they are inundated for shorter periods of time than low plots. Furthermore, the delayed effects of nutrients at the end of the growing season may be because there is a lag between nitrogen uptake and growth for *E. intestinalis* after nitrogen supply is rapidly changed (Fong et al. 1994). *Ectocarpus* cover did not significantly increase from nutrient enrichment, potentially because longer submergence reduced *Ectocarpus* nutrient limitation. Nutrient concentrations were targeting realistic increases in nutrient concentrations, but may not have been concentrated enough to promote large macroalgal blooms. Conversely, the low residence time of nutrients due to tidal flushing may have lowered assimilation rates by macroalgae. However, since I only measured macroalgal cover, it is unknown if nutrients increased macroalgal biomass.

Increased biomass in plots receiving nutrient enrichment may have caused macroalgae to persist longer due to facilitative clumping reducing desiccation stress (Bertness et al. 1999).

Although previous work has found that macroalgal cover can inhibit oyster growth and recruitment (Thomsen and McGlathery 2006), this study shows that the cover of seasonal macroalgae had limited effects on oyster fitness. Algal removal reduced the abundance of both oyster spat that settled the previous summer and adult oysters on natural reefs, indicating that macroalgal presence may actually help protect oysters (Bertness et al. 1999). Natural reefs may have been more susceptible to reductions in macroalgal biomass because natural reefs are less consolidated than restored reefs; in natural reefs, oysters are typically denser, reducing stressors associated with cold winter temperatures, like desiccation. Additionally, removals may have disturbed oyster aggregates on natural reefs because they are not cemented to the reef like most oysters on restored reefs. I also found no effects of seasonal macroalgae on oyster growth on the previous year's recruits. Body size can affect how susceptible oysters are to cover by epifauna, and oysters may have reached a size threshold where macroalgae cover did not limit food supply or water quality for oysters. Oyster growth in the winter is also lower due to reduced chlorophyll-a concentrations and metabolic rates, and may have contributed to no difference in growth. Macroalgae may have allowed sufficient water flow through to oysters because it is buoyant when submerged in water (Escartin and Aubrey 1995). Conversely, since I measured cover and not macroalgal biomass, macroalgae may not have reached a high enough biomass to reduce recruitment or growth (Thomsen and McGlathery 2006).

Standardizing elevation plots to restored reefs, which were originally restored on a higher elevation sandflat, prevented us from quantifying zones where *Ectocarpus* dominates

and reaches 100% cover at peak growing seasons. On many natural reefs, loose oyster shell substrate extends into the subtidal zone because natural reefs developed on lower substrate than restored sand flat reefs. If the non-living extension of the natural reef extends into the subtidal zone, *Ectocarpus* also extends beyond the critical depth for oyster reef growth (Ridge et al 2015) and correlates with areas where oysters do not survive. *Ectocarpus* has branched filaments that form a relatively dense canopy compared to the leafy canopy of *Ulva*, and may prevent flow and reduce fitness of oysters. However, further work is needed to understand how species morphology and biomass affect oyster fitness.

In conclusion, this experiment indicated that abiotic mechanisms, including water temperature and desiccation, are the dominant factors that regulate macroalgae cover on intertidal oyster reefs, and that at current abundances, macroalgae has limited negative effects on oyster fitness. Restored reefs had the highest macroalgal densities, the longest duration of algal cover, and were the only reefs that had increased macroalgal cover in response to nutrient enrichment. Continued nutrient enrichment may increase algal biomass and duration in the high intertidal on restored reefs, and thereby exerting negative effects on oysters (due to smothering). Although primarily abiotic mechanisms currently control seasonal macroalgal dynamics, future warming may cause changes in the associated food web. Estuaries will warm faster than offshore waters (Najjar et al. 2000), potentially causing temporal mismatch of nekton communities moving in from offshore. If nekton do not shift their recruitment timing as fast as warming affects macroalgal communities, there may be changes in trophic structure – reducing the top down control on mesograzers and causing accelerated declines in oyster-associated macroalgal communities. Warmer water temperatures would also increase metabolic rates, and in turn, increase herbivory and cause

more rapid seasonal declines (O'Connor 2009, Carr and Bruno 2013). I found higher cover of *Ectocarpus* on restored reefs, but were unable to examine the effects of high *Ectocarpus* cover on oyster fitness due to sampling design. Rapid growth allows *Ectocarpus* to be a successful competitor in sub optimal conditions, and may allow it to adapt to warming temperatures (Bolton 1983). As the effects of *Ectocarpus* on oyster fitness are unknown, phenologically-minded reef restoration, occurring after the seasonal decline in macroalgae, and above the elevation (Ridge et al 2015) where *Ectocarpus* grows will enhance reef growth.

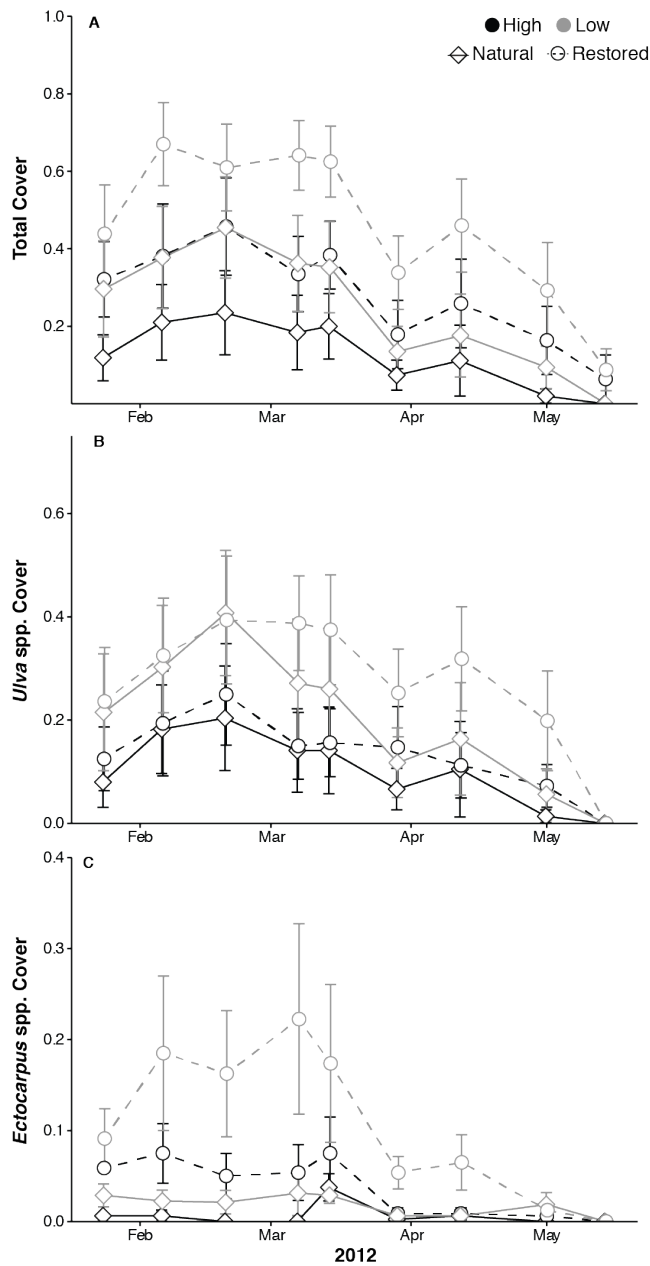


Figure 2.1 Seasonal macroalgal cover (proportion) of the (A) whole algal community, and the two most dominant species (B) *Ulva* spp. and (C) *Ectocarpus* spp. on intertidal oyster reefs at two elevations (low, high) and two restoration settings (restored, natural). Error bars represent ± 1 standard error.

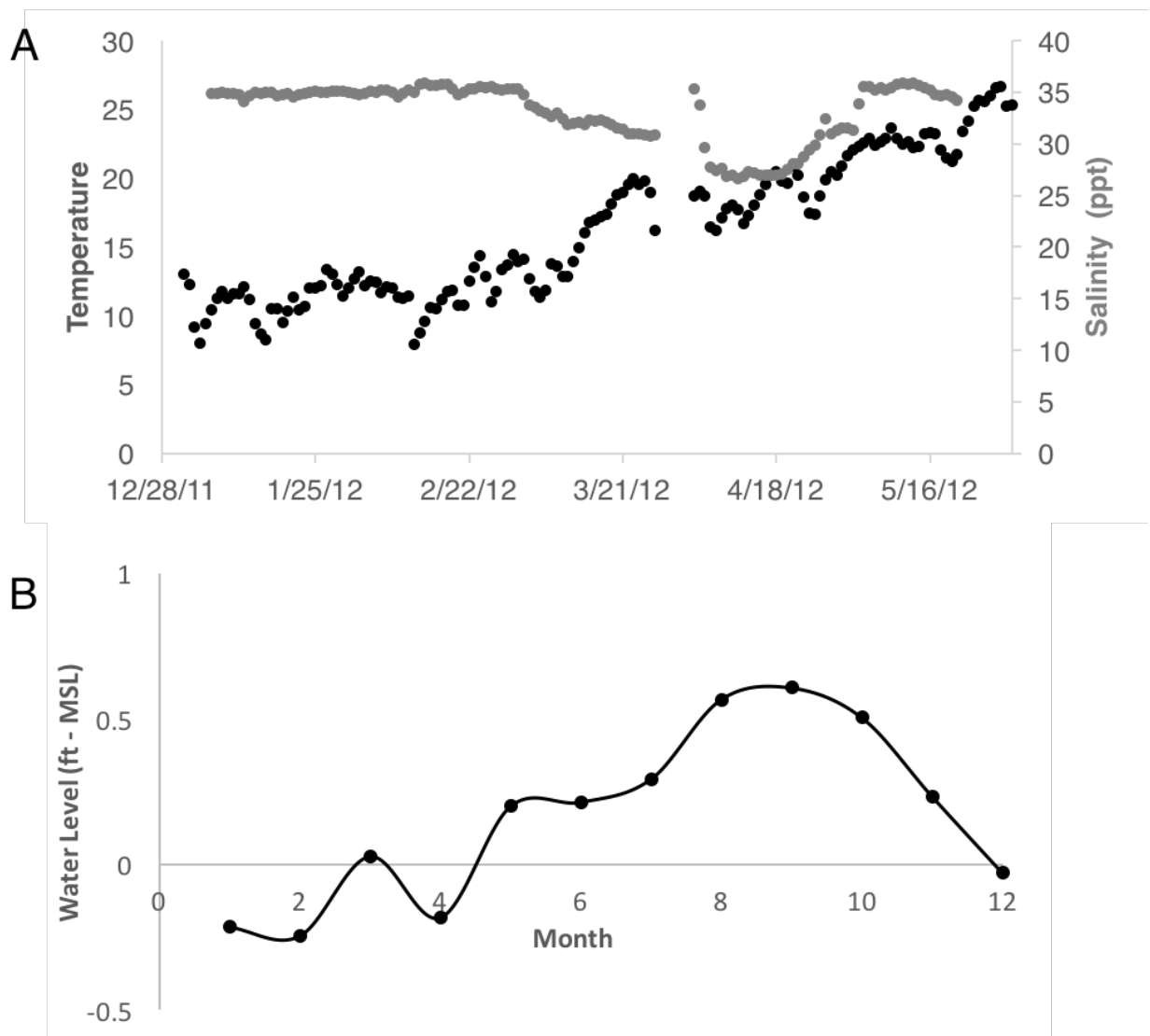


Figure 2.2 Water temperature (black) and salinity (gray) in Middle Marsh, Back Sound, NC during monitoring period (A) and monthly-averaged water level relative to Mean Sea Level (B) during 2012. Water quality data was unavailable from March 27 - April, 3, 2012 due to sonde calibration and deployment schedules.

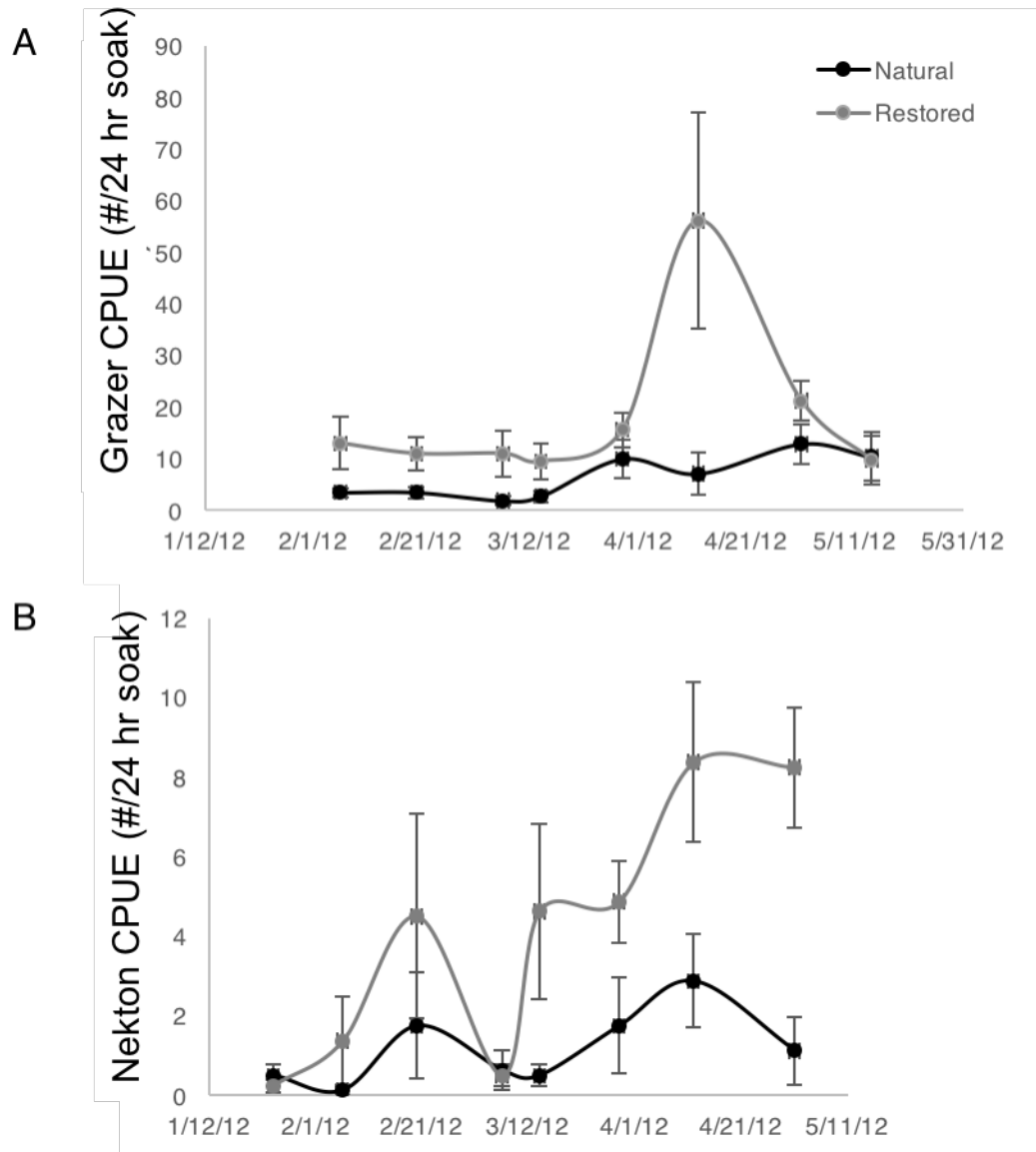


Figure 2.3 Catch per unit effort of grazers (A) and nekton (B) at restored and natural intertidal oyster reefs.

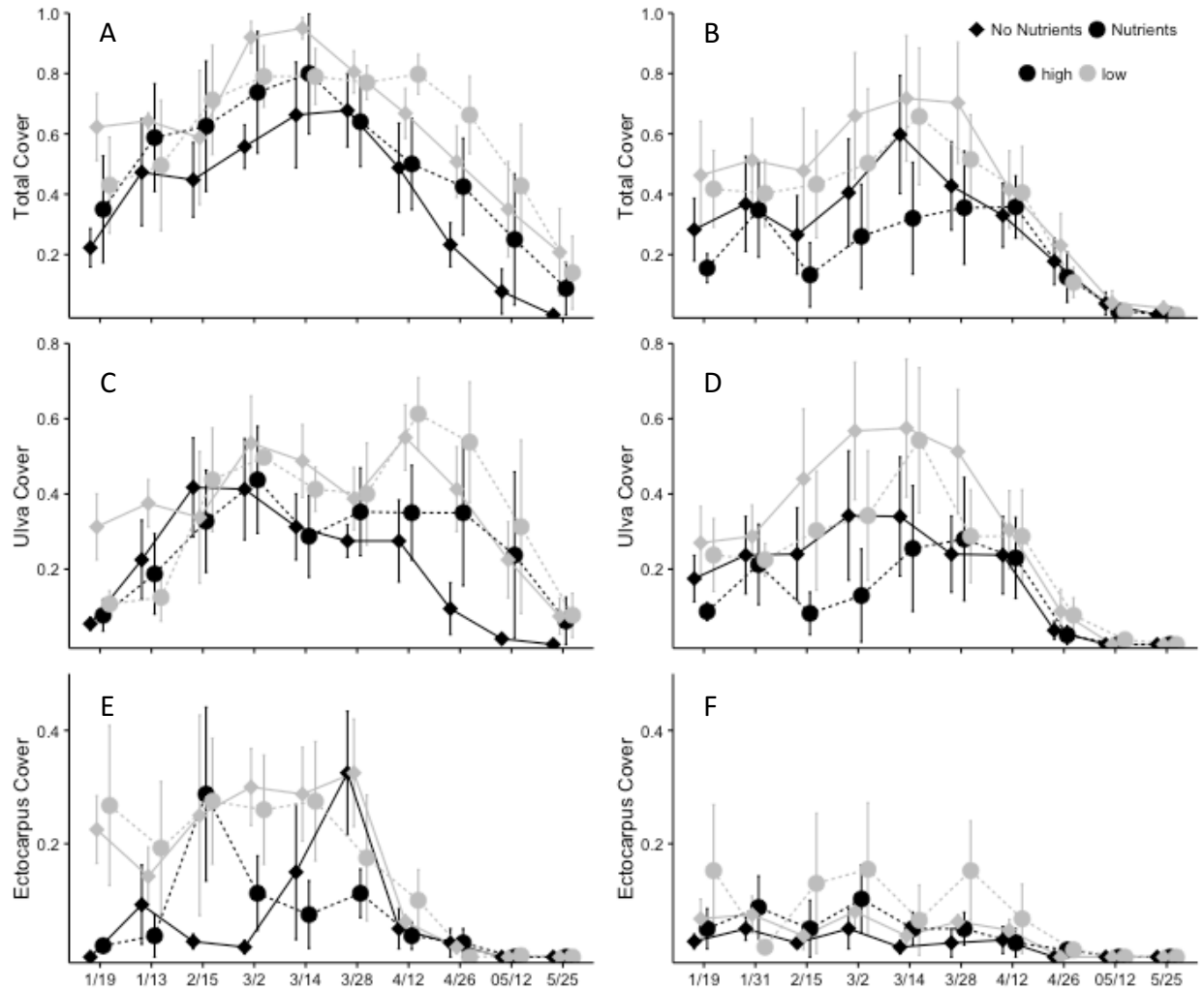


Figure 2.4 2011 seasonal cover (proportion) of macroalgae (A-B: total, C-D: *Ulva* spp, E-F: *Ectocarpus*) in response to experimental nutrient enrichment (ambient, +nutrients) at two elevations on restored (A, C, E) and natural (B,D,F) intertidal oyster reefs. Error bars represent ± 1 standard error.

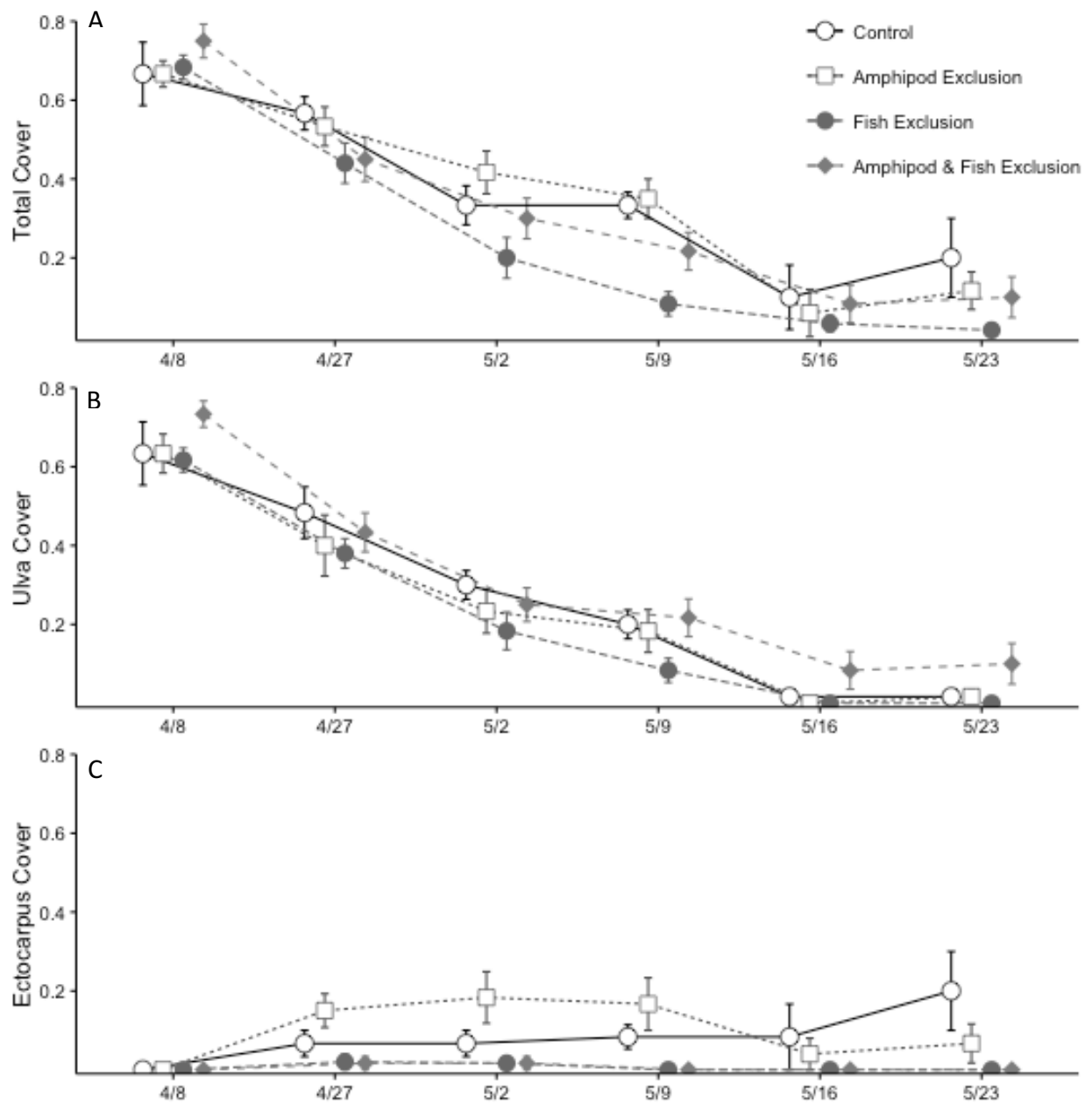


Figure 2.5 Proportional macroalgal cover of the (A) total community, and the two most abundant species (B) *Ulva* spp. and (C) *Ectocarpus* spp. when exposed to ambient nekton & grazers (white circle), grazer exclusion (white square), nekton exclusion (gray circle), and nekton and grazers exclusion (gray triangles). Error bars represent ± 1 standard error.

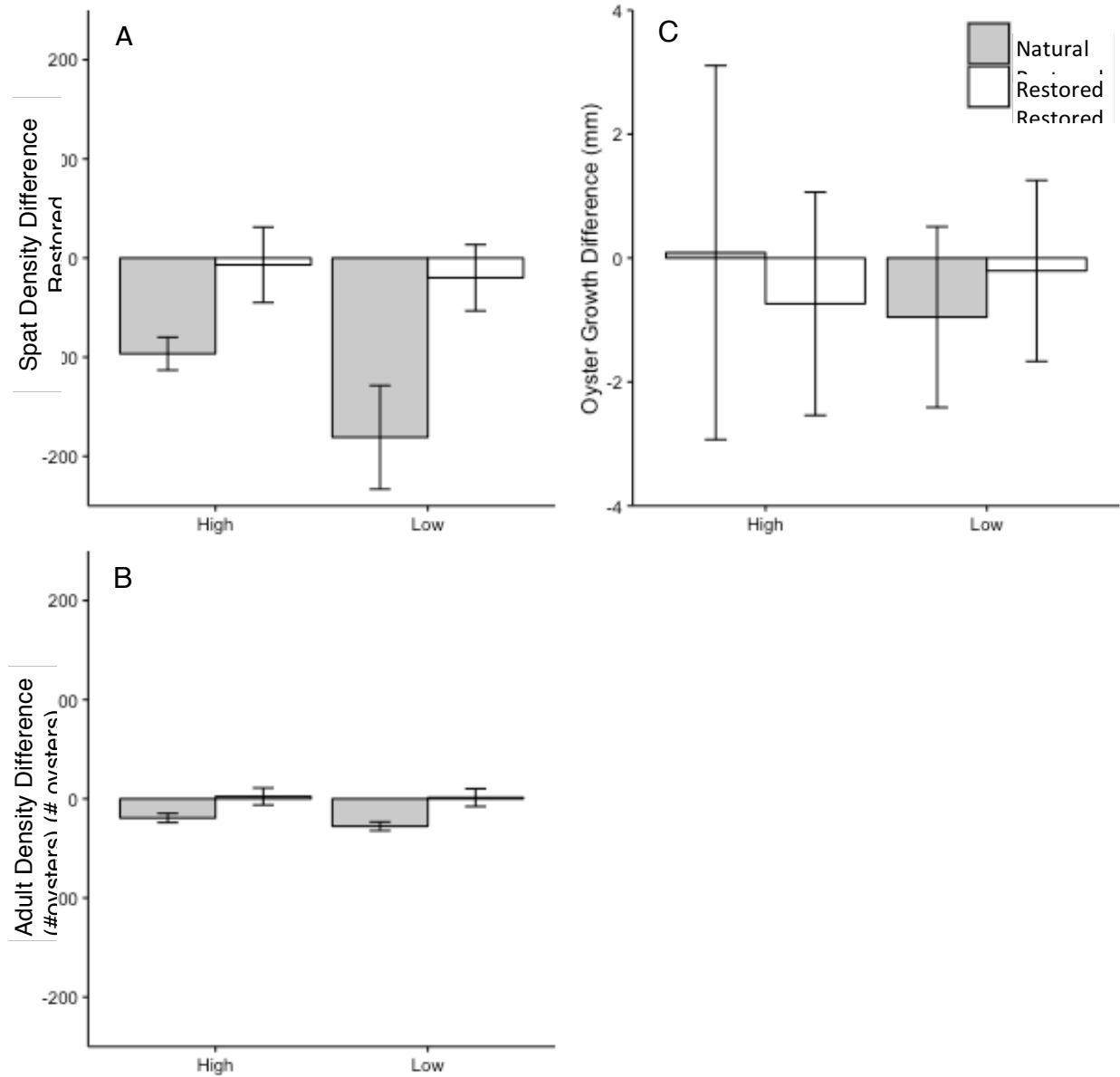


Figure 2.6 Effect of seasonal macroalgal cover on oyster fitness, measured as the difference in oyster density (spat A, adult B) or growth (C) between ambient macroalgae and macroalgal removal plots at two elevations (high, low) and two restoration settings (restored, natural). Error bars represent ± 1 standard error.

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CHAPTER 3: LANDSCAPE-LEVEL DIFFERENCES IN SETTLEMENT AND PREDATION REGULATE OYSTER REEF COMMUNITIES

Introduction

Biogenic habitats provide crucial nursery and foraging habitat, and are an important component of landscape mosaics (Lindsey et al. 2006, Boström et al. 2011). The development of habitat-building (foundation) species is regulated by the integration of settlement supply (Underwood and Fairweather 1989) and post-settlement processes, such as competition, predation and facilitation (Menge 2000, Wright and Steinberg 2001). Because foundation species are vital in supporting and sustaining ecological communities, it is important to understand which mechanisms regulate the development and persistence of biogenic habitats.

The development of biogenic habitats can be regulated by the balance of settlement and predation rates. The supply of settlers can control the abundance of a species in some systems (Roughgarden et al 1987). For instance, the wind velocity and wind pattern determines the quantity and location of where seeds are dispersed in grasslands (Soons et al. 2004). In other systems, varying predation pressure can dramatically alter and even reverse initial patterns in species densities that are established at settlement (Rilov and Schiel 2006, Fodrie et al. 2014). The relative importance of settlement and/or predation in controlling the growth of biogenic habitats is largely influenced by landscape setting (Shima et al. 2008) and environmental stress (Menge and Sutherland 1976, 1987) of the habitat.

The orientation of a habitat within a landscape mosaic can affect the degree to which settlement and predation influence the development of biogenic habitats. Estuarine landscapes are comprised of patches of seagrass meadows, salt marshes, biogenic reefs, and mangroves within a matrix of unvegetated bottom, and biogenic habitats develop in various arrangements. Within estuaries, Eastern oyster, *Crassostrea virginica*, reefs exhibit disparate development across gradients of salinities, habitat settings and aerial exposures (tidal emergence) resulting in differences in oyster demographics across scales from an individual reef to an entire estuary, presumably due to differences in settlement and predation (Baggett et al 2015, Walles et al 2016). Thus, oyster reefs can be used as a model system to examine how settlement and post-settlement processes affect biogenic habitat development.

In the euhaline portion of the estuary, oyster reefs develop predominantly in the intertidal zone, and previous work has found substantial variation in oyster settlement and adult densities within individual reefs. Oyster settlement decreases with increasing aerial exposure (due to lower submergence time) on intertidal reefs; however, the adult oyster density increases with greater aerial exposure (Fodrie et al. 2014). Previous work in Texas has found predation on oysters to be greater in the subtidal than intertidal in marine waters (Johnson and Smee 2014), suggesting that predation may have caused the inverse patterns of settlement and adult populations found on high salinity reefs in North Carolina (Fodrie et al. 2014). Additionally, in the low salinity regions of estuaries, where predators are less abundant (as a result of lower tolerance to fresher water), reefs can extend into the subtidal even though settlement is relatively lower than high salinity regions (Chestnut and Fahy 1953, Ortega 1981).

Within estuaries, oyster reefs develop either directly adjacent to a salt marsh or isolated on a sandflat, and there are differences in oyster populations between these landscape settings (Grabowski et al. 2005). The marsh edge can have higher predator abundance than isolated reefs due to high connectivity with adjacent habitats, thus may have higher predation rates than isolated habitats with relatively low connectivity (Micheli and Peterson 1999). Similar to the mismatch of juvenile and adult oyster densities along the intertidal gradient, adult oyster density across different landscape settings does may match patterns established after oyster settlement. Higher oyster settlement has been found on salt marsh fringing than isolated reefs, but isolated reefs have higher adult densities than fringing reefs (Carroll et al. 2015). Previous work has found limited differences in predation rates of oysters between reef landscapes (Micheli and Peterson 1999), thus, further work is needed to understand if predation may also be the mechanism accounting for differences between settlement and adult densities across landscapes.

The relative importance of settlement and predation, as well as the spatial scales at which these processes interact to control oyster reef development has not been fully explored. Previous work has identified that, in euhaline water, subtidal reefs have lower oyster density and biomass than intertidal reefs (Powers et al 2009, Fodrie et al 2014), and that reefs restored within the intertidal along saltmarshes typically have lower densities than isolated reefs (Grabowski et al 2005, Carroll et al 2015). However, I do not have a thorough understanding of how landscape setting and aerial exposure interact to affect settlement and post-settlement processes. For example, I know that predation of newly recruited oysters increases with increasing inundation (Johnson & Smee 2014), but I do not know if the magnitude or pattern of predation is equal across all landscapes settings. Additionally, I do

not understand how the structural complexity of the reef may affect the balance between settlement and predation, as the interaction of predator accessibility and foraging success will determine how biogenic habitats develop and persist (Turner 1989). Differences in structural complexity can affect predator foraging ability. On oyster reefs, high habitat structure increases prey survival (Grabowski 2004a) because it reduces predator foraging efficiency relative to unstructured habitats (Summerson and Peterson 1984). The role of *Xanthid* mud crabs (e.g., *Panopeus herbstii*) and blue crabs (*Callinectes sapidus*) as important predators of juvenile oysters has been identified, and have higher predation rates on fringing reefs than isolated reefs (Carroll et al 2016, Michelli & Peterson 1998). Conversely, little is known about the foraging behavior of predators of oysters such as the sheepshead, *Archosargus probatocephalus*, across landscapes or within the intertidal.

This study examined how settlement and predation affects intertidal oyster reef development across the aerial exposure gradient and between different landscape settings, as well as how structural complexity may modify these predation rates. Specifically, I asked: (1) How do landscape setting and aerial exposure affect oyster settlement across intertidal oyster reefs? (2) How do oyster reef landscape setting and aerial exposure affect predation rates, and do these patterns change with different habitat complexities? I expected that fringing reefs would have higher settlement rates than isolated oyster reefs similar to recent measurements comparing reef landscapes (Carroll et al 2016). Since aerial exposure may limit predation, I predicted predation rates to increase with increasing inundation time. I also expected nekton to have higher predation rates on saltmarsh reefs than isolated reefs because they are connected to habitat corridors, whereas isolated reefs are not, causing lower densities of oysters on fringing reefs. If mud crab predation is directly proportional to crab

density, then predation by resident crabs would be the greatest where there is highest oyster cluster mass.

Methods

I conducted a field experiment to understand how settlement and predation affect the vertical distribution of intertidal oysters across landscape settings and habitat complexities. I quantified oyster settlement rates and predation by different predator groups at three aerial exposures (mid intertidal to subtidal) on two intertidal reef seascape settings (isolated, fringing a salt marsh). Predation assays were conducted on isolated sandflat reefs and saltmarsh-fringing reefs in Middle Marsh (34° 41' 34.98" N 76° 36' 43.44" W) and Shackleford Banks (34° 41' 25.51" N 76° 35' 20.71" W), Back Sound, North Carolina. Middle Marsh and the marshes behind Shackleford Banks are representative of estuarine marsh complexes occupying a relic flood tidal delta and consist of a mosaic of natural and restored oyster reefs, seagrass meadows, salt marshes and sandflats.

Reef landscape characteristics

Oyster reef community characteristics were quantified at three elevations (-0.2, -0.35, -0.5 m) on isolated and fringing oyster reefs in 2014. Elevations were used as a proxy of aerial exposure and were measured in reference to the North American Vertical Datum established in 1988 (NAVD88), where local mean sea level is approximately -0.03 m NAVD88, and -0.42 m NAVD88 is mean low water. Oysters were excavated within a 0.01-m² quadrat (n=6), enumerated, and weighed. Simultaneously, crabs and all other organisms were manually collected during oyster reef excavations at each elevation to understand how

resident predators (e.g., small crabs including Xanthid crabs) are distributed along the aerial exposure gradient. All organisms were identified to species, counted, and measured.

Oyster settlement

To determine how landscape setting and aerial exposure affected oyster settlement, I deployed settlement mats across three elevations on fringing and isolated reefs. Settlement was quantified at three elevations that ranged from the top to the base of the reef, past the extent of living oysters. Since different reefs were used between the two years, the elevations in 2013 were -0.15, -0.5, and -0.75 m, while they were -0.2, -0.35, -0.5 m in 2014. Since oysters rapidly settle on new substrate and more frequently settle on the concave side of oyster shells (Ortega and Sutherland 1992), settlement mats were constructed by attaching three recycled oyster shells, concave side down, to Vexar® mesh. Attaching shells to Vexar® with the concave side down reduced access of predators to settlement shells. Settlement mats were secured to the surface of the reef at each elevation ($n = 6$) in June before oysters settled (Ortega and Sutherland 1992). After oyster settlement occurred, oysters and all community members were enumerated and measured on the concave side of the oyster shell in August of both years. In the first year, oyster settlement mats were deployed on June 6, 2013 and settlement quantified on August 8, 2013, while mats were deployed on June 14, 2014 and settlement quantified on August 30, 2014 in the second year.

Predation

I conducted a field experiment to quantify predation rates at three elevations on isolated and fringing intertidal oyster reefs with two different structural complexities. Since I was unable to deploy the complete 3-way design at once, I deployed the predation assays with the two complexities in consecutive years. While inter-annual variability may influence

settlement, the importance of structural complexity is known to be a mechanism in regulating species interactions, thus, running separate experiments and then making qualitative comparisons was deemed the best approach. In 2013, I conducted predation assays on low complexity tiles on recently restored isolated reefs built between the mid intertidal into subtidal depths (-0.15, -0.5, and -0.75 m) and a nearby fringing reef with corresponding elevations was used to match depths of the isolated reefs. In 2014, I conducted predation assays on high complexity tiles on natural reefs, and after surveying the elevations of natural reefs I found that reefs did not extend past mean low water (MLW). Therefore, I only conducted predation assays from the mid intertidal to the low subtidal (-0.2, -0.35, -0.5 m) in 2014.

Twenty spat (5-20 mm) were attached to predation assay plates with marine epoxy and five cage designs were used to identify which predators were consuming oysters (e.g., small crabs vs. large nekton). A predation assay plate with no cage was used to quantify total predation rate (all crabs and fish predators). To allow nekton (e.g., sheepshead) access to spat but prevent small resident crabs (e.g., mud crabs) from climbing into the cage, I used a topless 1-cm mesh cage with 4 inches of flashing tape around the top edge. Having a smooth surface surrounding the top of the cage prevented small resident crabs from climbing up and over into the topless cages, but allowed nekton to swim into the cage. A 6-cm mesh fully enclosed cage was used to only allow small crabs access to spat. A cage that fully encloses the oyster-spat plate with 1-cm mesh acted as a control for oyster attachment failure (no predation) and a cage with 1-cm mesh with three sides was used as a half cage control to account for predator attraction and changes in flow. A GoPro camera was deployed at an

open, topless, and wide-mesh cage in both years to verify predator groups with time lapse photography.

In September 2013, I deployed flat low complexity tiles (6 x 6 in) with spat attached with marine epoxy and evenly placed across the plate for the predation assay. Plates were deployed at low tide at three elevations from the top of the reef to below the living extent of living oysters (-0.15 m, -0.50 m, and -0.75 m NAVD88) on isolated sandflat reefs and a fringing reef in Middle Marsh. Oyster spat survival was quantified after four days. However, since nearly 100% of oysters in open plates were consumed at all elevations and landscapes at four days, I conducted an additional predation assay. I re-deployed the no-cage treatment across all landscapes and elevations at low tide to understand if there were any differences between landscapes and/or elevations. Survival was monitored for six hours (with checks at every 3 hours) until at least 50% predation had occurred on at least one treatment.

In September 2014, I used high complexity plates made by supporting 10 vertical oyster shells in a 10-cm x 20-cm concrete base to better mimic the structural complexity of an oyster reef. Spat were attached using marine epoxy to top and base of both sides of each shell on complex reef tiles to ensure that resident (i.e., mud crabs) and nekton (i.e., sheepshead) predators could both access oyster spat. Cages were deployed onto isolated and fringing reefs at each elevation at low tide in early September. Oysters were excavated to embed cages in the reef with shells flush with the surrounding oyster habitat, allowing oyster predators to access cages. The high complexity predation assay was deployed from the top to the base of the reef (-0.2 m, - 0.35 m, and -0.5 m NAVD88) during low tide.

Statistical analyses

Oyster biomass, mud crab density, oyster settlement, and spat survival in the low-

complexity no-cage deployment were analyzed with separate two-way ANOVA with elevation and reef landscape (isolated, fringing) as independent factors. Oyster settlement and oyster biomass were log transformed and mud crab density were Box-Cox transformed to meet assumptions of normality and homogeneity of variance. The effects of aerial exposure, cage type, and landscape setting on survival rates were tested using separate three-way ANOVAs for low and high complexity trials. Transformation did not make the variance of survival rates equal for either predation assay (low complexity in 2013 and high complexity in 2014) to meet the assumptions of homogeneity of variance, so a Kruskal-Wallis test was used to analyze the effect of landscape setting and elevation on spat survival for each year. Pairwise comparisons were made with Tukey's HSD test.

Results

Reef landscape characteristics

Oyster biomass increased with increasing elevation (Fig. 1A, elevation, $P < 0.001$) from $0.596 \pm 0.17 \text{ kg}/0.01\text{m}^2$ ($\mu \pm \text{SE}$) and $0.703 \pm 0.203 \text{ kg}$ at -0.20 m to $2.009 \pm 0.30 \text{ kg}/0.01\text{m}^2$ and $2.646 \pm 0.30 \text{ kg}/0.01\text{m}^2$ at -0.50 m on fringing reefs and isolated reefs, respectively. Isolated reefs had 20% higher oyster biomass than fringing reefs (landscape setting, $P = 0.039$).

Patterns of mud crab density (Fig. 1B) followed the same patterns as oyster density ($p = 0.002$, $R^2 = 0.248$). Isolated reefs had higher mud crab densities than fringing reefs (landscape setting, $P < 0.001$) and crab densities increased with decreasing elevation (elevation, $P = 0.024$). Fringing reefs had $5.1 \pm 1.1 \text{ crabs}/0.01\text{m}^2$ at -0.20 m to 1.8 ± 0.5

crabs/0.01m² at -0.50 m. Isolated reefs had 6.7 ± 0.8 crabs/0.01m² at -0.20 m and 2.7 ± 0.4 mud crabs/0.01m² at -0.50 m.

Oyster settlement

In 2013, there was a significant interaction between reef type and elevation ($P = 0.027$) that affected oyster settlement (Fig. 2A). Oyster settlement increased with decreasing elevations on isolated reefs with 3.1 ± 1.2 oysters/3-shells at high elevations and 35.3 ± 12.2 oysters/3-shells at low elevations. On fringing reefs, oyster settlement was lower overall and increased from 0.1 ± 0.2 to 9.9 ± 7.4 oysters/3-shells from at low elevations.

Overall settlement was higher in 2014 with a marginally significant interaction between landscape setting and elevation ($P = 0.076$, Fig 2B). Oyster settlement did not differ between elevations on fringing reefs and averaged 34.5 ± 7.5 oysters/3-shells¹, while settlement increased with decreasing elevation on isolated reefs (from 25.5 ± 6.0 oysters/3-shells at - 0.20 m to 56.1 ± 8.4 oysters/3-shells at -0.50 m).

Predation

On low complexity reef tiles in 2013 (Fig. 3A), cage type (predator identity) had a significant effect on survival of oyster spat ($P < 0.001$); however, reef landscape setting ($P = 0.777$) and elevation ($P = 0.247$) had no effect after four days (eight tidal cycles). When all predators had access to the spat, $100.0 \pm 0.0\%$ of the oysters were consumed within four days. Oysters in topless cages that only allowed nekton access also had a high mortality with only $3.6 \pm 1.8\%$ of oysters surviving. However, although not statistically significant, survival rates were marginally higher for topless cages and cage controls at high elevations. Sheephead were observed consuming oysters in topless and open cages using time-lapse photography. Conversely, large mesh cages, where small crabs only had access to consume

prey had high survival at $95.7 \pm 1.5\%$. Small mesh, fully enclosed cages also had high survival ($97.0 \pm 2.9\%$) indicating that consumption by predators, not glue failure, caused the spat loss.

Since 100% of spat were consumed in the low complexity, no-cage treatments after 4 days, I was unable to calculate a predation rate; therefore, I conducted an additional predation assay where I deployed tiles without cages again in 2013 and checked predation every three hours (Fig. 4). Very few spat were consumed after three hours, but after six hours there was a significant interaction between elevation and reef setting ($F = 3.317$, $P = 0.050$). Predation rates were highest on isolated reefs and there were no differences across elevations, with survival rates of $2.5 \pm 2.5\%$, $1.3 \pm 0.9\%$, and $0.8 \pm 0.8\%$ at -0.15 m, -0.50 m, and -0.75 m, respectively. Conversely, survival on fringing reefs increased with increasing elevation ($58.3 \pm 19.0\%$, $36.7 \pm 16.6\%$, and $1.7 \pm 1.1\%$ at high, mid and low elevations, respectively).

In high complexity reef tiles in 2014 (Fig. 3B), there was no effect of landscape setting on oyster survival, but there was an effect of cage type (predator identity, $P < 0.001$) on survival. Unlike 2013, there was no significant pattern in predation across elevations on topless cages, but there was a significant difference among elevations in the no-cage and large mesh cages, indicating mud crab predation. Predation was greatest at intermediate depths with only $42.1 \pm 24.4\%$ of oyster spat surviving at -0.35 m and was significantly different than survival at -0.50 m with $81.7 \pm 30.0\%$ of oyster spat surviving. Survival in full cages was 100%, indicating again that consumption by predators, not glue failure, caused the majority of spat loss. No nekton predators were observed on the time lapse photography, but mud crabs were observed within the high complexity blocks when survival was quantified at the end of the experiment.

Discussion

Settlement supply, not predation, was the best predictor of relative adult oyster densities between landscape settings on intertidal oyster reefs. Both settlement and adult oyster biomass were greatest on isolated reefs. When landscape setting was important in regulating predation rates, predation was also greatest on isolated reefs, indicating that predation did not reverse settlement patterns across different landscapes. Conversely, oyster settlement was a poor indicator of the patterns of adult densities across the intertidal gradient. Oyster spat abundance increased with increasing inundation time, while adult densities increased with decreasing inundation time (higher aerial exposure). Yet, the pattern in predation across the intertidal and the dominant predator was context-dependent. At low complexity, nekton (particularly sheepshead), were the primary predators and landscape setting determined the pattern of predation across the intertidal gradient. On fringing reefs, predation rates were a function of inundation time, increasing with decreasing aerial exposure. However, isolated reefs at low complexity had predation rates so high that I was unable to determine if there was an effect of aerial exposures. Predation assays on high complexity tiles yielded no difference in predation rates between fringing and isolated reefs and mud crabs were the dominant predators, consuming the most oyster spat at intermediate aerial exposures. Thus, predation may be an important driver of patterns across the intertidal, but the dominant predator and importance of landscape in driving predation rates is dependent on habitat structural complexity.

These results indicate that differences in the magnitude of oyster settlement between landscape settings reflects differences in adult oyster density on fringing and isolated reefs.

The landscape with the highest adult oyster density corresponded with highest settlement rates even though, when there were differences in predation between landscapes at low complexity, predation rates were actually higher on isolated reefs. The differences in adult abundances match those found on newly restored oyster reefs in North Carolina by Grabowski et al. (2005). The supply of larvae may be higher on isolated reefs because they have higher advection of water moving in and out with low tide, since there is no structure to buffer flow (Grabowski et al. 2005). Conversely, Carroll et al. (2015) in North Carolina have found higher settlement on fringing reefs than isolated reefs. However, settlement varies significantly across the intertidal and they did not measure settlement across the intertidal zone, thus, may have measured higher settlement on fringing reefs because they are lower than isolated reefs.

These findings are contrary to some other studies on predation across oyster reef landscapes. Previous work has found that predation on fringing reefs is greater than isolated reefs (Carroll et al 2015). The larger range of movement of sheepshead in this study may have caused isolated reefs to have higher predation rates than fringing reefs at low complexity. Because sheepshead are highly mobile fish, the distances between isolated habitats and other biogenic habitats probably did not inhibit their foraging behavior. Little is known about the movement of sheepshead, but they are often found surrounding pilings and on isolated reefs (Jennings 1985), indicating that they may not rely on habitat corridors for their movements within a landscape.

There were only differences in predation across the two landscapes at low complexity. Increasing complexity has been found to reduce predation, and if sheepshead are visual predators, the higher complexity tiles may have inhibited foraging. Predators at higher

trophic levels may have also had non-consumptive effects on mud crab foraging behavior, and reduce rates of predation on oysters (Grabowski 2004b). When sheephead were the dominant predators on low complexity tiles predation by mud crabs was absent. The presence of higher order consumers in oyster reef systems has been found to reduce intermediate predator foraging, having the same effect as direct consumptive effects top predators on intermediate predators (Grabowski 2004b). Since sheephead, and potentially other nektonic predators, were actively foraging on both fringing and patch reefs, mud crabs did not risk moving into a patch of open space to forage and that they only stayed within the reef matrix (Grabowski et al. 2008).

Mud crabs only foraged effectively on high structural complexity tiles and mud crab predation was absent at low complexity reefs across all elevations. Mud crabs are important predators of spat on intertidal oyster reefs (Carroll et al 2015) and I have found that their foraging rates are not only dependent on density of mud crabs, but also the integration of aerial exposure and structural complexity. On high complexity plates, the density of mud crabs increased with aerial exposure at both landscapes, but predation rates were highest at intermediate aerial exposures. Mud crabs typically forage during high tide (Kneib and Weeks 1990), thus, the interaction of mud crab densities and tidal inundation caused a maximum predation rate at intermediate depths on intertidal reefs. Since mud crab predation is a dominant mechanism for moderating oyster densities at high complexity in the low intertidal (< -0.35 m), other mechanisms like competition may be the most important for causing post-settlement mortality below mean low water (> -0.5 m, CHAPTER 1).

In conclusion, higher adult densities on isolated reefs are not due to lower predation rates relative to fringing reefs because predation rates were either equal (at high complexity)

or higher (at low complexity) on isolated reefs than those on fringing reefs. I found that settlement is an important driver of oyster density across oyster reef landscape settings, as the density of newly settled oysters was greater on isolated reefs than fringing reefs.

Additionally, habitat complexity affected which predator was dominant and the importance of landscape on predation rates. This has important implications for the development of the subtidal edge of intertidal oyster reefs. Edges of intertidal reefs are typically below the critical depth for growth (Ridge et al 2015) and comprised of unconsolidated shell due to low oyster survival. Thus, once a reef reaches a low complexity it is more susceptible to predation by sheepshead and may prevent any new settlement from surviving, especially if the reef is at an isolated landscape.

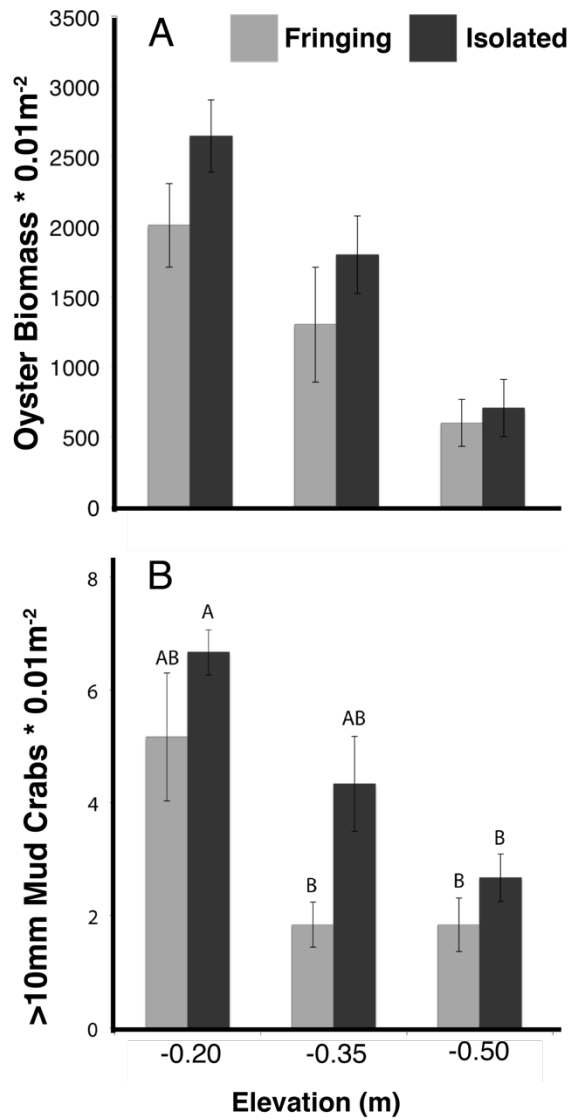


Figure 3.1. Oyster (A) biomass and (B) large mud crab density on intertidal oyster reefs at two settings (fringing, isolated) across three elevations. Error bars represent ± 1 standard error.

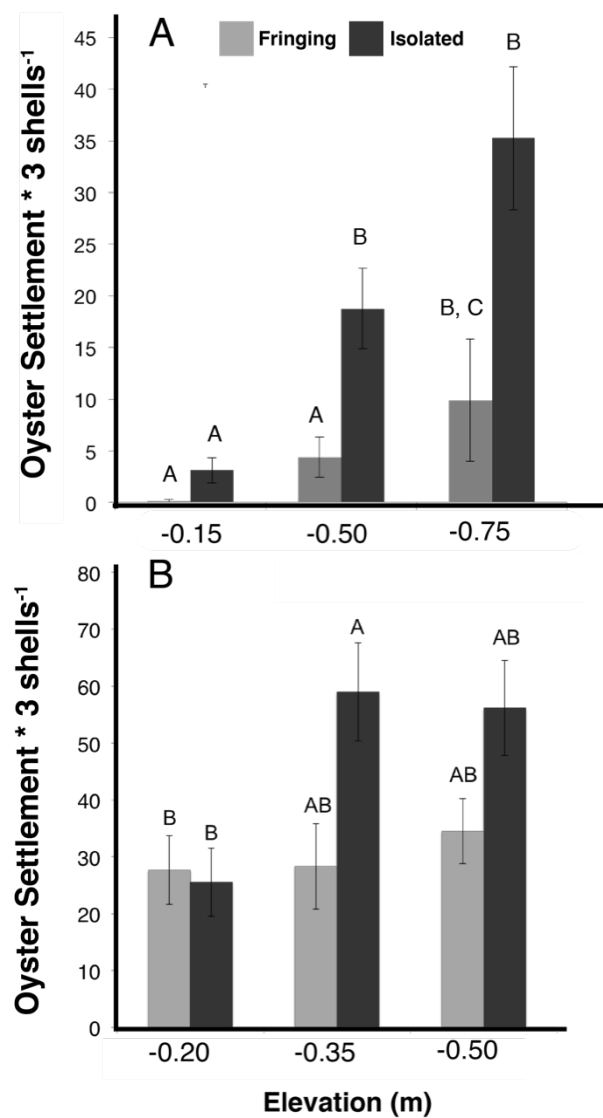


Figure 3.2. Oyster settlement on intertidal oyster reefs at two settings (fringing, isolated) across three elevations in (A) 2013 and (B) 2014. Error bars represent ± 1 standard error.

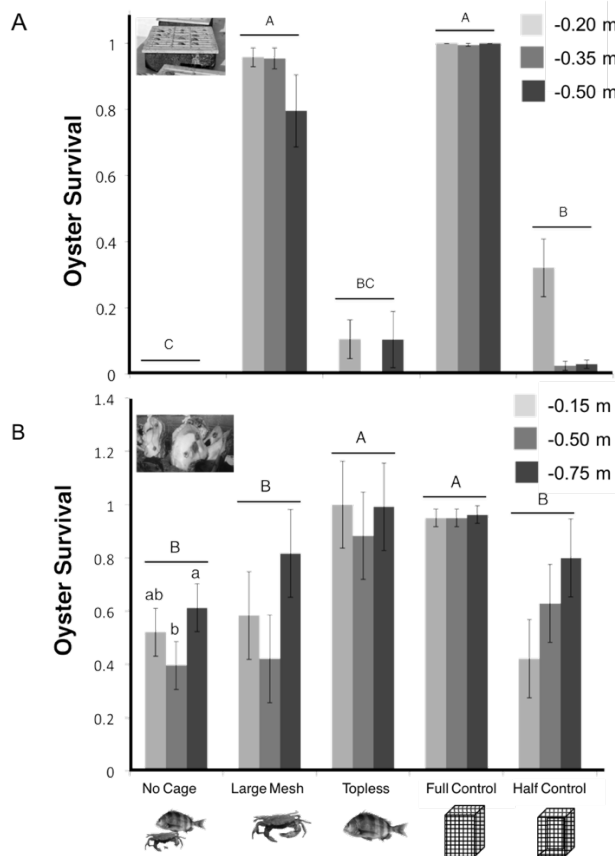


Figure 3.3. Survival of oyster spat after 4 days in (A) 2013 at low complexity and (B) 2014 high complexity for three elevations (high, mid, low) with one of five predator exclusion treatments (no-cage, large mesh cage, topless cage, small mesh full cage, cage control). “No-cage” treatment has a reef plate with no cage to quantify total predation rate, “Large Mesh” cage was a 6-cm mesh fully enclosed cage to only allow mud crab predators access to spat, and a “Topless” cage had 1-cm mesh cage without a top to allow nekton access to spat but prevent mud crabs from climbing into the cage. The “Full Control” cage fully encloses the oyster-spat plate with 1-cm mesh acted as a control for oyster attachment failure (no predation) and a “Half Control” cage with 1-cm mesh with three sides was used as a cage control to control for predator attraction and changes in flow. Error bars represent ± 1 standard error.

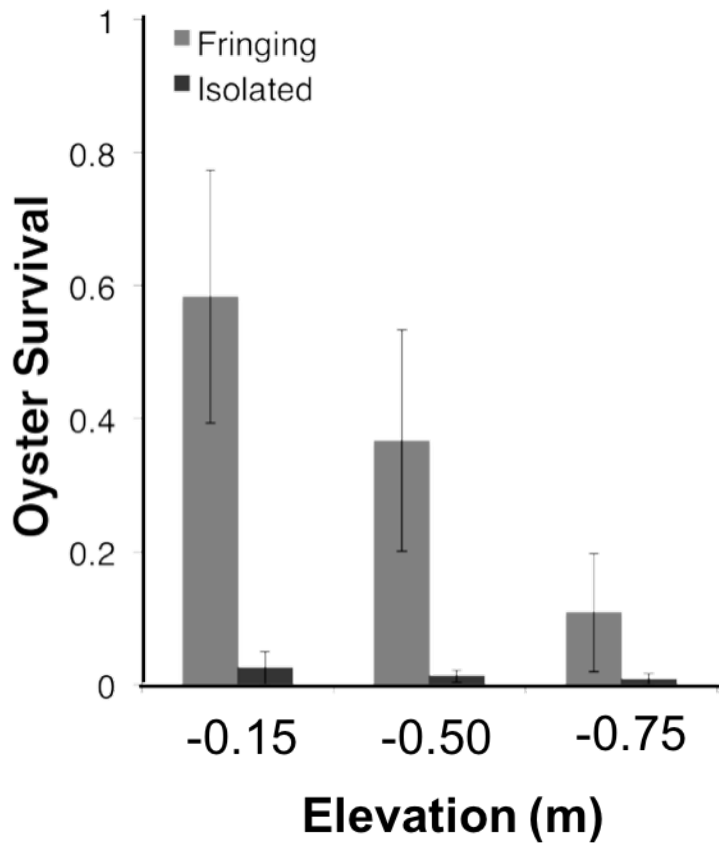


Figure 3.4. Total survival in 2013 of oyster spat low complexity predation tiles at three elevations and two landscape settings after a deployment of 6 hours. Error bars represent ± 1 standard error.

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CONCLUSIONS

Over the last century widespread disease, degraded water quality, and overfishing have dramatically reduced the abundance of oyster reefs and the ecosystem services they provide (Grabowski et al. 2012; Zu Ermgassen et al. 2012). To better restore reefs, models that predict where oyster reefs will develop successfully are being refined based on the evidence of oyster growth in specific settings without fully elucidating the contributing mechanisms (Fodrie et al. 2014; Ridge et al. 2015). Applying the ecological theory of processes that control habitat development across environmental stress gradients (Menge and Sutherland 1987), I isolated how interactions with community members affect the development of reefs. Additionally, I expanded on previous work on predation rates across tidal exposure and landscape settings (Johnson and Smee 2014; Carroll et al. 2015) by examining how oyster settlement and predation change across landscape settings. By understanding how ecological interactions control the development of biogenic habitats within the environmental mosaic, we can better restore and manage these essential ecosystems.

Interspecific competition with epibiota is a significant mechanism that causes oyster spat mortality with increasing tidal inundation (Chapter 1). Exploitative competition by encrusting epibiota, including barnacles, can dominate space in areas of high flow and overgrow newly settled oyster spat. Furthermore, interference competition with epifauna increases with decreasing aerial exposure causing reductions in growth at low aerial

exposures, presumably due to epibiota reducing both transport of food particles and local water quality. Thus, restoration practices should avoid areas where epibiota fouling is high. In euhaline waters, siting oyster restoration projects in the intertidal is one method to reduce mortality from competition. However, if the epibiotic communities are prolific, raising oyster spat to sizes larger than 15mm can significantly minimize the negative interactions with the epibiotic community.

Not all epibiota interactions on oyster reefs are negative as demonstrated by seasonal macroalgae. Surprisingly, seasonal macroalgal cover had no negative effects on oyster density or growth and had a slight positive effect on juvenile oyster abundance (Chapter 2). Thus, macroalgae facilitates oyster spat survival by protecting spat from exposure of cool winter water and air temperature. Eutrophication may increase *Ulva* spp. cover higher in the tidal range, but this work indicates that it will continue to protect intertidal oysters. It remains to be seen whether the dense *Ectocarpus* mats in the shallow subtidal have a greater smothering potential than *Ulva* for juvenile oysters, further limiting subtidal expansion in euhaline waters. Future work should also assess the mechanisms that control the seasonal development and decline of algae and how changes in climate may cause a temporal shift that could overlap and interfere with oyster settlement.

Predation was an important mechanism in reducing survival of oyster spat across the aerial exposure gradient (Chapter 2). This work indicates the role of post settlement processes like predation are dependent on landscape setting and relative structural complexity. On reefs with low structural complexity, nektonic predators including sheepshead have high predation rates on isolated reefs at all aerial exposures as compared to fringing reefs, where nekton predation increases with decreasing aerial exposure away from

the marsh edge. Within high complexity reefs, landscape setting is less important in determining predation, and resident crabs are responsible for predation that is greatest at intermediate aerial exposures. There may be a feedback mechanism of increased competition and predation in deeper areas that hinder the formation of structurally complex reef, facilitating further predation by nektonic predators.

While predation is an important factor in shaping reef development across the aerial exposure gradient, the differences observed in adult oyster density between landscape settings is likely due to magnitude of settlement. The density of newly settled oysters was greater on isolated reefs than fringing reefs and matched adult density patterns. The patterns of predation are complex and context dependent, as habitat complexity affected which predator was dominant and the importance of landscape on predation rates, but predation was also greater (at low complexity) or equal (at high complexity) on isolated reefs than those on fringing reefs. Conversely, in areas with limited settlement, predation may have a larger role in influencing oyster reef density.

Declining structural complexity due to overfishing and natural disturbances has negative implications for oyster reef recovery. Reduction in structural complexity shifts the dominant predator and increases predation rates across the aerial exposure gradient. Degradation of oyster reefs will increase predation rates of new recruits, further limiting the ability of a reef to recover after a disturbance. Thus, once a reef reaches a low complexity it is more susceptible to predation by sheephead and may prevent any new oyster recruits from surviving, especially if the reef is within an isolated landscape.

Future work should continue to examine how settlement, predation, and competition vary within different landscape settings and begin to explore if there are any interactive effects of predation and competition. Since predation actively reduces the abundance of new oyster and epifaunal recruits, it may have positive impact on the remaining oysters that may experience reduced competition. Additionally, examining how differences in recruitment of oysters across the intertidal zone will identify the role of intraspecific competition on oyster survivorship.

This dissertation investigated the role of post-settlement processes on the development of an essential estuarine foundation habitat. Interspecific competition and predation significantly reduce survival of oyster spat at low aerial exposures, however the degree these ecological interactions affect the development of reefs is context dependent. Holistically, my dissertation research provides a clearer picture of where euhaline oyster restoration will be successful. Restoring new substrate immediately before oyster settlement in the lower to mid-intertidal will minimize competition with epibiota and predation at either landscape setting, however, isolated landscape settings is recommended when the restoration goal is to maximize oyster abundance. Reducing species interactions through intertidal refuge will increase reef complexity and further increase survivorship from predation. The role of ecological interactions in controlling oyster reef growth will become increasingly important as sea level rise causes brackish areas to become more saline as estuaries migrate up river.

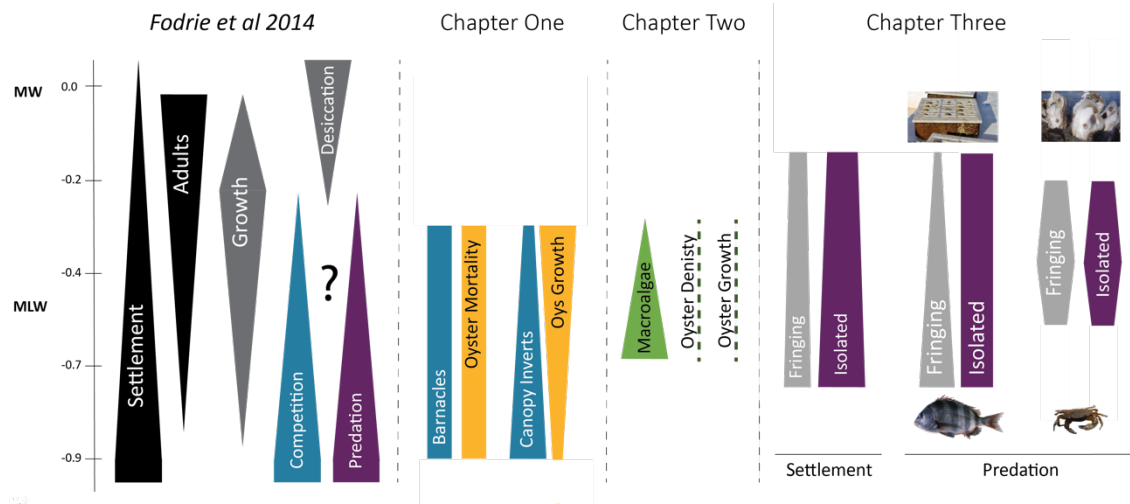


Figure 4.1. Conceptual model showing how post settlement processes including competition (Chapter 1 and 2) and predation (chapter 3) affect the growth and survival of oyster spat along the intertidal gradient to ultimately affect the development of oyster reefs in euhaline systems. Original figure adapted from Fodrie et al 2014.

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