PREDATOR-PREY RELATIONSHIPS BETWEEN *CRASSOSTREA VIRGINICA* AND SEVERAL SPECIES OF CRAB AFFECT OYSTER REEF FUNCTION

Luke Frederick Dodd

A dissertation submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Marine Sciences.

Chapel Hill
2015

Approved by:

Michael F. Piehler

Jonathan H. Grabowski

F. Joel Fodrie

Karl D. Castillo

Stephen R. Fegley

ABSTRACT

Luke Frederick Dodd: Predator-Prey Relationships between *Crassostrea virginica* and Several Species of Crab Affect Ecosystem Function
(Under the direction of Michael F. Piehler and Jonathan H. Grabowski)

Direct and indirect effects resulting from predator-prey interactions can affect ecosystem function. Understanding these relationships has been a central focus of community ecology for more than 50 years. However, human impacts on ecosystems during this period may have altered these relationships. Here I assess important impacts of ocean acidification, ocean warming, and land-use change on the predator-prey relationships between the oyster, *Crassostrea virginica*, and several species of crab. Calcifying marine taxa have been shown to respond differently to ocean acidification. Bivalves typically show more severe reductions in net calcification than the decapods that prey on them. This study found *C. virginica* and the crab, *Panopeus herbstii*, had generally reduced net calcification with decreasing calcite saturation states. However, both species maintained positive net calcification in undersaturated calcite conditions. In experiments, acidification significantly reduced the consumption rate of *C. virginica* by *P. herbstii*. Differences in net calcification did not explain consumption rates, rather behavioral change, manifest as reduced prey handling and persistence of the predator, were the likely cause. Ocean warming is driving pole-ward shifts in the ranges of numerous species, leading to the formation of novel communities. The stone crab, *Menippe mercenaria*, is one such species, but little is known about its ecology and thus its like impact on ecosystems in its new range. My data suggest that crabs between 70 and 90 mm are likely to have the largest effect on intertidal oyster

reefs due to higher consumption rates and an ability to consume all sizes of oyster. Smaller crabs are limited by the size of oyster they can consume and larger crabs demonstrated less interest in oyster as a prey item. Land-use change and accompanying habitat loss has driven increased interest in estuarine ecosystem services and functions. *C. virginica* relies on filtration to provide its outsized contribution to estuarine ecosystem function and services. Other species of bivalves reduce their filtration rates to reduce their predation exposure. However, *C. virginica* showed no such behavior in the presence of two common crab predators. This research highlights the dynamism of predator-prey relationships and their important role in current and future ecosystem function.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my co-advisors Dr. Michael Piehler and Dr. Jonathan Grabowski. Guys- Your willingness to let me wander across disciplines and to forge my own collaborations means the world to me. You have been the perfect pair to get me through to my Ph. D. Mike- Thank you for so much for putting up with all the not nutrient cycling I ended up doing. I never felt like I didn't belong and I credit that to you embracing the vagaries of co-advisement and enjoying the experience. You have always made yourself available to me, even if all I wanted to do was talk USMNT. It was your positive attitude, flexibility and flair for a summation sentence that made this thing go at all. Thank you. Jon- I will never forget the effort you put into our work: all the travel, all the phone calls, and dealing with my tendency for very infrequent status update emails. Your work was always a touchstone for my own and if I ever manage to become a competent science writer it entirely due to my time with you. Thank you.

The intellectual freedom that my advisors granted me would not have been meaningful without scientists interested in my ideas and working with me. To Dr. Justin Ries and Dr. Joel Fodrie thank you for collaborating or attempting to collaborate with me. Justin- You said yes to an idea pitched from a first year graduate student from a completely unrelated lab. I hope that I can emulate your openness and gumption in my own career. Joel- Our grant didn't get accepted but I learned more in those few weeks with you and Danielle than maybe any other point in my time at IMS. Your excellent sense of the broader picture has given me many ideas along the way.

To my committee past and present- Dr. Justin Ries, Dr. Joel Fodrie, Dr. Stephen Fegley, Dr. Karl Castillo- you have all put so much effort into your time with me. You have been shining examples of why we are called a scientific community. Thank you.

To the Piehler lab- You are very truly my science family and I expect emails for years to come on oyster ecology, project ideas, and gossip. To Suzanne Thompson, Caitlin White, Terri O'Meara, Ben Von Korff, Joe Caracappa, Paige Ferguson, Kate Walsh, Kaylyn Siporin, Walt Rogers- you have been both great companions and excellent laborers for me and my work. It was never clean and rarely easy. I will never forget you. To Ashley Smyth- It was your work and questions that began to make me see how I fit into the Piehler lab. I never knew how much you influenced me until long after you had moved on. You have picked up all of Mike's best qualities and have added your own. To Kathleen Onorevole- it was rough at first but we found our stride. You have been a great friend and I look forward to the work we have started together.

To those that have helped my science along the way- Travis Courtney, Cassandre Lazar, Isaac Westfield, Sara Coleman and to those that have kept me sane- Brett Froelich, Kar Howe, Nadine Potter- Thank you.

Finally to my family- this is a path that no one finds on their own. To those still with me and those now past. Thank you.

TABLE OF CONTENTS

LIST OF TABLES

Table

LIST OF FIGURES

Figure

1. INTRODUCTION

1.1 Predation

Predation has been a central theme of community ecology over the past half century, and more recently has been recognized for its important role in ecosystem dynamics and functioning (Hairston et al. 1960, Paine 1966, Carpenter et al. 1985, Sih et al. 1985, Werner & Peacor 2008, Schmitz et al. 2008). Specifically, the direct and indirect effects stemming from predator-prey interactions influence community structure (Sih et al. 1985), evolutionary processes (Vermeij 1982), and ecosystem functions (Schmitz et al. 2008). In fact, the threat of predation alone can change community function (Preisser et al. 2005, Laundre et al. 2010). Prey respond to the risk of predation by reducing their growth (Nelson et al. 2004), fecundity (Peckarsky et al. 1993, Creel et al. 2011), and time allocated for feeding (Smee & Weissburg 2006), as well as increased resource allocation toward defensive structures such as spines and shells (Côté 1995, Newell, Kennedy, et al. 2007). However, predator-prey relationships are not static and can be altered by environmental change (Mills et al. 2013). Anthropogenic environmental influences are increasingly affecting the natural world (Vitousek et al. 1997), including predator-prey interactions, and the effects on ecosystem functioning can be profound (Creel et al. 2011, Harley 2011).

1.2 Climate Change

Increasing anthropogenically derived carbon dioxide in the atmosphere is driving rapid and increasing change in global ocean chemistry. Ocean surface pH has fallen by 0.1 since 1800, and is projected to decrease 0.1 – 0.4 more by the end of the century (Brewer 1997, Sabine et al.

2004, Canadell et al. 2007, Hoegh-Guldberg et al. 2014). This would result in a nearly 50% reduction in the carbonate ion concentration of seawater and a corresponding decrease in its calcium carbonate saturation states. Projected shifts in calcium carbonate availability have prompted widespread concern over the fate of marine organisms that utilize biogenic calcium carbonate shells and skeletons, as well as, the far more numerous species that depend on these calcifiers for food or habitat (e.g., Gattuso et al. 1998, Langdon et al. 2000, Langdon 2005, Ries et al. 2009).

Climate change is also warming the world's waters and allowing species ranges to expand poleward (Sorte et al. 2010). Sea surface temperatures are expected to warm 1 to 4°C by the end of the century (Hoegh-Guldberg et al. 2014). This warming enables range shifts that can have the same magnitude of effect on community function as biological invasions (Sorte et al. 2010). For instance, the expansion of the sea urchin, *Centrostephanus rodgersii*, into the waters off of eastern Tasmania has resulted in reduced macroalgal cover and a minimum net loss of approximately 150 species in affected areas (Ling 2008). Additionally, documented range expansions in marine systems tend to occur at a much faster rate (19.0 km $year^{-1}$) than those in terrestrial systems (0.61 km $year^{-1}$). The speed and potential impact of marine range expansions increases the importance of close monitoring and study of species with expanding ranges.

1.3 Oyster Reefs

The eastern oyster, *Crassostrea virginica*, is widely recognized for its cultural, economic, and ecological values (Grabowski & Peterson 2007). The estimated monetary value of one hectare of unharvested oyster reef is in the tens of thousands of dollars per year, with additional nonmonetary portions associated with cultural and ecological values (Grabowski et al. 2012). Currently, oyster reef habitats are thought to be at approximately15% of historic global levels

(Beck et al. 2011, Wilberg et al. 2011, zu Ermgassen et al. 2012); with reefs in the mid-Atlantic and southeastern US similarly reduced (Rothschild et al. 1994, Frankenburg 1995, zu Ermgassen et al. 2013). Over the past 150 years, widespread disease, overfishing, and water quality degradation have significantly reduced the species' abundance and delivery of ecosystem services (Beck et al. 2011, zu Ermgassen et al. 2012). Additionally, large scale environmental change including increasing ocean acidification (Miller et al. 2009), bioinvasions (Burreson et al. 2000, Hollebone & Hay 2007), and range expansions (Sorte et al. 2010) are predicted to cause a wide range of new stressors on the coastal systems home to *C. virginica*. Sustaining and rehabilitating coastal habitats like *C. virginica* requires science-based management, especially give the diverse and substantial environmental stress in these areas.

1.4 Crab-Oyster Predator-Prey Interactions

Callinectes sapidus (blue crab), *Panopeus herbstii* (mud crab), and *Menippe mercenaria* (stone crab) are all important crustacean predators that prey upon various life stages of bivalves, including *C. virginica*, directly and indirectly affecting oyster population dynamics (Grabowski & Kimbro 2005, Hughes & Grabowski 2006, O'Connor et al. 2008, Fodrie et al. 2008, Grabowski et al. 2008).

Callinectes sapidus is an omnivorous swimming crab, exceeding 200 mm carapace width, which utilizes oyster reefs for both food and shelter (Williams 1984). It is a prominent predator on small to moderate sized oysters (Eggleston 1990), with strong influences on oyster population structure (O'Connor et al. 2008). *C. sapidus* is an economically and culturally important species throughout the coastal eastern and gulf U.S. states (Zohar et al. 2008). It is also an important predator of *P. herbstii*, exhibiting strong behavioral controls on mud crab foraging (Grabowski et al. 2008).

Panopeus herbstii is an omnivorous crab, ranging in size up to 60 mm carapace width (Williams 1984) and can reach densities in excess of 300 individuals m^{-2} on intertidal oyster reefs (unpublished data). It resides within oyster reefs, and is a prominent mesopredator that consumes juvenile oysters (McDermott & Flower 1952, Whetstone & Eversole 1981, Grabowski 2004). It has also been estimated as the most significant crustacean predator on *C. virginica* in North Carolina waters (Rindone & Eggleston 2011).

Menippe mercenaria is a primarily carnivorous, large, and heavy crab with a very strong crusher claw for breaking into shelled prey, such as bivalves (Gerhart & Bert 2008). Adult males can grow in excess of 130 mm carapace width (Rindone & Eggleston 2011). Small to moderate sizes of *M. mercenaria* dig burrows in soft and hard (i.e., oyster reef) substrates as shelter (Beck 1995). Stone crabs consume oysters (Menzel & Hopkins 1956, Menzel & Nichy 1958) and have been expanding northward in recent decades (Rindone & Eggleston 2011) where they will potentially impact oysters and other species dependent upon reef habitat.

1.5 Oyster reefs: Ocean Acidification

Ocean acidification studies investigating biogenic calcification have shown varied responses across a wide range of species, both in terms of magnitude and sign of the observed change (Gattuso et al. 1998, Langdon et al. 2000, Langdon 2005, Ries et al. 2009). This variability raises questions about community level effects if the balance of predator-prey relationships shifts among species with varying calcifying methods. For instance, calcification rates of *C. virginica* decrease linearly with decreasing pH (Gazeau *et al.* 2007; Ries *et al.* 2009; Beniash *et al.* 2010; Waldbusser *et al.* 2010), along with reduced shell hardness and fracture resistance (Beniash et al. 2010). In contrast, crustaceans exhibit the potential for neutral to increasing calcification rates (McDonald et al. 2009, Ries et al. 2009, Findlay et al. 2009, Long

et al. 2013). This pattern may include important estuarine predators, such as, *C. sapidus*, *P. herbstii*, and *M. mercenaria*. However, acidification can also cause behavioral changes through a number of potential pathways, including sensory cue deformation (Brown et al. 2002), increased metabolic costs (Briffa et al. 2012), and over stimulation of GABA neuroreceptors due to changes intracellular concentrations of Cl^- and/or HCO_3^- (Nilsson et al. 2012). Collectively, these effects could have wide ranging impacts on coastal ecosystems worldwide.

1.6 Oyster Reefs: Climate-induced range expansion

Over the past half century, *Menippe mercenaria* (stone crab) has extended beyond its previously recognized northern most range limit, Cape Lookout, North Carolina (Williams 1984), and is now found throughout Pamlico Sound, NC (Rindone & Eggleston 2011). Anecdotal evidence indicates that densities have also increased in North Carolina waters south of Cape Lookout (Rindone & Eggleston 2011). Stone crabs often live and feed on intertidal oyster reefs. Despite their presumed growing influence on this ecologically and economically important habitat, very little is known about stone crab abundance and ecology in intertidal reef ecosystems outside of Florida. Preliminary results of surveys conducted in 2012-13 near Morehead City, NC revealed stone crab densities up to 1 m^{-2}, an order of magnitude more than average densities reported across the subtidal Pamlico Sound (Rindone & Eggleston 2011) and on 1-year old constructed reefs in Back Sound, NC (Fodrie et al. 2014), but similar to previously reported maximum densities in Florida (Menzel & Hopkins 1956, Sinclair 1977).

Stone crabs consume oysters, and also are capable of altering the structure of their habitat by building burrows on the reef (Brown & Haight 1992). Quantifying the influence of this species requires careful assessment of their impacts on reefs to understand how their expansion has influenced oyster reef community structure.

1.7 Oyster Reefs: Predatory Influences on Ecosystem Functioning

Indirect effects that alter filtration in bivalves could have ecosystem-wide repercussions. Filtration underpins all potential bivalve ecosystem functions. Not only could changes in filtration potentially limit their growth, thereby influencing their value as a food source and ability to provide habitat, but filtration is also the direct mechanism for the delivery of numerous water quality services, such as nutrient processing (Newell et al. 2005), habitat restructuring (Hauxwell et al. 2003, Newell & Koch 2004), and the removal of phytoplankton from the water column (Grizzle et al. 2008, zu Ermgassen et al. 2013). Oyster reefs are a prominent provider of these services, reaching densities that can dramatically affect their environments (Newell et al. 2007). A significant response to predation risk could have far-reaching consequences for oyster-dominated environments.

1.8 Oyster Reefs Moving Forward

Further degradation of oyster reef habitat influences oyster reef communities directly and also impairs their delivery of key ecosystem functions and services, such as provision of nursery habitat (Coen et al. 1999, Peterson et al. 2003, Luckenbach et al. 2005, Grabowski et al. 2005, Tolley & Volety 2005), removal of anthropogenic nitrogen (Newell et al. 2002, Piehler & Smyth 2011), and shoreline stabilization (Meyer et al. 1997, Piazza et al. 2005, Scyphers et al. 2011). As an important mediating factor, the predator-prey relationship of crabs and oysters has a significant effect on the delivery of ecosystem functions and services. As such, this relationship warrants investigation in the context of current environmental factors including ocean acidification, climate-induced range expansions, and predatory influences on delivery of ecosystem functions. This information is critical for accurately assessing the stability and productivity of *C. virginica* stocks, and the quality of the reef habitat that they create. This

research is directly applicable to the management of *C. virginica* throughout its range, and also more broadly to scientists attempting to understand the consequences of future climate-driven changes on foundation species across the planet.

1.9 Study Objectives

1. Effect of Acidification on Calcification
    a. Objective: Quantify the effects of projected future levels of acidification on *Crassostrea virginica* & *Panopeus herbstii* calcification rates.
    b. Hypothesis: Acidification will negatively affect *Crassostrea virginica* calcification rates, whereas it will have no or a slight positive effect on *Panopeus herbstii* calcification rates.
2. Predator-Prey Relationships in Acidified Waters
    a. Objective: Determine how acidification influences the outcome of *Panopeus herbstii*-*Crassostrea virginica* predator-prey interactions
    b. Hypothesis: Acidification will negatively impact *Crassostrea virginica* calcification, thereby making them more susceptible to predation by *Panopeus herbstii.*
3. Prey Size Selection by *Menippe mercenaria*
    a. Objective: Determine prey selection of *Menippe mercenaria* on *Crassostrea virginica* across multiple crab size and densities.
    b. Hypothesis: Mechanical limitations will make smaller *Menippe mercenaria* choose smaller oyster prey. However, at some crab size mechanical limitations will cease and *Menippe mercenaria* will begin preying proportionally across oyster sizes.

4. The Effects of Predation Risk on *Crassostrea virginica* Filtration Rates

    a. Objective: Quantify the effect of predation risk on microalgal drawdown by *Crassostrea virginica.*

    b. Hypothesis: Predation risk will reduce microalgal drawdown, and the magnitude of this effect will be highly dependent on predator identity.

2. CALCIFICATION OF THE OYSTER CRASSOSTREA VIRGINICA AND THE CRAB PANOPEUS HERBSTII IN ACIDIFIED SEAWATERS

2.1 Abstract

Anthropogenic carbon emissions are driving global-scale ocean acidification, thus reducing the ocean's degree of saturation with respect to calcium carbonate ($CaCO_3$). This has been shown to alter the net calcification rates of many marine invertebrates, however, the magnitude, pattern, and even direction of the change differ widely across taxa. Juvenile *Crassostrea virginica* (eastern oyster) and adult *Panopeus herbstii* (Atlantic mud crab) were reared for 71 days at three pCO_2 levels (499, 785, 9274). Positive growth was observed in all treatments for both organisms, including the highest acidified treatment which was undersaturated with respect to calcite (0.8). The highest acidification significantly reduced net calcification in oysters compared to the control, but no other treatment showed a significant effect in either tested species. Stress-strain testing did not reveal any difference in oyster shell strength across treatments. Calcification in *C. virginica* was more affected by acidification than *P. herbstii*, but *C. virginica* maintained positive net calcification in all treatments despite the fact that some conditions were well below $CaCO_3$ saturation.

2.2 Introduction

Rising carbon dioxide concentrations in the atmosphere are driving rapid and increasing change in global ocean chemistry (Hoegh-Guldberg et al. 2014). Approximately one third of all anthropogenic CO_2 has been absorbed by the world's oceans, forcing ocean surface pH down by 0.1 since 1800 and will further reduce pH an estimated 0.1 – 0.4 more by end of century (Brewer

1997, Sabine et al. 2004, Canadell et al. 2007, Hoegh-Guldberg et al. 2014). If this prediction is realized, it will result in a nearly 50% reduction in the carbonate ion concentration and a corresponding decrease in calcium carbonate saturation states by 2100 (Brewer 1997). The projected shift in calcium carbonate availability has prompted widespread concern over the fate of marine organisms that utilize biogenic calcium carbonate shells and skeletons, as well as the far more numerous species that count on these calcifiers for food or habitat (Guinotte & Fabry 2008).

Studies investigating biogenic calcification under acidification have shown varied responses across a wide range of species, both in terms of magnitude and sign of the observed change (Gattuso et al. 1998, Langdon et al. 2000, Langdon 2005, Ries et al. 2009). These responses are heavily influenced by the form of calcium carbonate utilized (amorphous, aragonite, calcite) as well as the organism's capacity to modify or insulate the calcium carbonate depositional environment from ambient seawater (Ries et al. 2009). This variability in response to acidification raises questions about community level effects if the competitive balance shifts among species with varying calcifying methods.

Panopeus herbstii (Atlantic mud crab) and *Crassostrea virginica* (eastern oyster) share a prominent predator-prey relationship on oyster reefs of the eastern United States. *P. herbstii* is a major predator on juvenile oyster (Menzel & Nichy 1958, Meyer 1994) and may be the most significant crustacean oyster predator in the system (Rindone & Eggleston 2011). Oysters with slower growth rates or weaker shells due to the effects of ocean acidification should be vulnerable to predation by *P. herbstii* for longer periods, which could result in increased juvenile oyster mortality. Because eastern oysters are an important foundation species, prolonged

vulnerability to the ubiquitous *P. herbstii* could have significant effects throughout estuarine ecosystems.

Non-larval *C. virginica* primarily form shells with the relatively stable, low-magnesium calcite form of calcium carbonate, though portions are composed of a more porous higher magnesium form, thought to increase flexibility or maintain inner shell shape at a reduced metabolic cost (Korringa 1951, Taylor & Layman 1972, Carriker et al. 1991, MacDonald et al. 2010). *C. virginica* shells have a high level of contact with seawater, making them more vulnerable to dissolution. They also maintain their pallial fluid, their putative calcifying fluid, at lower pHs than ambient conditions leading to even further reduced availability of $CaCO_3$ at the calcification site (Crenshaw & Neff 1969, Crenshaw 1972). Despite this mix of risk factors, the shells of non-larval oysters are resistant to even extreme reductions in calcite saturation state when secondary stressors (salinity, temperature) are absent. Under single stressor conditions, net shell dissolution has yet to be observed with calcite saturation states (Ω_C) as low as 1.07-1.13 (Ries et al. 2009, Waldbusser et al. 2011) and positive net calcification has been observed at Ω_C as low as 0.7 (Waldbusser et al. 2011).

Less is known about *P. herbstii* calcification. Decapod crustacea typically produce their carapaces using high-magnesium calcite (5-12% magnesium content), and produce a hydrophobic epicuticle that reduces contact between seawater and mineralized portions of the carapace (Chave 1954, Plotnick et al. 1988, Ries et al. 2009). Species in the order decapoda are also thought to manipulate calcification site pH in favor of higher saturation states (Cameron 1989). These traits suggest a potential resistance to calcification complications brought on by acidification. This concept appears to be borne out by data for a number of decapods including the relatively closely related blue crab, *Callinectes sapidus*. *C. sapidus* showed a linear increase

in calcification rate with decreasing pH for values as low as 7.31, with the other decapods studied similarly experiencing positive calcification responses to CO_2-induced acidification (Ries et al. 2009).

Growth rates of settled oysters have been shown to be resilient to acidification, particularly in high salinity environments, despite exhibiting a number of risk factors for impaired calcification (Ries et al. 2009, Waldbusser et al. 2011). Decapods exhibit an even greater resilience, potentially increasing their calcification as waters acidify (Ries et al. 2009). To enhance our understanding of the effects of acidification on eastern oysters and their predators, adult *P. herbstii* and juvenile *C. virginica* were raised in a range of Ω_C (0.8, 5.1, 6.7) conditions manipulated through CO_2 bubbling. Included in our design was an undersaturated treatment in which inorganic calcite should dissolve. Oysters were grown both in tanks with, and without crabs present to assess the potential for induced defense through predator cues stimulating increased calcification (Newell et al. 2007).

2.3 Methods

Juvenile wild-strain *C. virginica* (18.7 ± 3.8 mm shell height) were obtained from Jonny Oyster Seed of St. Leonard, Maryland. Spat were separated from cultch shell using a diamond-embedded lapidary saw and as much of the excess shell was removed as possible. Spat were then individually attached to plastic microscope slides with cyanoacrylate epoxy. Thirty oysters were suspended 40 cm from the bottom of each tank on 1.7 mm diameter plastic cord. Adult *P. herbstii* (23-28 mm carapace width) were collected from Middle Marsh near Beaufort, North Carolina in early May 2011. Crabs were maintained in half of the tanks containing oysters and chambered during the growth period to control individual feeding rates and inhibit cannibalism while allowing for water and cue circulation. Oysters were raised in an orthogonal 3 x 2 design

with 3 acidification levels and 2 crab cue presence levels (present or absent), while crabs were raised in a 3 x 1 design with 3 acidification levels. All treatment combinations were replicated three-fold.

Crabs and oysters were raised in isolated 34 l tanks for 71 days in seawater with calculated $pCO_{2(gas-e)}$ values (±SD) of 499 (±114), 785 (±154), and 9274 (±2243) µatm (Table 2.1), corresponding to near-modern pCO_2, the predicted end-century pCO_2, and a level that exceeds the highest pCO_2 predicted to be experienced by these organisms. Although the high-pCO_2 treatment is higher than is predicted to occur in the atmosphere and open-ocean for the foreseeable future, comparable conditions already can occur in both healthy and degraded estuaries as high DIC from detrital organic matter, pollution, and stratification combine to elevate local pCO_2 in estuarine waters inhabited by both species (Cai & Wang 1998, Ringwood & Keppler 2002). Furthermore, our high-pCO_2 treatment was not formulated solely to target pCO_2 levels predicted for the foreseeable future, but rather to target pH levels and calcite saturation states that are predicted to occur over that timeframe. Because of the temporal and spatial variation in salinity (5 < salinity < 35 ‰) that occurs within estuarine waters that are inhabited by the investigated species, the pH (ca. 7.0) and calcite saturation states (ca. 0.8) that were maintained in the high-pCO_2 treatment are realistic for low to moderate salinity and correspondingly low-alkalinity (TA < 1000 µmol) estuarine waters equilibrated with an atmospheric pCO_2 of ca. 2400 µatm (predicted for year 2600, assuming a conservative annual increase of 3.5 µatm yr^{-1}). Furthermore, recent studies have revealed that estuaries such as the Chesapeake Bay (Waldbusser et al. 2011), Elkhorn Slough, California (Hofmann et al. 2011), and Charleston Harbor Estuary, South Carolina (Ringwood & Keppler 2002) currently experience significant annual (7.6 – 8.2, Chesapeake Bay) and tidal (7.4 – 8.1, Elkhorn Slough;

6.9 – 7.6, Charleston Harbor Estuary) pH variation due to fluctuations in salinity (and resulting total alkalinity) and local enrichments in DIC (resulting from seasonal re-mineralization of benthic organic matter). Lastly, experimental seawaters were formulated to encompass a range of carbonate system parameters (pH < 7 and undersaturation with respect to calcite) that were similar to those that were employed in recent studies on related subjects (e.g., Bibby et al. 2007, Dissanayake et al. 2010, de la Haye et al. 2011, 2012).

Partial pressures of CO_2 were established by mixing pure CO_2 with compressed air using *Aalborg* digital mass flow controllers. Experimental seawater was bubbled with microporous ceramic airstones into triplicate glass tanks. The pCO_2 of the mixed gases was measured with a *Qubit S151* infrared pCO_2 analyzer calibrated with certified air-CO_2 gas standards (precision = ± 2.0%; accuracy = ± 1.8%). Mean salinity (SD) was formulated at 31.72 (0.76) with *Instant Ocean Sea Salt* and deionized water and mean temperature (SD) was maintained at 25.97 °C (1.15) with 50 watt electric heaters. Although the trace elemental composition of *Instant Ocean Sea Salt* differs subtly from that of natural seawater, its major and minor elemental composition, as well as its carbonate chemistry, was the most similar to that of natural seawater when compared with eight other commercial sea salt mixes (Atkinson & Bingman 1998). Every two days, oysters were fed 14 ml per tank of a commercial algal blend containing *Nannochloropsis oculata*, *Phaeodactylum tricornutum*, and *Chlorella sp*. with cell sizes of 2–20 µm (DT's Live Marine Phytoplankton, Sycamore, IL, USA). Each crab was provided 50 mg ±7 mg dry weight of *Artemia* sp. (brine shrimp) on the same two-day feeding schedule. Temperature, pH, and salinity were measured every two days, while pCO_2 of mixed gases were measured weekly (sensu Ries et al. 2009) (Table 2.1; see Supplementary Data for all measured and calculated seawater parameters).

Seawater within each tank was continuously filtered (757 L h^{-1}) with a hanging power filter that contained a nylon-floss activated-carbon filter. Circulation and turbulence of seawater within each tank was enhanced with a 400 L h^{-1} powerhead. Each tank was covered with a transparent 3-mm plexiglass sheet and both the tank and the attached filtration system were wrapped with cellophane to promote equilibration between the gas mixtures and the experimental seawaters and to minimize evaporative water loss. Tanks were illuminated for 12 hours per day with standard white fluorescent lights (32 Watts, T8 6500K) to simulate oysters' and crabs' natural light cycle.

Temperature within experimental tanks was measured every other day with a NIST-calibrated partial-immersion organic-filled glass thermometer (precision ± 0.3%, accuracy ± 0.4%). Salinity was measured every other day with a *YSI* 3200 conductivity meter with a *YSI* 3440 cell (K=10) that was calibrated with seawater standards of known salinity provided by the laboratory of Prof. A. Dickson of Scripps Institute of Oceanography. Seawater pH was measured every other day with a *Thermo Scientific Orion 2 Star* benchtop pH meter with an *Orion* 9156BNWP pH probe, calibrated with 7.00 and 10.01 *Orion* NBS buffers traceable to NIST standard reference material (for slope of the calibration curve) and with seawater standards of known pH also provided by Prof. Dickson's laboratory (for y-intercept of the calibration curve). Seawater dissolved inorganic carbon (DIC) was measured via coulometry (*UIC 5400*) and total alkalinity (TA) was measured via closed-cell potentiometric Gran titration calibrated with certified Dickson TA/DIC standards. Measurement of DIC and TA of the certified reference materials (CRMs) were consistently within 0.3% of certified values. Differences between the measured and certified TA and DIC values of the CRMs were used to correct measurements of experimental seawater solutions.

Seawater pCO_2, pH, carbonate ion concentration ($[CO_3^{2-}]$), bicarbonate ion concentration ($[HCO_3^-]$), aqueous CO_2, and calcite saturation state (Ω_C) were calculated from measured DIC, TA, temperature and salinity with the program CO2SYS (Lewis & Wallace 1998), using Roy et al. (1993) values for K_1 and K_2 carbonic acid constant, the Mucci (1983) value for stoichiometric aragonite solubility product, and an atmospheric pressure of 1.015 atm.

Calcification rates of oysters and crabs were estimated using an empirically calibrated buoyant weight technique (Ries et al. 2009). Specimens were weighed at the beginning of the experiment and at 71 days. Each specimen was suspended by aluminum wire from a *Cole-Parmer* bottom-loading scale (precision ± 0.001; accuracy ± 0.002) at a depth of 10 cm in a tank filled with experimental seawater maintained at a temperature of 25 ºC and salinity of 33. A plastic-coated zinc mass standard was intermittently weighed to ensure consistency of the buoyant weight method throughout the duration of the experiment.

Buoyant weight-dry $CaCO_3$ weight relationships for oysters and crabs were empirically derived by plotting final dry $CaCO_3$ weights (after removal of organic matter) against final buoyant weights of 49 oysters and 18 crabs randomly selected from the three pCO_2 (control – 499 µatm, moderate – 785 µatm, high – 9273 µatm) treatments used in experiments. Oyster dry $CaCO_3$ weight was the dry weight (70 °C, 24 hours) of the shell after mechanical removal of soft tissue. Crab dry $CaCO_3$ weight was the dry weight of the crab carapace after organic matter was removed via combustion in a muffle furnace at 500 °C for 6 hours. Buoyant weight and dry $CaCO_3$ weights for individuals from all treatments were highly correlated (linear regression of: oyster: $R^2 = 0.9976$, $P < 0.001$; crab: $R^2 = 0.9828$, $P < 0.001$) and similar amongst treatments, indicating that densities of crab and oyster shells did not vary appreciably amongst treatments

(Ries et al. 2009). Consequently, a single linear equation for each species was used to convert buoyant weight to dry weight for purposes of estimating net calcification rates:

Oyster: Dry weight (mg) = 1.5996 * Buoyant weight (mg) - 0.5013;

Crab: Dry weight (mg) = 1.3411 * Buoyant weight (mg) - 0.0107.

To test shell strength, individual oyster shell valves were floated on a cyanoacrylate matrix and allowed to dry, providing uniform support for the shell and reducing the impact of shell morphology on testing. Shell strength testing was conducted using an Instron Lloyd LRX and a diamond coated bit. Testing parameters were set to preload to 0.5 N at a rate of 0.5 N min^{-1} and test to 85 N at a rate of 5 N min^{-1}. Stress-strain profiles were then analyzed across a number of metrics. Only oysters from treatments without crabs were tested.

2.4 Results

C. virginica displayed net positive calcification rates across all treatments (control: 11.056% ±0.996; moderate: 10.955% ±0.839; high: 9.028% ±0.712). The pCO_2 treatment significantly affected calcification rates but not crab presence or the interaction term (Table 2.2). Post hoc Ryan's Q tests showed significantly less calcification in the high *p*CO2 treatment as compared to both the control and moderate pCO_2 levels. No difference was found between control and moderate treatment levels. *P. herbstii* calcification was not affected by pCO_2 treatment (Table 2.2).

ANOVAs of *C. virginica* stress-strain profiles showed no differences across a number of metrics including initial slope (p = 0.981), total slope (p = 0.662), force (N) at shell break (p = 0.475), penetration depth at 75N (p = 0.361).

2.5 Discussion

My results show no change in *P. herbstii* calcification across all tested carbonate conditions. *C. virginica* calcification did not change in the moderate and control treatments. Net rates of *C. virginica* calcification were reduced in the very low saturation treatment but still maintained positive net calcification over the course of the study. Previous studies on oyster calcification have shown no change in calcification rates (Dickinson et al. 2012) or decreasing rates across a wide range of life stages (Gazeau et al. 2007, Miller et al. 2009, Ries et al. 2009, Beniash et al. 2010).

Oyster larvae utilize aragonite in shell development and as a result, are considered more vulnerable to ocean acidification. D-stage oyster larva of *C. virginica* reared by Miller et al. (2009) in seawater ranging from 1.2 to 0.6 Ω_A for twenty eight days showed linearly decreasing shell mass with decreasing Ω_A. Despite undersaturated aragonite conditions, oysters still displayed positive net growth. Under similar conditions larvae of another oyster, *Crassostrea ariakensis* showed no effect of Ω_A (1.3 - 0.6) on shell mass and displayed positive net calcification across all treatments (Miller et al. 2009).

Post-metamorphosis oysters transition to utilizing predominantly calcite for calcification. Three week post-metamorphosis oyster spat subjected to Ω_C of 1.4 and 8.4 showed reduced shell growth as well as reduced shell hardness for the latter treatment (Beniash et al. 2010). Juvenile *Crassostrea gigas* show a similar response to decreasing Ω_C, with short term exposures to Ω_C 5.7 – 2.0 resulting in linear decreases in net calcification rate (Gazeau et al. 2007). Extrapolation of this trend predicts positive net calcifications in undersaturated conditions similar to the findings of this study.

Adult *C. virginica* display the same response to decreasing Ω_C. Adults exposed to Ω_C 1.1 – 4.0 for sixty days showed strong linear decrease in net calcification, and extrapolation of the trend also predicts positive net calcification when waters are undersaturated (Ries et al. 2009). These studies either demonstrate capacity to calcify in undersaturated conditions or support a linearly decreasing relationship between $\Omega_{C/A}$ and calcification that, if overly extrapolated, mimic the same conclusions. The positive net calcification observed in undersaturated conditions for *C. virginica* is an important indicator of the resiliency of the species to acidification.

In contrast, Dickenson et al. (2012) found no significant difference in calcification rates across a wide range of Ω_C (1.6 – 9.1) for *C. virginica*. They suggested low net growth compared to variability as a cause of their finding of insignificance. It is possible that the lack of significant difference in calcification rates between moderate and control Ω_C in this study could also be attributed in part to this effect. This is particularity true for the calcification of *P. herbstii* where episodic growth patterns and potentially constrictive enclosures led to very high growth variability across treatments. Furthermore, previous research on crab calcification in acidified conditions has produced mixed outcomes (Ries et al. 2009, Long et al. 2013), possibly resulting from contrasting capacity for internal pH regulation across species and exposure times (Spicer et al. 2006, Pane & Barry 2007, Small et al. 2010).

Shell strength tests showed no differences across any of the acidification treatments. This result is in contrast to the findings of Beniash et al. (2010) who found higher Vickers microhardness values and fracture toughness for *C. virginica* reared in ambient conditions. Growth differences between acidification treatments were much larger in their experiment with ambient final shell weights nearly double that of the acidified treatment. Additionally, my

methods did not test shell strength properties at as fine a scale as that of Beniash et al. and as such may have been influenced by shell layers laid down under ambient conditions.

Salinity is an important factor that affects the health of oyster populations in estuarine waters. Salinity, as a proxy for alkalinity, has a strong influence on oyster calcification and is an indicator of the pH buffering capacity of a water body. Lower salinity conditions (15-16) have been shown to slow calcification rates and increase the metabolic cost of calcification (Waldbusser et al. 2011, Dickinson et al. 2012). This effect is compounded by the less favorable and less stable carbonate conditions inherent in estuarine environments. Counter to this trend of supportive calcification conditions in high salinity waters is the trend of increased oyster disease and predation in the same high salinity locations. Prevalence of oyster diseases increase with salinities of ~15 for MSX and ~10 for dermo (Haskin & Ford 1982, Paynter & Burreson 1991). Oyster predator diversity and predation mortalities show similar increases above salinity ~20 (Aronhime 2010). These patterns mitigate the value of high salinity oyster beds as a refuge habitat from acidification

C. virginica has a significant resilience to ocean acidification at a biological level. The magnitude of the ecological effect acidification threatens is unclear. The nonlethal effects of acidification, slowed growth and increased metabolic expense, could make oyster populations vulnerable to other factors such as disease, predation or other environmental stressors. Oysters may also lose suitable habitat as acidification makes low salinity waters less habitable and forces oysters toward more saline waters and higher threats from disease and predation. *C. virginica* appears more resilient to acidification than previously thought, as net calcification has now repeatedly been shown to remain comparable to ambient conditions even in waters undersaturated with respect to calcite (Waldbusser et al. 2011). However, acidification may still

have the effect of further weakening the species to the already present threats of predation, disease, pollution, and overharvesting.

2.6 Acknowledgements

I am grateful to Karl Castillo, Travis Courtney, and Cassandre Lazar for assistance with experimental implementation and to two anonymous reviewers for their valuable comments. Funding for this experiment was provided by National Science Foundation grant nos. OCE-1203859 (JHG), OCE-0961929 (MFP), and OCE-1357665 (JBR).

		Control	Control w/crab	Moderate	Moderate w/crab	High	High w/crab
CALCULATED PARAMETERS							
$pCO_{2(gas-e)}$	(ppm-v)	478	519	734	835	9567	8980
	SD	116	110	136	155	2261	2220
	Range	300 - 897	313 - 778	544 - 1023	619 - 1110	5913 - 14599	5164 - 13561
	n	33	33	33	33	33	33
pH		8.10	8.02	7.95	7.86	6.98	6.97
	SD	0.11	0.10	0.09	0.09	0.09	0.11
	Range	7.82 - 8.28	7.81 - 8.22	7.79 - 8.09	7.61 - 8.00	6.80 - 7.15	6.76 - 7.19
	n	33	33	33	33	33	33
$[CO_3^{2-}]$	(µM)	302	238	234	180	34	31
	SD	81	68	52	41	7	8
	Range	113 - 428	104 - 407	132 - 316	73 - 244	23 - 48	17 - 53
	n	33	33	33	33	33	33
$[HCO_3^-]$	(µM)	2168	1983	2401	2236	3258	3016
	SD	218	231	157	237	114	170
	Range	1585 - 2568	1466 - 2452	2024 - 2572	1679 - 2796	2866 - 3434	2747 - 3366
	n	33	33	33	33	33	33
$[CO_2]_{(SW)}$	(µM)	13	14	21	23	268	256
	SD	3	3	4	4	60	62
	Range	8 - 25	9 - 21	16 - 29	17 - 32	166 - 396	145 - 378
	n	33	33	33	33	33	33
Ω_C		7.3	5.7	5.6	4.3	0.7	0.6
	SD	2.0	1.7	1.3	1.0	0.2	0.2
	Range	2.6 - 10.4	2.4 - 9.9	31. - 7.6	1.7 - 5.8	0.5 - 1.1	0.3 - 1.1
	n	33	33	33	33	33	33
MEASURED PARAMETERS							
Salinity	(psu)	31.78	31.81	31.74	31.64	31.77	31.81
	SD	0.45	0.39	0.42	1.12	0.40	0.45
	Range	30.50 - 33.00	30.50 - 32.60	30.50 - 32.50	21.70 - 33.20	30.70 - 32.80	30.70 - 33.30
	n	99	99	99	99	99	99
Temp	(^{0}C)	25.7	26.4	25.4	25.8	26.2	25.4
	SD	0.8	1.2	0.7	1.0	1.2	0.6
	Range	24.2 - 28.8	24.4 - 30.4	24.4 - 28.3	23.5 - 29.8	23.2 - 30.5	24.4 - 28.4
	n	99	99	99	99	99	99
pH		8.15	8.01	7.97	7.87	6.96	6.91
	SD	0.07	0.11	0.06	0.08	0.11	0.07
	Range	8.09 - 8.54	7.92 - 8.35	7.96 - 8.25	7.79 - 8.22	6.84 - 7.98	6.90 - 7.22
	n	99	99	99	99	99	99
Alkalinity	(µM)	2865	2542	2940	2657	3335	3086
	SD	357	346	247	306	113	176
	Range	1865 - 3195	1731 - 3248	2342 - 3199	1861 - 3333	2960 - 3517	2801 - 3450
	n	33	33	33	33	33	33
DIC	(µM)	2484	2236	2656	2441	3560	3302
	SD	276	280	194	267	146	178
	Range	1716 - 2814	1590 - 2802	2185 - 2852	1784 - 3060	3073 - 3809	2963 - 3653
	n	33	33	33	33	33	33

Table 2.1. Calculated parameters ($pCO_{2(gas-e)}$, pH, $[CO_2^{2-}]$, $[HCO_3^-]$, $[CO_2]$, and Ω_C) and measured seawater parameters (salinity, temperature, pH, alkalinity, and dissolved inorganic carbon) for each treatment combination.

Source of variation	df	SS	MS	F	p
(a) **Oyster calcification**					
pCO_2	2	14.802	7.401	4.683	0.031*
Crab presence	1	1.776	1.776	1.124	0.310
pCO_2 x Crab presence	2	1.227	0.614	.388	0.687
Residual	12	18.964			
Total	17	36.941			
(b) **Crab calcification**					
pCO_2	2	214.224	107.112	3.337	0.106
Residual	6	192.620	32.103		
Total	8	406.843			

Table 2.2. (a) ANOVA of the effects on pCO_2 and crab presence on the percent change in calcification for oysters. (b) ANOVA of the effect of pCO_2 on the percent change in calcification for crabs. Significant p-values are denoted with an asterisk.

3. OCEAN ACIDIFICATION IMPAIRS CRAB FORAGING BEHAVIOR

3.1 Abstract

Anthropogenic elevation of atmospheric CO_2 is driving global-scale ocean acidification, which consequently influences calcification rates of many marine invertebrates and potentially alters their susceptibility to predation. Ocean acidification may also impair an organism's ability to process environmental and biological cues. These diverse impacts make it challenging to predict how acidification will alter species interactions and community structure. To examine effects of acidification on consumptive and behavioral interactions between mud crabs (*Panopeus herbstii*) and oysters (*Crassostrea virginica*), oysters were reared with and without caged crabs for 71 days at three pCO_2 levels. During subsequent predation trials, acidification reduced prey consumption, handling time, and duration of unsuccessful predation attempt. These negative effects of ocean acidification on crab foraging behavior more than offset any benefit to crabs resulting from a reduction in the net rate of oyster calcification. These findings reveal that efforts to evaluate how acidification will alter marine food webs should include quantifying impacts on both calcification rates and animal behavior.

3.2 Introduction

Predation has been a central focus of community ecology over the past several decades due to its importance in mediating community structure (Hairston et al. 1960, Sih et al. 1985, Werner & Peacor 2008). Predator-prey interactions can directly or indirectly manifest in a broad

range of lethal and sub-lethal effects, with far reaching consequences for community dynamics and evolutionary processes. Predation risk has resulted in the evolution of physical (e.g., coloration and morphological structures, such as spines and calcified exoskeletons), chemical (e.g., production of toxins in seaweeds; Taylor et al. 2002) and behavioral (e.g., refuge use and predator avoidance; Huffaker 1958, Werner & Peacor 2003) defenses among prey. Strong predator effects are often revealed when these "evolutionary arms races" (Dawkins & Krebs 1979) are shifted by events such as removal of top predators from a system (Estes & Palmisano 1974, Ripple & Larsen 2000) or introduction of non-native predator species (Green et al. 2012). Although these more immediate disturbances to ecosystems have greatly informed our understanding of the importance of top-down forcing, forecasting how longer-term perturbations such as environmental forcing will impact predator-prey interactions and community structure more broadly requires incorporating their potential impacts into ecological experiments.

It is well established that global change can significantly alter predator-prey interactions (Mills et al. 2013). For instance, rising carbon dioxide (CO_2) in the atmosphere is driving rapid, ubiquitous, and increasing change in global ocean chemistry and ecosystems (Walther et al. 2002, Orr et al. 2005). Ocean surface pH has already decreased by 0.1 since 1800 and is predicted to drop by an additional 0.1 – 0.4 units by end of century (Brewer 1997, Hoegh-Guldberg et al. 2014). This pH prediction for 2100 will result in a nearly 50% reduction in the carbonate ion concentration of seawater and a corresponding decrease in its calcium carbonate saturation states (Brewer 1997). The trend has raised concerns about the myriad calcifying marine organisms that construct their shells and skeletons from calcite and/or aragonite polymorphs of $CaCO_3$, and has prompted numerous studies investigating the potential effects of ocean acidification on rates of calcification. These studies have shown that marine calcifying

species exhibit differing responses to CO_2-induced ocean acidification (Gattuso et al. 1998, Langdon et al. 2000, Ries et al. 2009, Kroeker et al. 2010). Variation in these responses is largely due to their differing abilities to regulate protons at the site of calcification, the relative solubility of their skeletal mineral polymorphs, the extent to which they cover their shells or skeletons with protective organic layers, and whether they utilize photosynthesis that is fertilized by elevated pCO_2 (Ries et al. 2009).

Ocean acidification is expected to have largely negative impacts on bivalve species (Ries et al. 2009). For instance, calcification rates of *Crassostrea virginica* (eastern oyster) – along with shell hardness and fracture resistance – decrease linearly with CO_2-induced ocean acidification (Gazeau et al. 2007, Ries et al. 2009, Beniash et al. 2010, Waldbusser et al. 2011). Crustaceans, in contrast, exhibit the potential for neutral to increasing calcification rates (McDonald et al. 2009, Ries et al. 2009, Findlay et al. 2009, Long et al. 2013) with CO_2-induced ocean acidification. This pattern may include important estuarine predators, such as *Callinectes sapidus* (blue crab), *Panopeus herbstii* (mud crab), and *Menippe mercenaria* (stone crab). All of these crustacean species are important estuarine predators that prey upon various life stages of *C. virginica*; directly and indirectly affecting their population dynamics (Grabowski & Kimbro 2005, O'Connor et al. 2008, Grabowski et al. 2008). Additionally, oysters typically respond to predators by altering their calcification pattern to increase shell strength (Newell, Kennedy, et al. 2007). However, acidification has been shown to disrupt the induction of morphological defenses in *Littorina littorea* (Bibby et al. 2007), and may affect *C. virginica* similarly, further weakening its resistance to predation. Increased susceptibility of bivalves to predation, as suggested by opposing trends in calcification responses to acidification, could largely alter community dynamics throughout affected ecosystems.

Anthropogenically induced environmental changes throughout terrestrial and aquatic ecosystems often mediate changes in animal behavior (e.g., Pörtner & Peck 2010, Tuomainen & Candolin 2011). Behavior can become altered through three broad and often interacting pathways: information disruption, physical change, and avoidance of altered environments (Lurling & Scheffer 2007, Briffa et al. 2012). For avoidance to occur, disturbances must be relatively local and organisms must be able to immigrate to a more favorable location (Briffa et al. 2012). The relatively slow and ubiquitous advance of ocean acidification may limit the ability of organisms to find more favorable locations and, therefore, the utility of avoidance as a coping strategy.

Physical changes in response to ocean acidification are much more common and can include changes to metabolism (e.g., Dissanayake et al. 2010), calcification (e.g., Gazeau et al. 2007), and muscle strength (Wood et al. 2008, Landes & Zimmer 2012). The metabolic costs of survival in altered environments can result in reduced energy available for other behaviors, particularly for energetically expensive behaviors such as foraging and aggression (Dissanayake et al. 2010, Briffa et al. 2012). For instance, reduced calcification caused by ocean acidification causes *Littorina littorea* to compensate by increasing predator avoidance behavior (Bibby et al. 2007) and reduced claw strength in the crab *Carcinus maenas* at increased temperatures could result in altered prey selection (Landes & Zimmer 2012).

Information disruption affects behavior more directly, altering an organism's ability to perceive or process environmental information through a number of potential pathways (Pörtner & Peck 2010). For instance, information disruption has been shown to impair alarm responses in several fishes exposed to acidified waters (Leduc et al. 2003, Dixson et al. 2010). Laboratory evidence suggests that an alarm pheromone of these fishes suffers an irreversible change in

structure that renders it non-functional (Brown et al. 2002). This particular effect occurs completely external to the impaired organism. Growing evidence exists for widespread internal information disruption via interference with neurotransmitter function (Nilsson et al. 2012). In an effort to maintain internal acid-base homeostasis, some marine species alter intracellular concentrations of Cl^- and/or HCO_3^- (Wheatly & Henry 1992, Brauner & Baker 2009), which can lead to changes in ion gradients at neuron synapses and improper activity of some gamma-aminobutyric acid (GABA) receptors (Nilsson et al. 2012). GABA receptors are widespread in both vertebrates and invertebrates, making many marine species potentially vulnerable to this effect (Tsang et al. 2007). GABA receptor disruption has been shown to cause abnormal olfactory preferences and changes in swimming patterns in two coral reef fishes (Nilsson et al. 2012), as well as impaired predator escape behavior in a marine gastropod (Watson et al. 2014). These studies suggest that ocean acidification can negatively impact animal behavior, and in turn disrupt the transfer of energy to higher trophic levels.

To investigate how CO_2-induced ocean acidification will influence oyster reef communities, laboratory experiments were conducted to examine the impact of elevated pCO_2 on predator-prey interactions between *P. herbstii* and juvenile *C. virginica*. Experiments were designed to test the hypothesis that calcification rates of the oyster *C. virginica* are more negatively impacted by CO_2-induced ocean acidification than calcification rates of the crab *P. herbstii*, thereby increasing the oysters' susceptibility to mud crab predation. Alternatively, ocean acidification may disrupt the ability of mud crabs to locate or consume prey resources, thereby decreasing mud crab predation on oysters.

3.3 Methods

3.3.1 Growth conditions

Juvenile wild-strain *C. virginica* (18.7 ± 3.8 mm shell height) were obtained from Jonny Oyster Seed of St. Leonard, Maryland. Spat were separated from cultch shell using a diamond-embedded lapidary saw and as much of the excess shell was removed as possible. Spat were then individually attached to plastic microscope slides with cyanoacrylate epoxy. Thirty oysters were suspended 40 cm from the bottom of each tank on 1.7 mm diameter plastic cord. Adult *P. herbstii* (23-28 mm carapace width) were collected from Middle Marsh near Beaufort, North Carolina in early May 2011. Crabs were maintained in half of the tanks containing oysters and chambered during the growth period to control individual feeding rates and inhibit cannibalism while allowing for water and cue circulation. Oysters were raised in an orthogonal 3 x 2 design with 3 acidification levels and 2 crab cue presence levels (present or absent), while crabs were raised in a 3 x 1 design with 3 acidification levels. All treatment combinations were replicated three-fold.

Crabs and oysters were raised in isolated 34 liter tanks for 71 days in seawater with calculated $pCO_{2(gas\text{-}e)}$ values (±SD) of 499 (±114), 785 (±154), and 9274 (±2243) µatm (Table 3.1), corresponding to near-modern pCO_2, the predicted end-century pCO_2, and a level that exceeds the highest pCO_2 predicted to be experienced by these organisms. Although the high-pCO_2 treatment is higher than is predicted to occur in the atmosphere and open-ocean for the foreseeable future, comparable conditions already can occur in both healthy and degraded estuaries as high DIC from detrital organic matter, pollution, and stratification combine to elevate local pCO_2 in estuarine waters inhabited by both species (Cai & Wang 1998, Ringwood & Keppler 2002). Furthermore, our high-pCO_2 treatment was not formulated solely to target pCO_2 levels predicted for the foreseeable future, but rather to target pH levels and calcite

saturation states that are predicted to occur over that timeframe. And because of the temporal and spatial variation in salinity (5 < salinity < 35 ‰) that occurs within estuarine waters that are inhabited by the investigated species, the pH (ca. 7.0) and calcite saturation states (ca. 0.8) that were maintained in the high-pCO_2 treatment are realistic for low to moderate salinity and correspondingly low-alkalinity (TA < 1000 µmol) estuarine waters equilibrated with an atmospheric pCO_2 of ca. 2400 µatm (predicted for year 2600, assuming a conservative annual increase of 3.5 µatm yr^{-1}). Furthermore, recent studies have revealed that estuaries such as the Chesapeake Bay (Waldbusser et al. 2011), Elkhorn Slough, California (Hofmann et al. 2011), and Charleston Harbor Estuary, South Carolina (Ringwood & Keppler 2002) presently experience significant annual (8.2 – 7.6, Chesapeake Bay) and tidal (8.1 – 7.4, Elkhorn Slough; 7.6 – 6.9, Charleston Harbor Estuary) pH variation due to fluctuations in salinity (and resulting total alkalinity) and local enrichments in DIC (resulting from seasonal re-mineralization of benthic organic matter). Lastly, experimental seawaters were formulated to encompass a range of carbonate system parameters (pH < 7 and undersaturation with respect to calcite) that were similar to those that were employed in recent studies on related subjects (e.g., Bibby et al. 2007, Dissanayake et al. 2010, de la Haye et al. 2011, 2012).

Partial pressures of CO_2 were established by mixing pure CO_2 with compressed air using *Aalborg* digital mass flow controllers. Experimental seawater was bubbled with microporous ceramic airstones into triplicate glass tanks. The pCO_2 of the mixed gases was measured with a *Qubit S151* infrared pCO_2 analyzer calibrated with certified air-CO_2 gas standards (precision = ± 2.0%; accuracy = ± 1.8%). Salinity (SD) was formulated at 31.72 (0.76) with *Instant Ocean Sea Salt* and deionized water and temperature (SD) was maintained at 25.97 °C (1.15) with 50 watt electric heaters. Although the trace elemental composition of *Instant Ocean Sea Salt* differs

subtly from that of natural seawater, its major and minor elemental composition, as well as its carbonate chemistry, was the most similar to that of natural seawater when compared with eight other commercial sea salt mixes (Atkinson & Bingman 1998). Every two days, oysters were fed 14 ml per tank of a commercial algal blend containing *Nannochloropsis oculata*, *Phaeodactylum tricornutum*, and *Chlorella sp*. with a cell size of 2–20 µm (DT's Live Marine Phytoplankton, Sycamore, IL, USA). Each crab was provided 50 mg ±7 mg dry weight of *Artemia* sp. (brine shrimp) on the same two-day feeding schedule. Temperature, pH, and salinity were measured every two days, while pCO_2 of mixed gases were measured weekly (sensu Ries et al. 2009) (Table 3.1; see Supplementary Data for all measured and calculated seawater parameters).

Seawater within each tank was continuously filtered (757 L h^{-1}) with a hanging power filter that contained a nylon-floss activated-carbon filter. Circulation and turbulence of seawater within each tank was enhanced with a 400 L h^{-1} powerhead. Each tank was covered with a transparent 3-mm plexiglass sheet and both the tank and the attached filtration system were wrapped with cellophane to promote equilibration between the gas mixtures and the experimental seawaters and to minimize evaporative water loss. Tanks were illuminated for 12 hours per day with standard white fluorescent lights (32 Watts, T8 6500K) to simulate oysters' and crabs' natural light cycle.

Following the 71-day growth period, mud crabs and oysters were moved to tanks with a quartz sand substrate and with seawater chemistry that matched their respective experimental growth conditions. Twenty oysters, left attached to the plastic slides, were randomly selected then haphazardly arranged on the floor of the experimental tank. Two mud crabs were placed in each tank and allowed to prey upon these oysters for 48 hours or until oyster mortality exceeded 75%, whichever occurred first. Two mud crabs were used in each assay to incorporate effects of

acidification on conspecific aggression (McCormick et al. 2013), which can be an important component of *P. herbstii* foraging ecology (Grabowski & Powers 2004, Geraldi 2015). The number of oysters that was consumed was quantified every 2 hours for the first 12 hours and then sporadically for the remaining 36 hours.

3.3.2 Measurement and calculation of carbonate system parameters

Temperature within experimental tanks was measured every other day with a NIST-calibrated partial-immersion organic-filled glass thermometer (precision ± 0.3%, accuracy ± 0.4%). Salinity was measured every other day with a *YSI* 3200 conductivity meter with a *YSI* 3440 cell (K=10) that was calibrated with seawater standards of known salinity provided by the laboratory of Prof. A. Dickson of Scripps Institute of Oceanography. Seawater pH was measured every other day with a *Thermo Scientific Orion 2 Star* benchtop pH meter with an *Orion* 9156BNWP pH probe, calibrated with 7.00 and 10.01 *Orion* NBS buffers traceable to NIST standard reference material (for slope of the calibration curve) and with seawater standards of known pH also provided by Prof. Dickson's laboratory (for y-intercept of the calibration curve). Seawater dissolved inorganic carbon (DIC) was measured via coulometry (*UIC 5400*) and total alkalinity (TA) was measured via closed-cell potentiometric Gran titration calibrated with certified Dickson TA/DIC standards. Measurement of DIC and TA of the certified reference materials (CRMs) were consistently within 0.3% of certified values. Differences between the measured and certified TA and DIC values of the CRMs were used to correct measurements of experimental seawater solutions.

Seawater pCO_2, pH, carbonate ion concentration ($[CO_3^{2-}]$), bicarbonate ion concentration ($[HCO_3^-]$), aqueous CO_2, and calcite saturation state (Ω_C) were calculated from measured DIC,

TA, temperature and salinity with the program CO2SYS (Lewis & Wallace 1998), using Roy et al. (1993) values for K_1 and K_2 carbonic acid constant (Roy et al. 1993), the Mucci (1983) value for stoichiometric aragonite solubility product (Mucci 1983), and an atmospheric pressure of 1.015 atm.

3.3.3 Quantification of calcification rates via buoyant weighing

Calcification rates of oysters and crabs were estimated using an empirically calibrated buoyant weight technique (Ries et al. 2009). Specimens were weighed at the beginning of the experiment and at 71 days. Each specimen was suspended by aluminum wire from a *Cole-Parmer* bottom-loading scale (precision ± 0.001; accuracy ± 0.002) at a depth of 10 cm in a tank filled with experimental seawater maintained at a temperature of 25 ºC and salinity of 33. A plastic-coated zinc mass standard was intermittently weighed to ensure consistency of the buoyant weight method throughout the duration of the experiment.

Buoyant weight-dry $CaCO_3$ weight relationships for oysters and crabs were empirically derived by plotting final dry $CaCO_3$ weights (after removal of organic matter) against final buoyant weights of 49 oysters and 18 crabs randomly selected from the three pCO_2 (control – 499 µatm, moderate – 785 µatm, high – 9273 µatm) treatments used in experiments. Oyster dry $CaCO_3$ weight was the dry weight (70 °C, 24 hours) of the shell after mechanical removal of soft tissue. Crab dry $CaCO_3$ weight was the dry weight of the crab carapace after organic matter was removed via combustion in a muffle furnace at 500 °C for 6 hours. Buoyant weight-dry $CaCO_3$ weights for specimens from all treatments were highly correlated (linear regression of: oyster: $R^2 = 0.9976$, $P < 0.001$; crab: $R^2 = 0.9828$, $P < 0.001$) and similar amongst treatments, indicating that densities of crab and oyster shells do not vary appreciably amongst treatments (Ries et al. 2009).

Thus, a single linear equation for each species was used to convert buoyant weight to dry weight for purposes of estimating net calcification rates:

Oyster: Dry weight (mg) = 1.5996 * Buoyant weight (mg) - 0.5013;

Crab: Dry weight (mg) = 1.3411 * Buoyant weight (mg) - 0.0107.

3.3.4 Video analysis of behavior

Each feeding trial was recorded to explore the impacts of acidification on *P. herbstii* foraging behavior. Tanks were continuously illuminated to improve video quality. Predation in control pCO_2 treatments corresponds well to published predation rates under similar conditions, suggesting that the continuous using lighting was not detrimental to crab consumption (Rindone & Eggleston 2011). Two 30-minute segments of video were analyzed for each trial, with one starting point randomly selected from each of the following time intervals: 1.50 – 4.58 and 4.58 – 7.67 hours after the start of the experimental trial. Each video segment includes only active experimental time (i.e., before 75% oyster mortality was observed in a trial). Analysis of crab behavior included variables such as general activity (i.e., any movement of a claw), agonistic behavior (i.e., physical confrontations and delayed movements when in close proximity to each other during those confrontations), prey handling time, number of predation attempts, and average time spent in an unsuccessful predation attempt. Crabs often exhibited mild avoidance behavior, typically maintaining a minimum separation of approximately 8 – 10 cm, which was not considered agonistic behavior. Time spent handling prey included any use of the crabs' chela to grasp and manipulate an oyster. This definition encompasses all observed oyster manipulations except for brief pushing activity conducted with closed dactyls. An attempted predation event was defined as any generally continuous grasping contact regardless of periods

of inactivity. Small periods of non-contact (<20 s) were not considered a new event, as these events occurred infrequently and appeared to be either disengagement to perform brief displays of dominance or the result of the crab accidentally dropping the oyster, rather than intentionally terminating a predation attempt. Mean duration of unsuccessful predation attempts was quantified to examine how perseverant crabs were in attempting to consume oysters. The first five unsuccessful attempts identified in each tank were averaged to get a tank mean. If a replicate treatment did not include five attempts during the initial hour of analyzed video, additional segments were haphazardly selected from the two video analysis windows until a total of five unsuccessful attempts was reached. Several high pCO_2 replicates still failed to reach five attempts, and, as a result, this treatment was excluded from the analysis of mean duration of unsuccessful predation attempts.

3.3.5 Statistical analyses

Cochran's C test for heteroscedasticity of variances was conducted on all main effects in each analysis (Underwood 1981). Change in oyster buoyant weight was analyzed with a two-way ANOVA with pCO_2 (control, moderate and high) and crab cue (present vs. absent) as fixed factors. Change in crab buoyant weight was analyzed with a one-way ANOVA with pCO_2 as a fixed factor. Proportion of oyster consumed after 12 hours tank^{-1} was arcsine transformed and a two-way ANOVA was conducted with pCO_2 and crab cue as fixed factors. However, two trials were terminated prior to 12 hours due to prey depletion; therefore, the proportion of oysters consumed at time of termination was used in the analysis. Use of the consumption count at time of termination is a conservative estimate, as I assumed no additional predation following termination. Behavioral metrics of prey handling, general activity, and agonistic behavior were arcsine transformed, while prey encounters and mean duration of an unsuccessful predation

attempt were Box Cox transformed. All behavior metrics are values per tank and were analyzed with separate two-way ANOVAs with acidification and crab presence as fixed factors. All post-hoc tests were performed with Ryan's Q tests (Day & Quinn 1989).

3.4 Results

3.4.1 Calcification rates

Acidification negatively affected oyster calcification rates. The interaction between crab presence and pCO_2 ($F_{2,15} = 0.39$, $P = 0.687$) and the main effect of crab presence ($F_{1,15} = 1.12$, $P = 0.310$) were not significant, but pCO_2 did significantly affect calcification rates ($F_{2,15} = 4.68$, $P = 0.031$). Oyster calcification rates were significantly lower in the high pCO_2 treatment as compared to both the control and moderate treatments (Ryan's Q test, $P < 0.05$; Fig. 3.1), but the control and moderate treatments were not significantly different form each other. Crab calcification rates were not affected by pCO_2 treatment ($F_{2,6} = 0.70$, $P = 0.534$; Fig. 3.1).

3.4.2 Crab consumption of oysters

In addition to impacting oyster calcification rates, acidification reduced crab consumption of juvenile oysters. The interaction between crab presence and acidification ($F_{2,12} = 2.88$, $P = 0.095$) was not significant, but acidification did significantly influence consumption rates ($F_{2,12} = 42.7$, $P = <0.001$). The percentage of oysters consumed per tank was greatest in the control pCO_2 treatment (67.5±10%), intermediate in the moderate pCO_2 treatment (41 ± 7.5%), and lowest in the high pCO_2 treatment (1 ± 1%; Ryan's Q test: $P < 0.05$ for all pairwise comparisons of acidification treatments; Fig. 3.2a). Meanwhile, crab presence during the growth period did not affect crab consumption of oysters ($F_{1,12} = 1.39$, $P = 0.26$).

3.4.3 Crab behavior

Acidification impacted crab foraging behavior. Prey handling did not vary with the interaction of acidification or predator presence ($F_{2,12}$ = 1.97, P = 0.182), nor with the main effect of crab presence ($F_{1,12}$ = 0.16, P = 0.695). However, prey handling was significantly different across acidification treatments ($F_{2,12}$ = 6.08, P = 0.015), with the control and high treatments significantly differing from each other (Fig. 3.2b). There was a similar pattern for mean duration of an unsuccessful predation attempt, with no effect of the interaction between acidification and crab presence ($F_{1,8}$ = 1.23, P = 0.300) or the main effect of crab presence ($F_{1,8}$ = 1.13, P = 0.320). However, there was a significant effect of acidification ($F_{1,8}$ = 10.63, P = 0.012; Fig. 3.2c), with moderate acidification reducing the mean duration of unsuccessful predation attempts by 84.6% compared to the control acidification treatment.

There was no effect of acidification or crab presence on the number of predation attempts by crabs: acidification x crab presence interaction ($F_{2,12}$ = 0.53, P = 0.600), crab presence ($F_{1,12}$ = 0.04, P = 0.842), or acidification ($F_{2,12}$ = 2.87, P = 0.096). Yet, there was a trend of decreasing prey encounters for the high pCO_2 treatment, with that treatment averaging approximately 1/3 the encounters of the other pCO_2 treatments and 11 out of 15 observed high pCO_2 encounter events occurring in a single replicate.

General activity of crabs did not vary with the interaction of acidification and crab presence ($F_{2,12}$ = 0.14, P = 0.875), nor with either the main effects of acidification ($F_{2,12}$ = 2.16, P = 0.158) or crab presence ($F_{1,12}$ = 1.00, P = 0.336). Similarly, agonistic behavior did not vary significantly with the interaction term ($F_{2,12}$ = 2.59, P = 0.116), the main effect of acidification ($F_{2,12}$ = 0.78, P = 0.482), or the main effect of crab presence ($F_{1,12}$ = 0.42, P = 0.528).

3.5 Discussion

Ocean acidification reduced *P. herbstii* (mud crab) predation on juvenile *C. virginica* (eastern oyster). This finding was counter to our initial hypothesis that potentially differential effects of ocean acidification on crab and oyster calcification (Ries et al. 2009) would facilitate crab consumption of oysters. This counterintuitive result cannot be explained by negative effects of ocean acidification on crab shell mass because acidification did not significantly influence *P. herbstii* calcification rates. Furthermore, *C. virginica* calcification differed only between control and high pCO_2 treatments. Despite oyster calcification rates in the intermediate pCO_2 treatments being statistically indistinguishable from the control pCO_2 treatments, several metrics of crab behavior differed significantly between these two treatments. Furthermore, the reduced calcification rates of oysters in the high treatment likely rendered them more vulnerable to predation by mud crabs, which is counter to what was observed in the behavioral assays. Thus, the negative effect of acidification on the ability of mud crabs to prey upon oysters more than offset any advantage conferred to the crabs from the decline in net rate of oyster calcification under the highest pCO_2 treatment.

Other experiments investigating the impact of CO_2-induced ocean acidification on predator-prey dynamics of oysters or crabs either support the hypothesis that acidification either increases the predation risk for oysters and other calcifying crab prey or has no effect. Amaral et al. (2012) found that oysters exposed to low pH due to acidic runoff from sulphatic soils are more susceptible to drill predation than oysters from reference sites lacking acidic runoff (Amaral et al. 2012). Meanwhile, Landes and Zimmer (2012) found no increase in predation on *Littorina littorea* by the green crab *Carcinus maenas* under acidified conditions (Landes & Zimmer 2012). Differences between our experimental results and those of Amaral et al. (2012)

and Landes and Zimmer (2012) may stem from the latter studies conducting predation trials at control pH conditions, whereas I conducted trials at the acidification levels under which the organisms were originally reared. Furthermore, Amaral et al. (2012) used predators reared in non-acidified water, whereas both predators and prey in the present experiment were reared under the same suite of control and acidified treatments. These unexpected findings highlight the importance of assessing the impact of acidification on predator behavior, in addition to its impact on calcification rate and shell/skeletal properties.

To further explore why acidification reduced crab foraging rates, the impacts of acidification on each of the different crab behavior and oyster consumption metrics were evaluated. Behaviors that remain consistent even in the high pCO_2 treatment can be considered resilient to changes in seawater pH. Crab general activity, agonistic behavior, and number of predation attempts did not differ as a function of crab presence or acidification. Previous studies on the effects of acidification on the levels of general activity in crustaceans are mixed, with conflicting results concerning the mechanism driving the behavioral change. For example, acidification had no effect on general activity levels of two species of crayfish (Tierney & Atema 1986). However, acidification reduced the time that a hermit crab (*Pagurus bernhardus*) spent in motion when either presented with an improved shell choice (~25%; de la Haye et al. 2011) or exposed to prey cues (~40%; de la Haye et al. 2012), and reduced the swimming ability of a penaeid shrimp (~30%; Dissanayake & Ishimatsu 2011).Similar variation in activity response to pCO_2 is present in fishes, both across species and within species across temperatures (Cripps et al. 2011, Devine et al. 2012a, Nowicki et al. 2012). The impact of pCO_2 on activity appears to be highly variable, but when changes in activity are induced by acidification, they are likely to have significant effects on predator-prey dynamics and community structure.

Agonistic behavior was generally low across all treatments (~1% of time observed) except in the two control and moderate pCO_2 replicate tanks with low rates of crab consumption of oysters, where agonistic interactions accounted for 8-13% of the time observed (Fig. 3.3). Acidification has been shown to invert the aggression and competitive dominance relationships between two species of damselfish competing for space on a coral reef (McCormick et al. 2013) and conspecific agonistic interactions may be similarly susceptible to the influence of acidification. However, no such effect was observed in *P. herbstii*. Agonistic interactions observed in our study were mostly displays of dominance and very brief physical confrontations in which a single crab was consistently dominant. The experiment was explicitly designed to avoid resource depletion, and consequently may have dampened agonistic interactions among crabs, thereby making it challenging to identify differences in aggression across treatments. Additional experiments are needed to better understand the effect of acidification on *P. herbstii* aggression.

Although there was no effect of acidification on prey encounter rates, encounter rates in the high pCO_2 treatment were approximately one-third those in the other two treatments. De la Haye et al. (2012) found that hermit crabs in acidified conditions were less successful at locating prey scent than those in the control treatment (de la Haye et al. 2012). In their study, a non-food object was soaked in prey cue and then presented to the crab, thereby isolating scent as the only cue available to identify the food source. Multiple sensory cues have been shown to compensate for loss of olfaction in damselfishes (Devine et al. 2012b), and the present study maintained visual and tactile cues in addition to scent cues. Habitat choices made by settling damselfishes were significantly altered by acidification when only scent cues were presented, but those differences disappeared when a broader suite of sensory cues were provided (Devine et al.

2012b). Multiple senses appear to be capable of compensating, to some degree, for potential reduction in chemosensory ability. The present study suggests that *P. herbstii* encounter rates of *C. virginica* are largely resilient to near-term acidification but may be reduced under extreme conditions.

Although encounter rates did not differ amongst the acidification treatments, acidification reduced total predator handling of prey and decreased the average time that predators spent unsuccessfully attempting to consume prey. These findings suggest that although acidification has minimal effect on the ability of predators to locate prey, predators are less persistent when they attempt to consume prey under acidified conditions. This could be a response to increased metabolic requirements associated with acidification. Penaeid shrimp were unable to maintain swimming efforts under highly acidified conditions (Dissanayake & Ishimatsu 2011). Thus, crabs in acidified water may lack the capacity for prolonged predation attempts. However, I found no effect of acidification on several other energetically expensive behaviors (e.g., locomotion, aggression). A potential alterative mechanism explaining reduced crab handling of oysters could stem from GABA receptor excitation in *P. herbstii*, which could be disrupting cost-benefit processes. Suboptimal resource utilization after encountering prey has been previously observed in a hermit crab species (de la Haye et al. 2011). De la Haye et al. (2011) found that a hermit crab reduced shell switching from inferior to optimal shells in elevated pCO_2 and concluded that acidification disrupts resource assessment and decision making processes (de la Haye et al. 2011). The role of GABA disruption as a mediating factor remains unclear as haemolymph Cl^- increased in acidified waters, which is the inverse of what has been observed in fish (Brauner & Baker 2009 and references therein) and mollusks (Parker et al. 2013 and references therein). Regardless of the mechanism, our results suggest that acidification disrupts

the ability of predators to consume prey, and could consequently reduce transfer of energy to higher trophic levels.

It is unclear to what extent *P. herbstii* and *C. virginica* will adapt to future ocean acidification, which could result in greater or reduced mud crab foraging success on oysters under acidified conditions. Over sufficiently long timescales and barring extinction of either species, both species may adapt to CO_2-induced ocean acidification. In the near term, however, our results suggest that harm caused by ocean acidification to oysters and the reefs that they form may be at least partially offset by behavioral impairment of their crustacean predators. Yet these results also suggest that the crustacean predators that demonstrated resilience (relative to mollusks) in early ocean acidification studies, because they are more capable of calcifying under acidified conditions, may indeed be vulnerable to acidification in other ways.

Acidification has been found to strongly impact calcification rates of individual organisms (Gattuso et al. 1998, Langdon et al. 2000, Gazeau et al. 2007, McDonald et al. 2009, Ries et al. 2009, Findlay et al. 2009, Kroeker et al. 2010, Beniash et al. 2010, Waldbusser et al. 2011, Long et al. 2013). Generalizing the impacts of ocean acidification at population, community, and ecosystem levels will require incorporating how other processes, such as predator foraging behavior and prey avoidance of predators (Amaral et al. 2012, Landes & Zimmer 2012), are impacted by ocean acidification. Our study explores some of these other key processes and demonstrates that acidification-induced impairment of *P. herbstii* foraging on *C. virginica* offsets any potential benefit to the crabs that results from preying upon more weakly calcified oysters under acidified conditions. These findings have important implications for the management of crustacean fisheries and oyster reefs, which provide valuable ecosystem services such as providing nursery ground for economically valuable fishery species, stabilizing

shorelines, and removing anthropogenic nitrogen from eutrophied estuaries (Meyer et al. 1997, Peterson et al. 2003, Piehler & Smyth 2011).

3.6 Acknowledgements

I am grateful to Karl Castillo, Travis Courtney, and Cassandre Lazar for assistance with experimental implementation and to two anonymous reviewers for their valuable comments. Funding for this experiment was provided by National Science Foundation grant nos. OCE-1203859 (JHG), OCE-0961929 (MFP), and OCE-1357665 (JBR).

Table 3.1. Mean (standard deviation) seawater parameters for pCO_2 treatments: calculated pCO_2 of mixed gases in equilibrium with experimental seawaters [$pCO_{2\ (gas\text{-}e)}$]; calcite saturation state (Ω_C); pH; and dissolved inorganic carbon (DIC). Full seawater parameters available as Supplementary Data.

	Control-pCO_2	**Moderate-pCO_2**	**High-pCO_2**
$pCO_{2\ (gas\text{-}e;\ ppm\text{-}v)}$	499 (114)	785 (154)	9274 (2243)
Ω_C	6.7 (2.0)	5.1 (1.3)	0.8 (0.2)
pH	8.20 (0.11)	8.04 (0.08)	7.05 (0.09)
DIC	2360 (303)	2549 (256)	3432 (207)

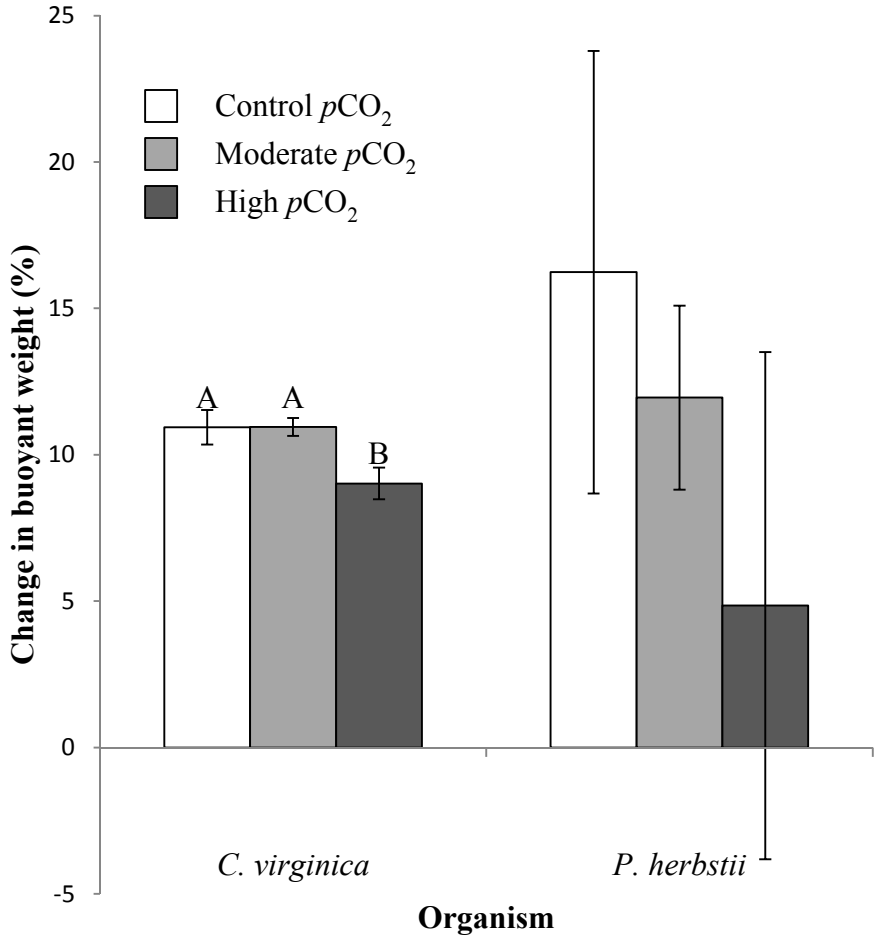

Fig. 3.1. Mean (± SE) change in buoyant weight for *P. herbstii* and *C. virginica* after 71 days.

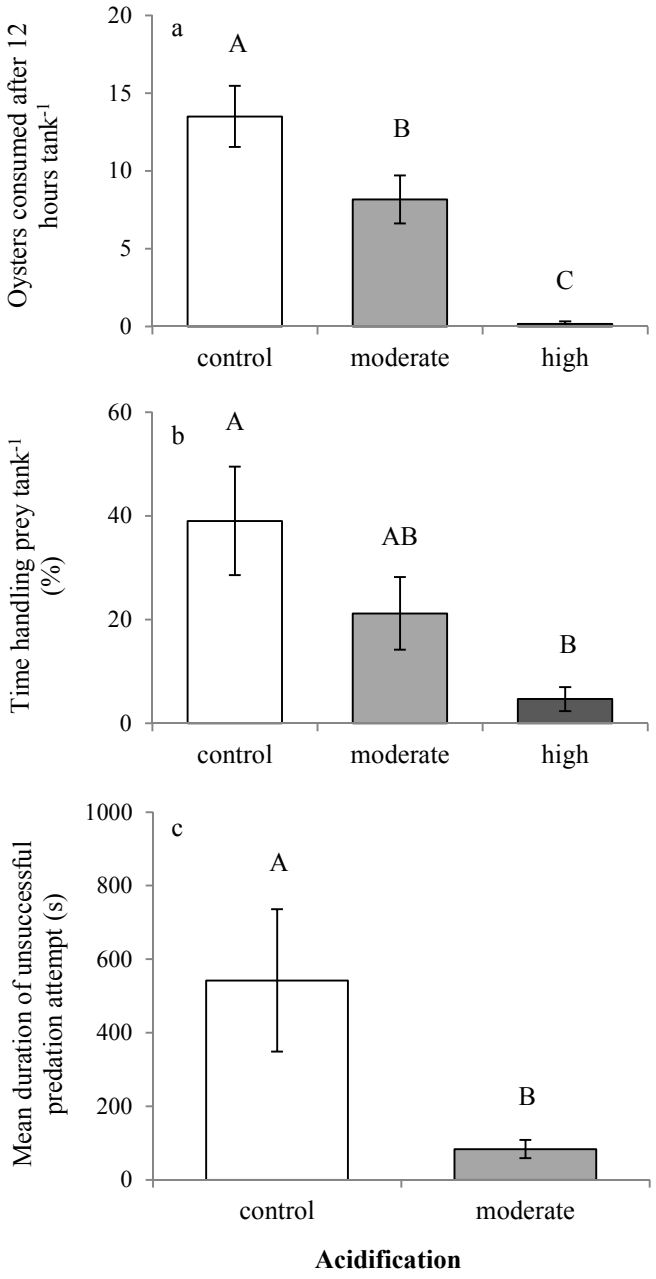

Fig. 3.2. (a) Oyster consumption, (b) prey handling, and (c) predator persistence at different acidification treatments. Data are untransformed means ± SE; letters denote significant differences from Ryan's Q post-hoc tests. Several high pCO_2 replicates failed to reach five unsuccessful predation attempts; therefore, the high acidification treatment was excluded from (c).

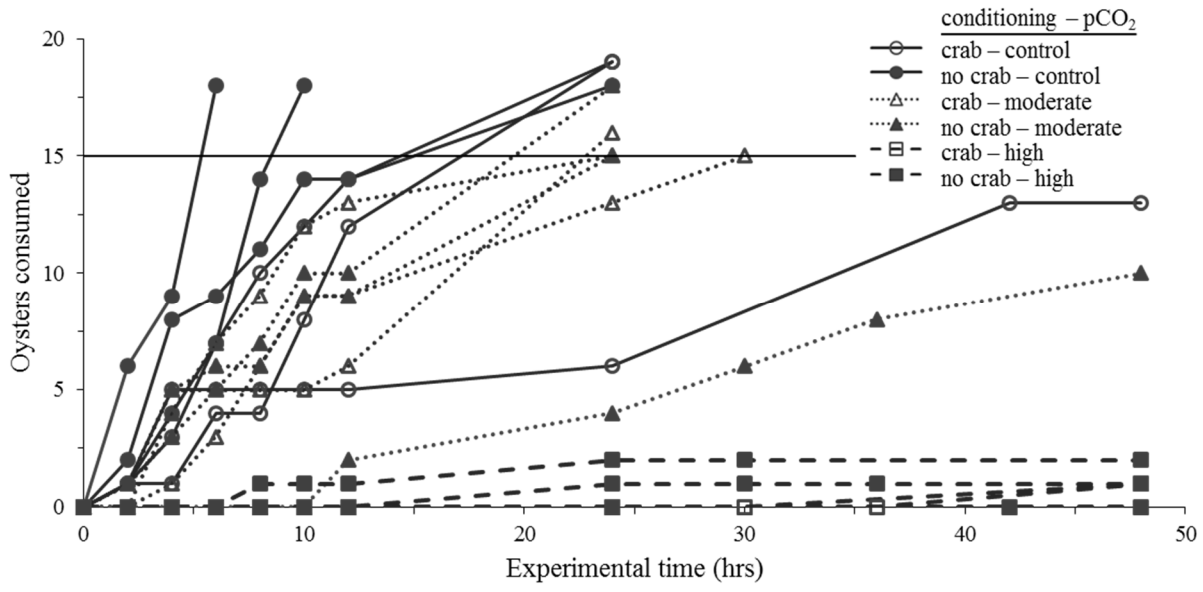

Fig. 3.3. Oysters consumed vs. time for each replicate predation trial. Reference line at 15 oysters consumed marks 75% mortality and trial termination.

4. SIZE SELECTION OF OYSTER PREY BY THE STONE CRAB, MENIPPIE MERCENARIA

4.1 Abstract

The stone crab *Menippe mercenaria* is a specialist predator on hard shelled prey including the oyster *Crassostrea virginica*. *M. mercenaria* is a resident of *C. virginica* reefs and is capable of significantly altering reef structure. Recent evidence indicates a poleward range expansion and increasing densities in the northernmost portions of its range. Little is known about how their prey size selection changes with crab size or density. Three sizes of crab, alone or in conspecific pairs, were allowed to prey on natural clusters of oysters for 24 hours. Oyster mortality by count and biomass were recorded and oyster size selected by crabs was analyzed using a relativized electivity index (E*). Oyster mortality by count did not vary across treatments but biomass was significantly different as larger crabs consumed larger oysters. Crabs larger than 70 mm prey on oysters in sizes nearly proportional to their abundance, while smaller crabs significantly select smaller oyster prey. Crabs larger than 95 mm consumed much less oyster biomass than crabs 70-90 mm unless starved for an extended period; possibly indicating a dietary shift in this size class crab. *M. mercenaria* 70-90 mm were the most capable and willing oyster predators and therefore could have the largest per capita influence on oyster reef community structure.

4.2 Introduction

Prey selection is a central theme of ecology; having been codified into Optimal Foraging Theory (OFT) (Emlen 1966, MacArthur & Pianka 1966, Pyke et al. 1977). OFT predicts how

predators invest their time and activities in the pursuit of acquiring adequate metabolic energy. The wide array of options in behavior and prey selection constituting an optimal decision makes predicting what a predator will do difficult, particularly as environmental complexity increases (Schluter 1981). Organisms are expected to maximize some relevant currency, often net energy intake but potentially anything of value to the organism, such as time or a specific nutrient, under a set of physical, biological, and ecological constraints. However, the questions of what currency is maximized and what constraints may be significant can vary from species to species and environment to environment.

Crustacean predation on mollusks is an illustration of the complexity inherent to OFT. Crustaceans generally select smaller molluscan prey than the maximum size they are physically capable of consuming (Juanes 1992). This may reflect optimal selection when handling time increases for larger prey to the extent that maximum profitability (tissue consumed/handling time) occurs at a smaller size range (e.g., Hughes & Seed 1981). However, crustacean predators often select smaller prey than even OFT would predict (Juanes 1992). Instead of maximizing net energy intake, crustaceans seem to be either minimizing the potential for claw damage or minimizing their own predation risk (Juanes 1992, Yamada & Boulding 1998, Aronhime & Brown 2009). Potential constraints on this system include a range of physical (e.g., current speed (Finelli et al. 2000)), biological (e.g., claw strength (Yamada & Boulding 1998)), and ecological factors (e.g., prey density (Elner & Hughes 1978); presence of competitors (Wong et al. 2010, Chakravarti & Cotton 2014)). However, environmental constraints are not frequently manipulated in selection experiments and as a result remain poorly identified across many systems.

Specialist shell-breaking crustaceans do not show the same general preference for smaller molluscan prey (Yamada & Boulding 1998). Degree of specialization can be determined by diet and claw biomechanics, measured as the mechanical advantage (MA) of each claw (Yamada & Boulding 1998). For instance, the generalist crab, *Hemigrapsus nudus*, has a broad diet and a mean MA of 0.28, while the specialist crab *Cancer productus*, feeds primarily on shelled prey and has a mean MA of 0.39 (Yamada & Boulding 1998). This difference manifests in a strong contrast between the two species' prey size preference for the snail, *Littorina sitkana*. *H. nudus* strongly preferred the smallest size class offered (4-5 mm), while *C. productus* consumed all but the largest size class equally (4-13 mm) (Yamada & Boulding 1998).

The closely related stone crabs, *Menippie mercenaria* and *Menippie adina*, are highly specialized, with a diet dominated by hard-shelled prey and a strong claw MA of 0.50 (*M. mercenaria*). These crabs are capable of producing an average torque force ten times that of the co-occurring generalist crab, *Callinectes sapidus* (strong claw MA 0.29) (Yamada & Boulding 1998, Brown et al. 2009). Large *M. adina* that were offered oysters *Crassostrea virginica* (mean ±SE; carapace width (CW) 94.5 ±3.5 mm) and mussels *Ischadium recurvum* (CW 83.6 ±2.7 mm) exhibited no prey-size preferences, and in both cases, there were no differences in profit (tissue consumed/handling time) across prey sizes (Brown & Haight 1992, Aronhime & Brown 2009). In these studies, prey were individually separated and presented without physical structure. In contrast, *M. mercenaria* (CW 96 ±12 mm) presented with clusters of *C. virginica* in a low structure environment exhibited a strong size selection for 25-70 mm oyster (Rindone & Eggleston 2011). Profitability was not accounted for in this study, however the ratio of oyster sizes selected is not consistent with that identified by Brown and Haight (1992). Several factors

varied between these studies, including habitat complexity, crab size, and crab species, so that the mechanisms driving the observed differences in selectivity is not clear.

Competitive interactions can also alter expectations of predatory efficiency and prey selection (Milinski 1982, Svanback & Bolnick 2007). The effect of conspecific interactions between specialist crabs on their predatory behavior is highly variable. *Carcinus maenas* (strong claw MA 0.36; Warner et al. 1982) increases its feeding rate in the presence of a conspecific competitor, possibly risking an increased chance of claw damage to outcompete other individuals (Chakravarti & Cotton 2014). *M. mercenaria* interactions with conspecifics have been shown to vary with environmental setting. Crabs presented a single size of clam prey consumed more biomass than expected from a multiplicative model on a sand substrate but performed as expected on a hard bottom substrate (Wong et al. 2010). When presented multiple sizes of clam prey, conspecific pairs consumed all available prey sizes proportionally on sand bottom but selected significantly fewer large clams on hard bottom (Wong et al. 2010).

The unique structure of oyster reefs may also influence prey selection. Because oysters settle preferentially on conspecifics, reefs form a highly complex matrix of sizes that may or may not be individually accessible. Protection from being individually targeted by a large predator is likely size dependent. However, the risk of collateral mortality is likely to be significant for oyster sizes below a target prey size. This may contribute to a weakening of size dependent selection for predators whose diet includes large oysters.

The stone crab is a substantial predator on oysters, capable of altering reef structure through burrow creation and localized extirpation (Brown & Haight 1992, O'Connor et al. 2008, Fodrie et al. 2008, Rindone & Eggleston 2011). There is also evidence that its range and densities may be increasing northward due to the warming effects of climate change (Rindone &

Eggleston 2011). The impacts of range expansion in marine ecosystems can be just as large as those caused by invasions (Sorte et al. 2010). *C. virginica* reefs that provide shelter and foraging habitat for stone crabs are well recognized for ecosystem functions with cultural and socioeconomic value (Meyer et al. 1997, Peterson et al. 2003, Piehler & Smyth 2011, Grabowski et al. 2012). As such, the potential ecological impacts of expanding predator populations, such as *M. mercenaria*, on this highly valuable habitat could influence efforts to manage coastal resources.

To test how crab size affects stone crab selection of oyster prey on oyster reefs, three distinct size classes of the stone crab were offered natural clusters of the eastern oyster, *C. virginica*. Additionally, combinations of crabs across the three size classes were introduced together to investigate the effects of conspecific interactions on crab feeding rates and size selection.

4.3 Methods

Intermolt stone crabs were collected by hand and with crab pots from Bogue Sound, NC in July and August 2013-2014. Crabs from three discrete size classes, small (45 – 65 mm CW), medium (70 – 90 mm CW), and large (95 – 115 mm CW), were kept separated in flow through tanks and fed fish ad libitum. They were maintained in these tanks for at least 24 hours and no more than 7 days with food followed by a starvation interval, prior to experimental trials. Oyster clusters were collected from one reach of Hoop Hole Creek, Bogue Sound (Fig. 4.1). Clusters were separated into clusters of 5 to 40 oysters, and all oysters were measured. Each cluster was cleaned of all epiphytes and attached upright to a brick using Z-Spar Marine Epoxy (Splash Zone A-788). Clusters were kept in flow-through tanks with unfiltered Bogue Sound water until the

epoxy was fully cured according to the manufacturer's instructions and the clusters subsequently used in the experiment.

Laboratory experimental tanks measuring 65 cm x 90 cm, were filled to a depth of 30 cm with filtered Bogue Sound water (salinity ~32). The tanks were kept in a 12 hour day-night light cycle with water temperatures maintained between 25 and 27 °C. One 25 cm deep PVC burrow (5.1 to 15.2 cm diameter) was provided for each crab in a trial based on preliminary trials of burrow diameter preference. From 8 to 15 clusters, containing mean (SD) count of 168 ± 9 oysters were placed into each tank. Loose oyster shells were added up to the height of the bricks. All clusters were completely submerged at the start of the trials. Tanks were covered with mesh to prevent crabs from escaping. Trials were started near midday and lasted 24 hours. The experiment contained seven treatments with four replicates each (Table 4.1). Small (S), medium (M), and two large crab treatments (L-48; L-96) consisted of one crab per trial. The other three treatments consisted of two small crabs (S+S), two medium crabs (M+M), and one small and medium crab together (S+M). All crabs were starved for 48 hours prior to the start of each trial, except for one large single crab treatment where the individual crabs were starved for twice as long (L-96).

Following each trial, clusters were remeasured and missing oysters were identified. No attempt was made to identify targeted versus collateral mortality. An oyster was considered killed if tissue was exposed following trial completion. Oyster biomass was determined by converting shell length to dry weight using a relationship derived from intertidal *C. virginica* (Copeland & Hoese 1966, White et al. 1988, Powell et al. 1995). Oyster mortality and biomass data were Box-Cox transformed and then analyzed using one-way ANOVAs. Tukey's post hoc tests were utilized to perform pairwise comparisons of treatment levels to test for significant

differences. Expected oyster count and biomass consumption for the paired crab treatment levels were calculated using the multiplicative risk model (Wilbur & Fauth 1990, Soluk 1993, Sih et al. 1998): $C_{ab} = P_a + P_b - (P_aP_b)$. Where C_{ab} is the predicted proportion of individuals or biomass consumed by predator a and predator b when foraging together, P_a is the observed proportion consumed in isolation by predator a and P_b is the observed proportion consumed in isolation by predator b.

Because oyster size distributions differed among treatments and total prey counts were not identical, a metric designed to account for the variation in prey among replicates was used in the analysis. Stone crab preference for different size classes of oysters (1-25 mm, 26-50 mm, 51-75 mm, 76-100 mm, 100+ mm) were calculated using a relativized electivity index, E* (Vanderploeg & Scavia 1979). E* integrates the relative abundance of each individual prey type as well as the total abundance of all prey. This index permits the analysis of less stringently controlled prey communities. E* values range from -1, indicating that the predator does not consume the prey item, to 1, indicating exclusive preference of one prey type from a pool of infinite prey types. The maximum possible E* value for 5 categories, as used in our study, is 0.667. To correct for this asymmetry between potential positive and negative values, positive E* values were multiplied by 1.5, so that the maximum possible value was 1. A value of 0 indicates consumption proportional to the relative abundance in the prey community. Trendlines of E* plotted against prey size class were fitted to each treatment using linear regression in JMP. Trendlines were selected by treatment across all five prey size categories, and separately, across just the first four size categories, excluding the often unutilized 100+ size bin. The four prey size category abbreviated slope analysis allows for consideration of proportional predation below the size threshold of 100 mm, while still calculating E* across all five categories. The slopes were

then used to indicate preference for small oysters (negative slope), large oysters (positive slope), or no size preference (slope of 0). A slope of 0 is possible through size-proportional predation, as well as, by total lack of consumption. Due to this convergence of values for very disparate ecological circumstances, the single replicate where crabs did not consume any oyster were excluded from the analysis. Thus, the E* slope analysis indicates stone crab prey preferences when predation occurs. E* slopes were Box Cox transformed and analyzed by one-way ANOVA. Tukey-Kramer post hoc tests were used to identify significant differences across treatments.

4.4 Results

Oyster mortality by count was not significantly different across treatments ($F_{6,21} = 0.962$; $P = 0.474$; Fig. 4.2a). However, oyster mortality by biomass was significantly affected by treatment ($F_{6,21} = 4.227$; $P = 0.006$; Fig. 4.2b). Tukey post hoc tests revealed that the L-96 treatment differed from the three treatments with the smallest biomass consumed: S+S, S, and L-48. Full 5-point E* slopes significantly differed across treatments (Table 4.2; $F_{6,20} = 8.20$; $P = 0.0001$). The L-96 E* slope significantly differed from those of the S+S, S, and L-48 treatments, whereas the M treatment slope differed from those of the S and L-48 treatments (Table 4.2; Tukey post hoc tests, $p < 0.05$). Abbreviated 4-point E* slopes also differed across treatments ($F_{6,20} = 5.45$; $P = 0.002$), with differences among the L-96, M, and M+M treatments as well as the L-48 and S treatments (Table 4.2; Tukey post hoc tests, $p < 0.05$).

4.5 Discussion

Stone crab size and density had a significant effect on feeding rates and size selection on oyster prey. The lack of difference in the number of oysters consumed, coupled with the strong

differences in biomass, highlight the importance of prey size selection in determining community level effects of predation.

Stone crabs larger than 70 mm are very capable oyster predators. Both the M and L-96 treatments showed E* slopes not different from zero, indicating feeding in proportion to prey abundance. A correction for profit (tissue consumption/handling time) was not attempted in this study, as the incidental mortality observed would obscure profit calculations by size. However, prey profit has been previously shown not to vary by size in *C. virginica* for *M. adina* (Brown & Haight 1992). The probable lack of a strong profit signal by size and the strong co-occurrence of multiple sizes of oyster would likely drive feeding selection toward proportional feeding, as observed in this study.

Starvation time had a strong effect on prey selection and biomass consumption for crabs larger than 95 mm, with greater starvation resulting in crabs consuming more oyster biomass. The difference in biomass consumed between the L-48 and L-96 treatments may indicate that large stone crabs do not prefer oysters, but that they may switch to consuming oysters when other foods are scarce. In the wild, dexterity could become a limiting factor in their ability to prey on highly structured reefs with large densities of living adult oysters. However, the clusters used in this experiment, while highly structured, were well spaced to increase the potential for individual prey selection, which may have reduced potential dexterity constraints.

In the L-48 treatment, stone crabs preferred smaller oysters and consumed less biomass. This result agrees in part with Rindone & Eggleston (2011), who starved stone crabs for 48 hours and found that they avoid consuming large adult oysters. While their range of crab sizes included both the medium and large size classes used in this study, their mean size falls within the large crab size class (Rindone & Eggleston 2011). In other crab species, claw breaking strength can be

lower within their largest size ranges, and individuals appear to compensate behaviorally by restricting their consumption of larger prey, thereby reducing their applied breaking force and the probability of damaging their claws (Taylor et al. 2000). This effect could manifest in stone crabs as a reduced preference for very strongly armored prey items such as adult oysters. However, starvation may induce crabs to alter their foraging behavior and consume larger prey.

Small stone crabs preferred oysters less than 25 mm. Outside of their burrow creation behavior, small stone crabs may be ecologically redundant to the numerous other oyster predators in the system (O'Connor et al. 2008). However, ontogenetic shifts in prey preference would be expected in an organism with such a large size range of 2-142 mm (Gerhart & Bert 2008), and stone crabs apparently transition from a high degree of size selectivity when they are small to very little selectivity when they reach 65 to 70 mm CW. This transition likely has strong effects on reef structure; as crabs grow larger they become capable of reducing or eliminating structure through their foraging behavior. Biomass consumption by larger crabs also was more than 20 times greater than that of smaller crabs. These results suggest that crab size largely influences their types of effects on oyster reef habitat and community structure, with individuals between 70 and 90 mm CW having the largest per capita effect on reef community structure.

Evidence of conspecific interference was present in all sizes. Crabs in the S+S treatment consumed fewer oysters but equal biomass as expected based on the multiplicative risk model compared to the S treatment (Fig. 4.2). Changes in size selectivity toward greater utilization of the 26-50 mm and 51-75 mm oyster size classes partly explain the increase in consumed biomass (Fig. 4.1). Interference among smaller stone crabs may be limited because individuals reduced their prey selectivity, so that a larger prey field was available. Stone crabs typically transition to sexual maturity while in the small crab size class used in this study (Gerhart & Bert 2008).

Aggression between juveniles has not been well studied, but immature individuals may experience fewer and less intense encounters (Sinclair 1977).

When small and medium stone crabs were together, they consumed fewer oysters than expected based on the multiplicative model by both count and biomass (Fig. 4.2), but crab density did not influence their prey selectivity (Fig. 4.1). The larger of the two crabs would likely be competitively dominant, and could be restricting the foraging behavior of the smaller crab (Sinclair 1977). Additionally, both count and biomass consumed are not different than that observed for a single medium crab. Therefore, the medium-sized crab may still interfere with the smaller crab's foraging behavior.

Medium-sized crabs when together also consumed fewer oysters than predicted based on both count and biomass, and had the largest difference between observed and predicted for both metrics (Fig. 4.2). Differences in size selectivity between the M and M+M treatments are visible in both the E* values (Table 4.2) and graphical representation (Fig. 4.1), with both showing an increase in crab preference when together with a conspecific for the 26-50 mm size class and a decrease for the 100+ mm oyster size class. Conspecific interference was expected to be highest in this treatment, as all crabs will have reached sexual maturity.

Increased densities of adult stone crabs will likely reduce individual feeding rates on oyster reefs. Dispersion of *Menippe spp.* hybrids is theorized to be partially driven by resource availability (Lindberg et al. 1990). However, on oyster reefs where resources levels are high, dispersion may become more dependent upon foraging space rather than the resource itself. Burrow occupation is common to all stone crab size classes, but burrow creation behavior decreases at ~60 mm CW as crabs become less vulnerable to predators (Beck 1995). Oyster reefs

with high densities of living oysters may reduce the foraging arenas of stone crabs due to the increased stability of burrows.

M. mercenaria has expanded its range poleward in recent decades and anecdotal evidence indicates that densities may also be increasing in northern portions of its range (Rindone & Eggleston 2011). Increased stone crab densities will likely have an effect on oyster reef habitat and community structure. However, stone crab interference interactions will likely reduce their per capita effects on oyster survival. However, strong localized crab impacts may be possible under some density, demographic, and burrow stability conditions (Rindone & Eggleston 2011). Further work should be conducted on feeding behaviors of the largest stone crab size class to investigate the possibility of a dietary transition away from oyster prey and the ecological impacts such a shift may have.

ID	Crabs	Starve time (hrs)
S	Small x1	48
M	Medium x1	48
L-48	Large x1	48
L-96	Large x1	96
S+S	Small x2	48
M+M	Medium x2	48
S+M	Small x1; Medium x1	48

Table 4.1. Treatment identifications with corresponding crab populations by size class and starvation times.

	1-25	26-50	51-75	76-100	100+	Full 5-point E* slope				Abbreviated 4-point E* slope		
L-96	0.11	-0.35	-0.42	-0.09	0.11	-0.03†	A			-0.07†	A	
M	0.22	-0.49	-0.15	-0.18	-0.62	-0.14†	A	B		-0.09†	A	
M+M	0.15	0.43	-0.48	-0.16	-1.00	-0.29	A	B	C	-0.19†	A	
S+M	0.35	-0.01	-0.30	-0.56	-0.65	-0.26†	A	B	C	-0.30†	A	B
S+S	0.50	0.14	-0.47	-1.00	-1.00	-0.41		B	C	-0.51	A	B
S	0.80	-0.24	-0.74	-1.00	-1.00	-0.44			C	-0.59		B
L-48	0.30	-0.19	-0.86	-1.00	-1.00	-0.45			C	-0.61		B

Table 4.2. Mean E* index values for each treatment by oyster size. Untransformed mean E* slopes for full 5-point E*slope and abbreviated 4-point E* slope. Letters denote significance based on Tukey-Kramer post hoc testing; † indicates a slope not different from zero based on 95% confidence intervals.

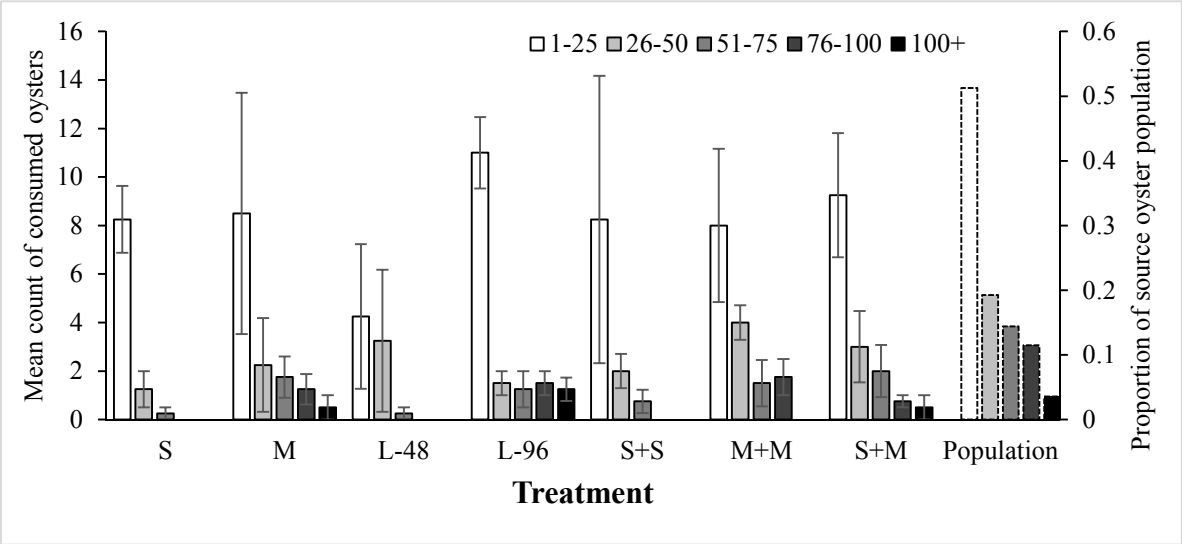

Figure 4.1. Average count of oysters consumed by size class and treatment (left axis) and proportion of oyster size classes in the natural population (right axis). Error bars ±SE.

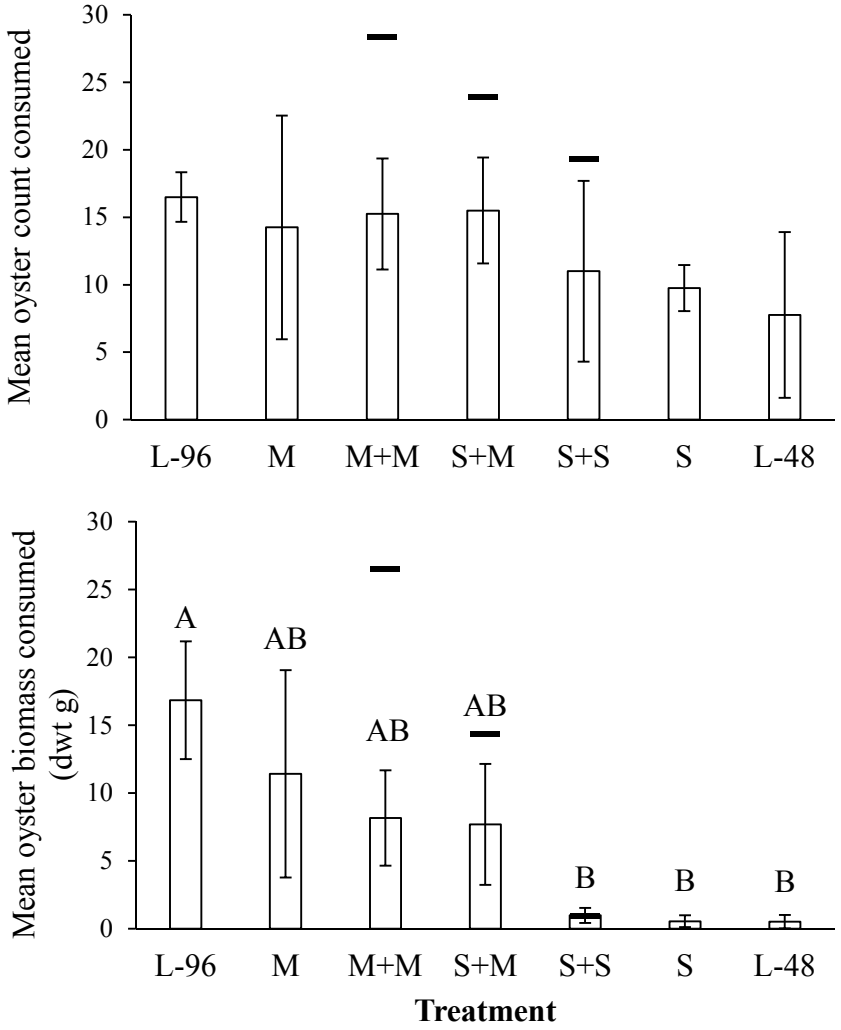

Figure 4.2. (a) Count of oysters consumed by treatment, (b) biomass of oysters consumed by treatment. Error bars ±SE. Solid horizontal dashes indicate expected consumption based on multiplicative risk model. Letters denote significant differences among treatments based on Tukey's post hoc test.

5. PREDATOR IMPACTS ON OYSTER CHLOROPHYLL A DRAWDOWN

5.1 Abstract

Biotic interactions can have strong influences on the provision of ecosystem functions. As ecosystem engineers, bivalves often provide disproportionate contributions to ecosystem function. They also utilize numerous morphological and behavioral responses to reduce predation, which can include changes in their filtration rates. To test the response of *Crassostrea virginica* reef filtration rates to the presence of predators, groups of oysters from two size classes were exposed to varying degrees of predation risk from *Callinectes sapidus* and *Panopeus herbstii* in outdoor mesocosms. Water column chlorophyll *a* concentrations and crab behavior were measured over the duration of the experiment. Predation risk had no effect on oyster reef drawdown of chlorophyll *a*. Our results suggest that this important ecosystem function of oyster reefs is not mediated by behaviorally induced predator effects. Therefore, efforts to model how oyster predators influence filtration rates and associated ecosystem services should focus primarily on the factors that influence oyster mortality rather than predator effects on oyster behavior.

5.2 Introduction

Through indirect interactions, predators can influence not only community structure (Hairston et al. 1960, Paine 1966, Menge 1995, Grabowski & Kimbro 2005) but also ecosystem functioning (Chapin, III et al. 1997, Schmitz 2008, Schmitz et al. 2008). For instance, when in

the presence of *Carcinus maenas* (green crab) effluent, the trophic efficiency of *Nucella lapillus* (dogwhelk) feeding on *Semibalanus balanoides* (acorn barnacle) is reduced by 44% to 76% (Trussell et al. 2006). In this case, the indirect effect of predator cue presence reduced energy transfer from the basal resource (barnacle) to higher trophic levels. However, not all predator effects necessarily influence ecosystem functioning. Identifying the effects that are of critical importance to ecosystem functioning is necessary to determine when biotic and physical processes are integrally coupled and to help inform efforts to manage these systems (Levin 1992, Kremen 2005).

As ecosystem engineers and filter feeders, bivalves perform a wide array of ecosystem functions (Jones et al. 1994). For instance, similar to herbivores, they promote trophic transfer and nutrient cycling. They also support aquatic-specific functions like enhancing benthic-pelagic coupling and water clarity. But some species, such as oysters and mussels, also influence a third suite of functions by creating habitat, which in turn promotes secondary and tertiary productivity, as well as, stabilizes sediments by baffling water flow. The vast majority of these functions stem directly or indirectly from oysters filtering the water because this process affects oyster growth and habitat provisioning (Grabowski & Peterson 2007). Therefore, factors that influence bivalve filtration are likely to have disproportionately large effects on the ecosystem functions that they provide.

Predation risk is one factor known to potentially affect filtration in bivalves (Kulakovskii & Lezin 2002, Smee & Weissburg 2006, Naddafi et al. 2007). Bivalves employ a wide range of behavioral and morphological responses to the risk of predation. Generally, induced morphological changes result in prey organisms that are more challenging for predators to capture and handle (Elner & Hughes 1978, Hughes & Seed 1995), while behavioral changes

result in more cryptic or inaccessible individuals that are less likely to be identified and caught by predators (Griffiths & Richardson 2006, Smee & Weissburg 2006). Morphological change in bivalves primarily includes thicker or stronger shells and byssal threads (Côté 1995, Reimer & Harms-Ringdahl 2001, Newell, Kennedy, et al. 2007, Neo & Todd 2011, Johnson & Smee 2012).

Behaviorally, bivalves respond to the threat of predation in several ways. For instance, predators induce *Macoma balthica* (Baltic clam) to burrow deeper, thereby reducing their risk of being captured (Griffiths & Richardson 2006). They also found that clam burrowing depth changes with predator identity and predator diet, with the strongest response stemming from exposure to crab predators that have consumed *M. balthica* (Griffiths & Richardson 2006). Mussels also modify their behavior when exposed to predation risk: *M. edulis* responds to the threat of lobster predators by aggregating (Côté & Jelnikar 1999), and *Brachidontes variabilis* (black mussel) occupies smaller shelters in the presence of injured conspecifics (Shin et al. 2008). In addition, bivalves alter their filtration rates to avoid being detected by predators. For instance, *M. balthica* decreases filter feeding when exposed to injured conspecifics or blue crabs, thereby by decreasing the production of its odoplume (Smee & Weissburg 2006). Furthermore, exposure to predators induces *M. edulis* and *Dreissena polymorpha* (zebra mussel) to reduce filter feeding (Kulakovskii & Lezin 2002, Naddafi et al. 2007). Meanwhile, predation risk reduced *D. polymorpha* drawdown of phytoplankton biomass by 25% (Naddafi et al. 2007). Thus, by modifying prey filter feeding, predators indirectly affect linkages among food web dynamics, animal behavior and ecosystem processes (Schmitz et al. 2008).

C. virginica is a reef forming bivalve and a prominent provider of many ecosystem services such as removing excess nitrogen, providing habitat for commercially and recreationally

valuable species, and stabilizing shorelines (Breitburg et al. 2000, Mann 2000, Coen & Luckenbach 2000, Newell et al. 2002, Peterson et al. 2003, Newell, Kemp, et al. 2007, Piehler & Smyth 2011). *C. virginica* is capable of decreasing water column chlorophyll *a* concentrations by more than 75% (Dame et al. 1984) and can increase average denitrification rates two to six times the rate on unstructured mudflats (Piehler & Smyth 2011). These and other water quality services have been estimated to contribute as much as half of the total ecosystem service value provided by *C. virginica* (Grabowski et al. 2012). If *C. virginica* responds in the same way to predator cues as the similarly conspicuous zebra mussel, predators could have a dramatic effect on the ecosystem functions and services that oysters provide. To investigate the effect predators have on the filtration rate of *C. virginica*, oyster removal of chlorophyll *a* from the water column was quantified when oysters were exposed to varying types of *Callinectes sapidus* (blue crab) and *Panopeus herbstii* (mud crab) cues (physical manipulation, predator scents, unconfined blue crab, unconfined mud crab, and unconfined blue crab and mud crab; Table 5.1), the two dominant oyster predators in this system (O'Connor et al. 2008, Rindone & Eggleston 2011). By measuring oyster drawdown of phytoplankton biomass across two oyster size classes in the presence and absence of predator cues, I aimed to quantify the magnitude of effects that these predators may have on oyster filtration, and consequently their potential influence on associated ecosystem services.

5.3 Methods

Juvenile individual *C. virginica* (27.8 ± 5.1 mm) were obtained from the Horn Point Hatchery, MD and adult *C. virginica* were collected from Hoop Hole Creek, NC (91.5 ± 11.4 mm). Adult oysters were separated and scraped clean, and then placed in a flow through tank supplied with unfiltered seawater from Bogue Sound, NC along with the juvenile oysters. *P.*

herbstii (35 ± 4 mm) were obtained from intertidal oyster reefs at Hoop Hole Creek, NC and *C. sapidus* (123 ± 12 mm) were caught in crab pots from Bogue Sound, NC. All crabs were collected in July of 2013 and maintained in flow-through tanks for at least 24 hours before being used in experiments. All species were housed in separate tanks to prevent predation while acclimatizing. Crabs were fed shucked oysters ad libitum every other day with a final feeding ending approximately 12 hours before the start of a trial. All organisms were measured and the crabs sexed.

A total of seven treatments were used in this experiment: predator and oyster free (i.e., settlement, oyster only, physical manipulation, predator scents, unconfined blue crab, unconfined mud crab, and unconfined blue crab and mud crab together; Table 5.1). The settlement treatment contained no oysters or predators and served as a control for the natural loss of chlorophyll *a* through settling. The oyster only and physical manipulation treatments contained only oysters, with the manipulation treatment also receiving physical disturbance without predator scent to simulate tactile predatory stimuli. This was accomplished by lightly dragging a weighted polyester glove over all oysters, with manipulation rates determined from crab-oyster contact rates in preliminary trials. The scent treatment contained both blue and mud crabs caged separately for the duration of the experiment. The unconfined mud crab, unconfined blue crab, and unconfined mud crab and blue crab consisted of uncaged crabs of a single or mixed species. Each treatment was replicated three times for each of the oyster size classes, resulting in a total of 42 individual trials.

Trials were conducted in tanks (90cm x 90cm x 22.5cm) filled to a uniform depth of 14cm. Tank bottoms were covered with a layer of clean oyster shell to provide shelter and a more natural substrate for the crabs. Oysters were secured to ceramic tiles (11cm x 11cm) with

Z-Spar Marine Epoxy (Splash Zone A-788), which were then attached to cement pavers (15 cm x 15 cm) using silicone glue. The tiles were kept in flow-through tanks while the epoxy cured for a minimum of 12 hours. Crabs were placed in the experimental tanks approximately 12 hours before the start of a trial to saturate the mesocosms with the scent of predators. During this period, water flow through the tank was approximately 4 l min^{-1}. Blue crabs (1 $replicate^{-1}$) were put in plastic mesh cages during this period to prevent predation on mud crabs, but released to roam freely within the tank at the inception of each trial. Mud crabs (8 $replicate^{-1}$) were placed in their respective tanks. For the scent only treatment, blue crabs and mud crabs were put into separate plastic mesh cages for the entire duration. Water flow was discontinued immediately prior to starting a trial, and an aquarium pump (160 gallons min^{-1}) was used to circulate and oxygenate water. Juvenile oyster trials contained 54 oysters in each replicate, whereas adult oyster trials contained 10 oysters in each.

Treatments were run concurrently for each oyster size class. Trials began approximately 30 minutes after sunrise. Oyster tiles were placed in their respective tanks, the appropriate crabs were released from their cages, and mesh coverings (2 cm stretch length) were clipped to the rim of the tanks to prevent escape. While each treatment was running, a water sample was taken approximately 5 cm below the water surface every sixty minutes. The manipulation treatment was handled every thirty minutes as determined by preliminary trials. When water sampling and manipulation were scheduled to occur at the same time, the water sample was taken first to reduce any effect of resuspension on the sample. After four hours, crabs were removed and oyster mortality was recorded.

Chlorophyll *a* drawdown was quantified through repeated water sampling of experimental trials through time. Using a 0.7 µm GFF filter, 100 mL of each water sample was filtered. Filters

were extracted in 7 mL of 90% acetone solution and analyzed for chlorophyll- *a* using a fluorometer (Welschmeyer 1994). Chlorophyll *a* concentrations were normalized by converting them to proportion remaining from T_0. A line was fitted through time for these points for each replicate and the slopes (proportion chlorophyll *a* removed $hour^{-1}$) were analyzed in a two-way ANOVA with treatment and oyster size as fixed factors. Slopes passed Levene's test for equality of variances. Two orthogonal a priori contrasts were established using the available degrees of freedom from the two-way ANOVA. First, the settlement control was compared against all treatments containing oysters to verify that removal of chlorophyll *a* by oysters was different than background removal. Then, I tested for differences among all treatments containing oysters.

5.4 Results

Predator cues did not reduce removal of water column chlorophyll *a* by oysters. The interaction between oyster size and predator treatment ($F_{6,28} = 1.37$, $P = 0.260$), as well as, the main effect of oyster size ($F_{1,28} = 1.78$, $P = 0.193$) were not significant. There was a significant effect of treatment ($F_{6,28} = 14.87$, $P = <0.001$) on chlorophyll *a* removal. A priori contrasts revealed a significant difference between the settlement treatment and all treatments with oyster present ($F_{1,28} = 88.50$, $P = <0.001$). However, there was no difference among any of the treatments with oyster present ($F_{5,28} = 0.10$, $P = 0.992$, Table 5.2).

Oyster mortality in medium oyster treatments occurred in three of the six predator treatments. Mean (SE) percent oyster mortality was 0.6% (±0.6) in the oyster only treatment, 0.6% (±0.6) in the blue and mud crab treatment, and 4.9% (±0.6) in the mud crab treatment. There was no oyster mortality in the large oyster treatments

5.5 Discussion

Effects of predators on filtration rates have been demonstrated in several bivalve species including hard clams, blue mussels, and zebra mussels (Kulakovskii & Lezin 2002, Smee & Weissburg 2006, Naddafi et al. 2007). Contrary to this evidence, I found that predators had no impact on oyster removal of chlorophyll *a* from the water column. My results suggest that the near-term delivery of ecosystem functions and services by oyster reefs may be decoupled from non-consumptive predator-prey interactions (i.e., predator avoidance behavior).

The normal feeding behavior of bivalves includes varying degrees of long-term temporal variation. For instance, over a 26-hour period, *Argopecten irradians* (bay scallop) maintains a constant or steadily decreasing then stabilizing filtration rate, characterized by minor variation and no periods when it ceases filter feeding (Palmer 1980). Alternatively, both *Crassostrea gigas* and *C. virginica* exhibit much higher variation, including periods of negligible filtration activity (Palmer 1980, Gerdes 1983). Hourly variation for the mussels *Choromytilus meridionalis* and *M. edulis* is in between these two extremes (Griffiths 1980, Kulakovskii & Lezin 2002). The high degree of filtration rate variation oysters are capable of may contribute to the lack of a response to predator presence. In addition, short-term disruptions in oyster filter feeding rates may be compensated for by later increasing filtration rates.

Predator and prey identities can determine the outcome of biotic interactions (O'Connor et al. 2008). For instance, the hard clam is cryptic and is typically found in low densities, so that a clam that responds to the threat of predation by reducing its filtration rate will likely enhance its chance of surviving (Smee & Weissburg 2006), and although mussels are capable of forming reefs and are found in high densities, they are also more likely to occur in small aggregations and rely on their physical environment for protection (Uryu et al. 1996). In contrast, oysters are conspicuous and typically found in high densities. To realize an increase in survival through

restricting filtration activity, all oyster, regardless of size, would have to stop filtering on a given section of reef and as the dominate predators are not capable of consuming the largest oyster, this may be unlikely. In addition, predatory crabs utilize oysters for both food and shelter. This close spatial association may also erode the value of a fear response. In contrast, as a slow moving predator that relies heavily on olfaction to locate prey, *Asterias rubens* (common starfish) may be more likely to illicit the fear response observed in blue mussels (Kulakovskii & Lezin 2002).

Reduction of filtration rates by oysters smaller than those used in this study are unlikely to greatly reduced total reef filtration. The relative contributions to total reef filtration by oyster size can be estimated using local intertidal reef survey data (supplemental data), an intertidal oyster length to dry weight relationship (Copeland & Hoese 1966, White et al. 1988, Powell et al. 1995), and a dry weight to filtration rate relationship (Riisgård 1988). From this it can be estimated that, filtration by oysters smaller than 20 mm accounted for only 5% of total reef filtration despite representing 56% of oysters by count. If oysters of this size class were to decrease filtration rates in response to the presence of a predator similar to that exhibited by zebra mussels (25% reduction), total reef filtration would decrease by only ~1% (Naddafi et al. 2007). Given the high degree of predator residency on these reefs, as opposed to the predators used in any comparable study (Kulakovskii & Lezin 2002, Griffiths & Richardson 2006, Smee & Weissburg 2006, Naddafi et al. 2007), and the ubiquity of conspecific settlement in oysters leading to a scent saturated and conspicuous environment, even this small reduction seems unlikely.

C. virginica grow quickly, thereby reducing predation risk by achieving greater size. They may exhibit defensive behaviors in the presence of other predators, but I found no evidence

that they respond to either tactile or chemosensory cues of two common predatory crabs. While individual oysters may yet display a change in filtration in the presence of predators, my results suggest that predators do not appear to be impact oyster filter feeding at larger scales (i.e., oyster reef patches).

In restoration and ecosystem service contexts, average filtration is often assumed to be a constant based on a number of environmental and demographic variables (Riisgård 1988, Dame 1993, Newell & Langdon 1996). If my finding that oysters do not reduce filtration to avoid predation holds for other oyster predators, the direct effects of predators on oyster filter feeding behavior do not need to be accounted for in reef filtration estimates. However, top predators such as toadfish and blue crabs that induce mud crabs to hide and consume fewer oysters (Grabowski 2004, Grabowski et al. 2008) may indirectly affect oyster long-term filtration rates on reefs, especially if reduced predation eventually results in greater adult oyster biomass on these reefs. Oyster reefs are also credited for performing a wide diversity of services that may be dependent on each other, such as serving as nursery habitat for juvenile fishes and mobile invertebrates like blue crabs and those services related to filter feeding. My results suggest that oyster predators and filter feeding behavior are decoupled. This finding implies that the nursery function of *C. virginica* reefs does not impair their potential to provide other key ecosystem services such as excess nitrogen removal and enhanced water quality.

My findings highlight the primacy of direct predation in mediating existing oyster reef filtration rates, including any effects that may indirectly modulate predation on oysters. The effects of predators present in this system (Grabowski 2004, Grabowski et al. 2008) do not extend to oyster behavior or effect their near-term delivery of resulting ecosystem function and services. However, any predator behaviors that have a direct or indirect effect on oyster

demographics, including changes in predation (Grabowski 2004), growth (Johnson & Smee 2012), fecundity (Johnson & Smee 2012), or recruitment, will affect reef filtration, and therefore, delivery of ecosystem function and services.

	Oyster	Chemical Cues	Physical Manipulation	Possible Predation
Blue and Mud Crabs	Yes	Both	Yes	Yes
Blue Crab	Yes	Blue Crab	Yes	Yes
Mud Crab	Yes	Mud Crab	Yes	Yes
Scent	Yes	Both	No	No
Oyster Only	Yes	No	No	No
Physical Manipulation	Yes	No	Yes	No
Settlement	No	No	No	No

Table 5.1. Presence of predation stimuli that may affect filtration rates by treatment.

		df	SS	MS	F	p
Size		1	0.002	0.002	1.78	0.193
Treatment		6	0.114	0.019	14.89	<0.0001
	all oyster vs settlement	1	0.114	0.114	88.50	<0.0001
	among oyster	5	0.001	0.001	0.10	0.992
Size x Treatment		6	0.011	0.002	1.37	0.260
Error		28	0.036	0.001		
Total		41	0.163			

Table 5.2. Two-way ANOVA of oyster size and predator treatment on filtration rates. *A priori* analysis (in gray) is based on values from the two-way analysis.

6. CONCLUSION

6.1 Summary of Results

Coastal systems are under increasing anthropogenic influence, including ocean acidification, warming, nutrient pollution, and land-use change (Vitousek et al. 1997). The human-induced changes are altering the function of coastal systems and may result in significant negative ecological impacts. Predator-prey relationships can be an important mediating factor of environmental change and ecosystem function. However, not all predator-prey relationships will have a significant influence on ecosystem function. Determining which relationships are the most important to ecosystem function can help identify representative relationships and behaviors (chapters 3, 4, & 5), contribute to predictions of community and ecosystem level effects (chapters 2 & 3), and manage ecosystem functions and services (chapters 3 & 5) in the face of environmental change.

Net calcification by *C. virginica* decreases with increasing acidification, however, *C. virginica* retains a positive net calcification rate even in undersaturated carbonate conditions as demonstrated in this dissertation and by Waldbusser et al. (2011). Furthermore, oyster shell strength appears unaffected by the acidification levels I tested. *P. herbstii* net calcification did not show an effect of acidification. This was partially due to the high variability observed in the calcification data, likely influenced by the stochastic growth patterns common to crustaceans. As a result, at the onset of the predation experiment (chapter 3), no great difference is present between the net calcification relationships of oyster and crabs in any treatment. That is to say, the

presumed relative strength of oyster and crabs appears comparable across acidification treatments during the predation phase and, as a result, change in calcification does not appear to influence predation rates.

Predation rates of *P. herbstii* on *C. virginica* were strongly affected by acidification. Significant differences existed between all acidification treatments and the reduced consumption coincided with less time spent handling prey and decreased attack persistence by *P. herbstii*. The decrease in consumption is likely a result of neurotransmitter dysfunction caused by increasing intracellular pH (Nilsson et al. 2012). This effect has been observed for a wide variety of behaviors across a number of taxa. The ubiquity of the effect, both in terms of affected behaviors and species, is of great concern for global food production and ecosystem function.

Stone crab predation on oysters appears to change with crab size. Smaller crabs, less than ~70 mm, are not capable of consuming the largest available oysters and, as a result, must pick and choose suitable prey from the wider available pool. Larger crabs are capable of breaking open any oyster they encounter and select oysters in a less discriminate way. I also observed reluctance in the largest crabs to prey on oysters, which may indicate a dietary shift in that size class away from oysters as a prey item. Because the medium crab size class in this study, 70 – 90 mm, preyed on oysters of all sizes, it may have a disproportionate effect on oyster reef structure and the ecosystem services they provide.

Oyster reef filtration is not affected by the presence of *C. sapidus* or *P. herbstii*, two common oyster predators. Numerous other bivalve species do exhibit the behavior of reducing filtration in the presence of predators in order to reduce scent plums and avoid attracting the predator. However, *C. virginica* seems to lack this behavior; possibly relying on the protection of

larger oysters to defend against predation. This is significant because filtration underpins all oyster ecosystem functions and services. If fear was a significant influence on filtration rates some functions, such as being a nursery for crustaceans and fishes, would hinder the delivery of other functions, such as nitrogen removal or shoreline stabilization. Instead oyster filtration rates can be considered a relatively stable variable, dependent on oyster demographics and physical factors such as temperature, water flow, and seston availability.

6.2 Variable Influence of Predator-Prey Relationships on Ecosystem Function

Predator-prey relationships can be an important mediating factor on the delivery of ecosystem functions and services. However, not all relationships will have a significant effect on all aspects of environment. Furthermore, persistence anthropogenic effects have altered environments in ways that may change how predator-prey relationships fit into the broader ecosystem and may alter their effect on ecosystem functions and services. The studies presented in this dissertation highlight the importance and variability of predator-prey relationships in an ecosystem context.

For instance, the presence of predators had no effect on *C. virginica* filtration, and therefore no effect on their delivery of numerous ecosystem functions. In contrast, the mud crab-oyster predator-prey relationship is critical to understanding the effect ocean acidification has on trophic efficiency in this system. Hypotheses formed on the basis of the biological effects of acidification, primarily of calcification but also metabolism, would suggest foraging outcomes either neutral or the opposite sign of those observed in this dissertation (chapter 3). The relative influence of predation on ecosystem functions can also vary significantly within a predator species as the presence of ecological redundancy and predation behaviors change across predator

sizes, as shown in the case of stone crab predation on oyster. Small stone crab behave much like a large mud crab and large stone crab show some evidence of not preferring oyster as a prey item. Alternatively, medium stone crab are very capable and willing oyster predators and can quickly reduce local reef complexity, an important factor in several ecosystem functions. Precise knowledge of the predator-prey relationships highlight areas where predators are likely to have significant effects on ecosystem function and where the effects of predation might not be as consequential.

6.3 Summation

My dissertation examined the importance of predator-prey relationships on a critical estuarine foundation habitat and considered how significant changes such as ocean acidification and warming might affect these interactions. The direct measurement of a reduction in consumption by a predator as a result of increased pCO_2 is the first result of that kind in marine systems. Clearly additional research is needed to corroborate and broaden my findings and to test a larger range of organisms. I found that acidification had a significant but relatively minor influence on calcification of oysters, but that behavioral change in the predatory crab was of far greater influence on the predator-prey dynamic of these species. Determining the "losers and winners" in a more acidic ocean is an ongoing effort and my work will help advance the consideration of ecological factors such as changing interactions in food webs. Oysters are being counted on to help restore coastal ecosystems. My findings regarding the influence of predators on oyster filtration and the importance of stone crabs (which are moving their range, likely in response to warming) present important examples of factors that must be considered when weighing the ability of habitats to affect ecosystem function. As a whole, my dissertation research provides a clearer picture of the future for oyster reefs in a fast-changing ocean.

7. APPENDIX

7.1 Supplementary data: Ocean acidification impairs crab foraging behavior

		Control-pCO_2	Moderate-pCO_2	High-pCO_2
MEASURED PARAMETERS				
pCO_2 (gas)	(ppm-v)	497	798	9487
	SD	6	13	63
	Range	490-506	776-819	9381-9586
Sal		31.79	31.69	31.79
	SD	0.42	0.84	0.43
	Range	30.50 - 33.00	21.70 - 33.20	30.70 - 33.30
T	(°C)	26.1	25.6	25.8
	SD	1.0	0.9	1.0
	Range	24.2 - 30.4	23.5 - 29.8	23.2 - 30.5
pH_m		8.20	8.04	7.05
	SD	0.11	0.08	0.09
	Range	7.92 - 8.54	7.79 - 7.79	6.84 - 7.98
TA	(µM)	2704	2799	3211
	SD	385	311	193
	Range	1731 - 3248	1861 - 3334	2801 - 3517
DIC	(µM)	2360	2549	3432
	SD	303	256	207
	Range	1590 - 2815	1784 - 3060	2963 - 3809
CALCULATED PARAMETERS				
pCO_2 (gas-e)	(ppm-v)	499	785	9274
	SD	114	154	2243
	Range	300 - 897	544 - 1110	5164 - 14600
pH_c		8.18	8.03	7.09
	SD	0.11	0.10	0.10
	Range	7.93 - 8.40	7.73 - 8.21	6.88 - 7.31
$[CO_3^{2-}]$	(µM)	270	207	32
	SD	81	54	8
	Range	104 - 428	73 - 316	17 - 53
$[HCO_3^-]$	(µM)	2076	2319	3137
	SD	242	216	188
	Range	1466 - 2568	1679 - 2796	2747 - 3435
$[CO_2]$ (SW)	(µM)	14	22	262

	SD	3	4	60
	Range	8 - 25	16 - 32	145 - 396
$\mathbf{\Omega_C}$		6.7	5.1	0.8
	SD	2.0	1.3	0.2
	Range	2.6 - 10.6	1.8 - 7.8	0.4 - 1.3

Supplement 1. Average measured and calculated parameters for pCO_2 treatments. Average measured pCO_2 of the mixed gases sparged into the experimental seawaters [$pCO_{2\,(gas)}$], salinity (Sal), temperature (T), pH (pH_m), total alkalinity (TA), and dissolved inorganic carbon (DIC). Average calculated pCO_2 of the mixed gases in equilibrium with the experimental seawaters [$pCO_{2\,(gas\text{-}e)}$], pH (pH_c), carbonate ion concentration [CO_3^{2-}], bicarbonate ion concentration [HCO_3^-], dissolved carbon dioxide ($[CO_2]_{SW}$), and calcite saturation state (Ω_C). "SD" is standard deviation of the mean.

REFERENCES

Amaral V, Cabral HN, Bishop MJ (2012) Effects of estuarine acidification on predator-prey interactions. Mar Ecol Prog Ser 445:117–127

Aronhime BR (2010) Predator-prey interaction in estuarine bivalves: size selection, effects of salinity, and indirect interactions. Louisiana State University

Aronhime BR, Brown KM (2009) The roles of profit and claw strength in determining mussel size selection by crabs. J Exp Mar Bio Ecol 379:28–33

Atkinson MJ, Bingman C (1998) Elemental composition of commercial seasalts. J Aquaric Aquat Sci 8:39–43

Beck MW (1995) Size-specific shelter limitation in stone crabs: A test of the demographic bottleneck hypothesis. Ecology 76:968–980

Beck MW, Brumbaugh RD, Airoldi L, Carranza A, Coen LD, Crawford C, Defeo O, Edgar GJ, Hancock B, Kay MC, Lenihan HS, Luckenbach MW, Toropova CL, Zhang G, Guo X (2011) Oyster reefs at risk and recommendations for conservation, restoration, and management. Bioscience 61:107–116

Beniash E, Ivanina A, Lieb NS, Kurochkin I, Sokolova IM (2010) Elevated level of carbon dioxide affects metabolism and shell formation in oysters *Crassostrea virginica*. Mar Ecol Prog Ser 419:95–108

Bibby R, Cleall-Harding P, Rundle S, Widdicombe S, Spicer J (2007) Ocean acidification disrupts induced defences in the intertidal gastropod *Littorina littorea*. Biol Lett 3:699–701

Brauner CJ, Baker DW (2009) Patterns of acid-base regulation during exposure to hypercarbia in fishes. In: Glass ML, Wood SC (eds) Cardio-Resporatory Control in Vertebrates. Springer Berlin Heidelberg, Berlin, Heidelberg, p 43–63

Breitburg DL, Coen LD, Luckenbach MW, Mann R, Posey M, Wesson JA (2000) Oyster reef restoration: convergence of harvest and conservation strategies. J Shellfish Res 19:371–377

Brewer PG (1997) Ocean chemistry of the fossil fuel CO_2 signal: the haline signal of "business as usual". Geophys Res Lett 24:1367–1369

Briffa M, la Haye K de, Munday PL (2012) High CO_2 and marine animal behaviour: potential mechanisms and ecological consequences. Mar Pollut Bull 64:1519–28

Brown GE, Adrian, Jr. JC, Lewis MG, Tower JM (2002) The effects of reduced pH on chemical alarm signalling in ostariophysan fishes. Can J Fish Aquat Sci 59:1331–1338

Brown SC, Cassuto SR, Loos RW (2009) Biomechanics of chelipeds in some decapod

crustaceans. J Zool 188:143–159

Brown KM, Haight ES (1992) The foraging ecology of the Gulf of Mexico stone crab *Menippe adina* (Williams et Felder). J Exp Mar Bio Ecol 160:67–80

Burreson EM, Stokes NA, Friedman CS (2000) Increased virulence in an introduced pathogen: *Haplosporidium nelsoni* (MSX) in the eastern oyster *Crassostrea virginica*. J Aquat Anim Health 12:1–8

Cai W-J, Wang Y (1998) The chemistry, fluxes, and sources of carbon dioxide in the estuarine waters of the Stilla and Altamaha Rivers, Georgia. Limnol Oceanogr 43:657–668

Cameron JN (1989) Post-moult calcification in the blue crab, *Callinectes sapidus*: timig and mechanism. J Exp Biol 143:285–304

Canadell JG, Quere C Le, Raupach MR, Field CB, Buitenhuis ET, Ciais P, Conway TJ, Gillett NP, Houghton RA, Marland G (2007) Contributions to accelerating atmospheric CO_2 growth from economic activity, carbon intensity, and efficiency of natural sinks. Proc Natl Acad Sci

Carpenter SR, Kitchell JF, Hodgson JR (1985) Cascading trophic interactions and lake productivity. Bioscience 35:634–639

Carriker MR, Swann CP, Prezant RS, Counts, III CL (1991) Chemical elements in the aragonitic and calcitic microstructural groups of shell of the oyster *Crassostrea virginica*: a proton probe study. Mar Biol 109:287–297

Chakravarti LJ, Cotton PA (2014) The effects of a competitor on the foraging behaviour of the shore crab Carcinus maenas. PLoS One 9:e93546

Chapin, III FS, Walker BH, Hobbs RJ, Hooper DU, Lawton JH, Sala OE, Tilman D (1997) Biotic control over the functioning of ecosystems. Science (80-) 277:500–504

Chave KE (1954) Aspects of the biogeochemistry of magnesium 1. Calcareous marine organisms. J Geol 62:266–283

Coen LD, Luckenbach MW (2000) Developing success criteria and goals for evaluating oyster reef restoration: ecological function or resource exploitation? Ecol Eng 15:323–343

Coen LD, Luckenbach MW, Breitburg DL (1999) The role of oyster reefs as essential fish habitat: a review of current knowledge and some new perspectives. In: American Fisheries Society Symposium.p 438–454

Copeland BJ, Hoese HD (1966) Growth and mortality of the American oyster, *Crassostrea virginica*, in high salinity shallow bays in Central Texas. Publ Inst Mar Sci 11:149–158

Côté IM (1995) Effects of predatory crab effluent on byssus production in mussels. J Exp Mar

Bio Ecol 188:233–241

Côté IM, Jelnikar E (1999) Predator-induced clumping behaviour in mussels (*Mytilus edulis* Linnaeus). J Exp Mar Bio Ecol 235:201–211

Creel S, Christianson DA, Winnie, Jr. JA (2011) A survey of the effects of wolf predation risk on pregnancy rates and calf recruitment in elk. Ecol Appl 21:2847–2853

Crenshaw MA (1972) The inorganic composition of molluscan extrapallial fluid. Biol Bull 143:506–512

Crenshaw MA, Neff JM (1969) Decalcification at the mantle-shell interface in molluscs. Am Zool 9:881–885

Cripps IL, Munday PL, McCormick MI (2011) Ocean acidification affects prey detection by a predatory reef fish. PLoS One 6:e22736

Dame RF (1993) The role of bivalve filter feeder material fluxes in estuarine ecosystems. In: Dame RF (ed) Bivalve Filter Feeder in Estarine and Coastal Ecosystem Processes. Springer-Verlang, Berlin, Heidelberg, p 254–269

Dame RF, Zingmark RG, Haskin E (1984) Oyster reefs as processors of estuarine materials. J Exp Mar Bio Ecol 83:239–247

Dawkins R, Krebs JR (1979) Arms races between and within species. Proc R Soc B 205:489–511

Day RW, Quinn GP (1989) Comparisons of treatments after an analysis of variance in ecology. Ecol Monogr 59:433–463

Devine BM, Munday PL, Jones GP (2012a) Homing ability of adult cardinalfish is affected by elevated carbon dioxide. Oecologia 168:269–276

Devine BM, Munday PL, Jones GP (2012b) Rising CO_2 concentrations affect settlement behaviour of larval damselfishes. Coral Reefs 31:229–238

Dickinson GH, Ivanina A V., Matoo OB, Pörtner HO, Lannig G, Bock C, Beniash E, Sokolova IM (2012) Interactive effects of salinity and elevated CO_2 levels on juvenile eastern oysters, *Crassostrea virginica*. J Exp Biol 215:29–43

Dissanayake A, Clough R, Spicer JI, Jones MB (2010) Effects of hypercapnia on acid-base balance and osmo-/iono-regulation in prawns (Decapoda: Palaemonidae). Aquat Biol 11:27–36

Dissanayake A, Ishimatsu A (2011) Synergistic effects of elevated CO_2 and temperature on the metabolic scope and activity in a shallow-water coastal decapod (*Metapenaeus joyneri*; Crustacea: Penaeidae). ICES J Mar Sci 68:1147–1154

Dixson DL, Munday PL, Jones GP (2010) Ocean acidification disrupts the innate ability of fish to detect predator olfactory cues. Ecol Lett 13:68–75

Eggleston DB (1990) Functional responses of blue crabs *Callinectes sapidus* Rathbun feeding on juvenile oysters *Crassostrea virginica* (Gmelin): effects of predator sex and size, and prey size. J Exp Mar Bio Ecol 143:73–90

Elner RW, Hughes RN (1978) Energy maximization in the diet of the shore crab, *Carcinus maenas*. J Anim Ecol 47:103–116

Emlen JM (1966) The role of time and energy in food preference. Am Nat 100:611–617

Estes JA, Palmisano JF (1974) Sea otters: their role in structuring nearshore communities. Science (80-) 185:1058–1060

Findlay HS, Kendall MA, Spicer JI, Widdicombe S (2009) Post-larval development of two intertidal barnacles at elevated CO_2 and temperature. Mar Biol 157:725–735

Finelli CM, Pentcheff ND, Zimmer RK, Wethey DS (2000) Physical constraints on ecological processes: a field test of odor-mediated foraging. Ecology 81:784–797

Fodrie FJ, Kenworthy MD, Powers SP (2008) Unintended facilitation between marine consumers generates enhanced mortality for their shared prey. Ecology 89:3268–3274

Fodrie FJ, Rodriguez AB, Baillie CJ, Brodeur MC, Coleman SE, Gittman RK, Keller DA, Kenworthy MD, Poray AK, Ridge JT, Theuerkauf EJ, Lindquist NL (2014) Classic paradigms in a novel environment: inserting food web and productivity lessons from rocky shores and saltmarshes into biogenic reef restoration. J Appl Ecol 51:1314–1325

Frankenburg D (1995) Report of North Carolina Blue Ribbon Advisory Council on Oysters. North Carolina Department of Enviornmen, Health, and Natural Resources, Raleigh, NC

Gattuso J-P, Frankignoulle M, Bourge I, Romaine S, Buddemeier RW (1998) Effect of calcium carbonate saturation of seawater on coral calcification. Glob Planet Change 18:37–46

Gazeau F, Quiblier C, Jansen JM, Gattuso J-P, Middelburg JJ, Heip CHR (2007) Impact of elevated CO_2 on shellfish calcification. Geophys Res Lett 34:L07603

Geraldi NR (2015) Prey size structure diminishes cascading effects by increasing interference competition and predation among prey. Ecology

Gerdes D (1983) The Pacific oyster *Crassostrea gigas* Part I. Feeding behaviour of larvae and adults. Aquaculture 31:195–219

Gerhart SD, Bert TM (2008) Life-history aspects of stone crabs (genus *Menippe*): size at maturity, growth, and age. J Crustac Biol 28:252–261

Grabowski JH (2004) Habitat complexity disrupts predator-prey interactions but not the trophic cascade on oyster reefs. Ecology 85:995–1004

Grabowski JH, Brumbaugh RD, Conrad RF, Keeler AG, Opaluch JJ, Peterson CH, Piehler MF, Powers SP, Smyth AR (2012) Economic valuation of ecosystem services provided by oyster reefs. Bioscience 62:900–909

Grabowski JH, Hughes AR, Kimbro DL (2008) Habitat complexity influences cascading effects of multiple predators. Ecology 89:3413–3422

Grabowski JH, Hughes AR, Kimbro DL, Dolan MA (2005) How habitat setting influences restored oyster reef communities. Ecology 86:1926–1935

Grabowski JH, Kimbro DL (2005) Predator-avoidance behavior extends trophic cascades to refuge habitats. Ecology 86:1312–1319

Grabowski J, Peterson C (2007) Restoring oyster reefs to recover ecosystem services. In: Theoretical Ecology Series.

Grabowski JH, Powers SP (2004) Habitat complexity mitigates trophic transfer on oyster reefs. Mar Ecol Prog Ser 277:291–295

Green SJ, Akins JL, Maljković A, Côté IM (2012) Invasive lionfish drive Atlantic coral reef fish declines (SJ Goldstien, Ed.). PLoS One 7:e32596

Griffiths RJ (1980) Filtration, repiration and assimilation in the black mussel *Choromytilus meridionalis*. Mar Ecol Prog Ser 3:63–70

Griffiths CL, Richardson CA (2006) Chemically induced predator avoidance behaviour in the burrowing bivalve *Macoma balthica*. J Exp Mar Bio Ecol 331:91–98

Grizzle RE, Greene JK, Coen LD (2008) Seston removal by natural and constructed intertidal eastern oyster (*Crassostrea virginica*) reefs: a comparison with previous laboratory studies, and the value of in situ methods. Estuaries and Coasts 31:1208–1220

Guinotte JM, Fabry VJ (2008) Ocean acidification and its potential effects on marine ecosystems. Ann N Y Acad Sci 1134:320–342

Hairston NG, Smith FE, Slobodkin LB (1960) Community structure, population control, and competition. Am Nat 94:421–425

Harley CDG (2011) Climate change, keystone predation, and biodiversity loss. Science (80-) 334:1124–1127

Haskin HH, Ford SE (1982) *Haplosporidium nelsoni* (MSX) on Delaware Bay seed oyster beds: a host-parasite relationship along a salinity gradient. J Invertebr Pathol 40:388–405

Hauxwell J, Cebrián J, Valiela I (2003) Eelgrass *Zostera marina* loss in temperate estuaries: relationship to land-derived nitrogen loads and effect of light limitation imposed by algae. Mar Ecol Prog Ser 247:59–73

Hoegh-Guldberg O, Cai R, Poloczanska ES, Brewer PG, Sundby S, Hilmi K, Fabry VJ, Jung S (2014) 2014: The Ocean. In: Barros V, Field C, Dokken D, Mastrandrea M, Mach K, Bilir T, Chatterjee M, Ebi K, Estrada Y, Genova R, Girma B, Kissel E, Levy A, MacCracken S, Mastrandrea P, White L (eds) Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part B: Regional Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, united Kingdom and New York, NY, USA, p 1655–1731

Hofmann GE, Smith JE, Johnson KS, Send U, Levin LA, Micheli F, Paytan A, Price NN, Peterson B, Takeshita Y, Matson PG, Crook ED, Kroeker KJ, Gambi MC, Rivest EB, Frieder CA, Yu PC, Martz TR (2011) High-erequency dynamics of ocean pH: a multi-ecosystem comparison (W-C Chin, Ed.). PLoS One 6:e28983

Hollebone AL, Hay ME (2007) An invasive crab alters interaction webs in a marine community. Biol Invasions 10:347–358

Huffaker CB (1958) Experimental studes on predation: dispersion factors and predatory-prey oscillations. Hilgardia 27:343–383

Hughes AR, Grabowski JH (2006) Habitat context influences predator interference interactions and the strength of resource partitioning. Oecologia 149:256–264

Hughes RN, Seed R (1981) Size selection of mussels by the blue crab *Callinectes sapidus*: energy maximizer or time minimizer? Mar Ecol Prog Ser 6:83–89

Hughes RN, Seed R (1995) Behavioural mechanisms of prey selection in crabs. J Exp Mar Bio Ecol 193:225–238

Johnson KD, Smee DL (2012) Size matters for risk assessment and resource allocation in bivalves. Mar Ecol Prog Ser 462:103–110

Jones CG, Lawton JH, Shachak M (1994) Organisms as ecosystem engineers. Oikos 69:373–386

Juanes F (1992) Why do decapod crustaceans prefer small-sized molluscan prey? Mar Ecol Prog Ser 87:239–249

Korringa P (1951) On the nature and function of “chalky” deposits in the shell of *Ostrea edulis* Linnaeus. Proc Calif Acad Sci 27:133–158

Kremen C (2005) Managing ecosystem services: what do we need to know about their ecology? Ecol Lett 8:468–479

Kroeker KJ, Kordas RL, Crim RN, Singh GG (2010) Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. Ecol Lett 13:1419–1434

Kulakovskii EE, Lezin PA (2002) Filtration activity of the blue mussel, *Mytilus edulis* (Mytilda, Mytilidae), under influence of the starfish, *Asterias rubens* (Forcipulata, Asteriidae). Zool Zhurnal 81:292–297

la Haye KL de, Spicer JI, Widdicombe S, Briffa M (2011) Reduced sea water pH disrupts resource assessment and decision making in the hermit crab *Pagurus bernhardus*. Anim Behav 82:495–501

la Haye KL de, Spicer JI, Widdicombe S, Briffa M (2012) Reduced pH sea water disrupts chemo-responsive behaviour in an intertidal crustacean. J Exp Mar Bio Ecol 412:134–140

Landes A, Zimmer M (2012) Acidification and warming affect both a calcifying predator and prey, but not their interaction. Mar Ecol Prog Ser 450:1–10

Langdon C (2005) Effect of elevated pCO_2 on photosynthesis and calcification of corals and interactions with seasonal change in temperature/irradiance and nutrient enrichment. J Geophys Res 110:C09S07

Langdon C, Takahashi T, Sweeney C, Chipman D, Goddard J, Marubini F, Aceves H, Barnett H, Atkinson MJ (2000) Effect of calcium carbonate saturation state on the calcification rate of an experimental coral reef. Global Biogeochem Cycles 14:639–654

Laundre JW, Hernandez L, Ripple WJ (2010) The landscape of fear: ecological implications of being afraid. Open Ecol J 3:1–7

Leduc AOHC, Noseworthy MK, Adrian JC, Brown GE (2003) Detection of conspecific and heterospecific alarm signals by juvenile pumpkinseed under weak acidic conditions. J Fish Biol 63:1331–1336

Levin SA (1992) The problem of pattern and scale in ecology: the Robert H. MacArthur Award Lecture. Ecology 73:1943–1967

Lewis, Wallace (1998) CO2SYS: Program developed for CO_2 system calculations, ORNL/CDIAC-105.

Lindberg WJ, Frazer TK, Stanton GR (1990) Population effects of refuge dispersion for adult stone crabs (Xanthidae, *Menippe*). Mar Ecol Prog Ser 66:239–249

Ling SD (2008) Range expansion of a habitat-modifying species leads to loss of taxonomic diversity: a new and impoverished reef state. Oecologia 156:883–894

Long WC, Swiney KM, Harris C, Page HN, Foy RJ (2013) Effects of ocean acidification on juvenile red king crab (*Paralithodes camtschaticus*) and tanner crab (*Chionoecetes bairdi*)

growth, condition, calcification, and survival (S Dupont, Ed.). PLoS One 8:e60959

Luckenbach MW, Coen LD, Ross PGJ, Stephen JA (2005) Oyster reef habitat restoration: relationships between oyster abundance and community development based on two studies in Virginia and South Carolina. J Coast Res 40:64–78

Lurling M, Scheffer M (2007) Info-disruption: pollution and the transfer of chemical information between organisms. Trends Ecol Evol 22:374–379

MacArthur RH, Pianka ER (1966) On Optimal Use of a Patchy Environment. Am Nat 100:603–609

MacDonald J, Freer A, Cusack M (2010) Alignment of crystallographic *c*-Axis throughout the four distinct microstructural layers of the oyster *Crassostrea gigas*. Cryst Growth Des 10:1243–1246

Mann R (2000) Restoring the oyster reef communities in the Chesapeake Bay: a commentary. J Shellfish Res 19:335–339

McCormick MI, Watson S-A, Munday PL (2013) Ocean acidification reverses competition for space as habitats degrade. Sci Rep 3:3280

McDermott JJ, Flower FB (1952) Preliminary studies on the common mud crabs on oyster beds of Delaware Bay. Natl Shellfish Assoc Conv Addresses:47–50

McDonald MR, McClintock JB, Amsler CD, Rittschof D, Angus RA, Orihuela B, Lutostanski K (2009) Effects of ocean acidification over the life history of the barnacle Amphibalanus amphitrite. Mar Ecol Prog Ser 385:179–187

Menge BA (1995) Indirect effects in marine rocky intertidal interaction webs : patterns and importance. Ecol Monogr 65:21–74

Menzel RW, Hopkins SH (1956) Crabs as predators of oysters in Louisiana. Proc Natl Shellfish Assoc 46:177–184

Menzel RW, Nichy FE (1958) Studies of the distribution and feeding habits of some oyster predators in Alligator Habor, Florida. Bull Mar Sci 8:125–145

Meyer DL (1994) Habitat partitioning between the xanthid crabs *Panopeus herbstii* and *Eurypanopeus depressus* on intertidal oyster reefs (*Crassostrea virginica*) in southeastern North Carolina. Estuaries 17:674–679

Meyer DL, Townsend EC, Thayer GW (1997) Stabilization and erosion control value of oyster cultch for intertidal marsh. Restor Ecol 5:93–99

Milinski M (1982) Optimal foraging: the influence of intraspecific competition on diet selection. Behav Ecol Sociobiol 11:109–115

Miller AW, Reynolds AC, Sobrino C, Riedel GF (2009) Shellfish face uncertain future in high CO_2 world: influence of acidification on oyster larvae calcification and growth in estuaries (Z Finkel, Ed.). PLoS One 4:e5661

Mills LS, Zimova M, Oyller J, Running S, Abatzoglou JT, Lukacs PM (2013) Camouflage mismatch in seasonal coat color due to decreased snow duration. Proc Natl Acad Sci 110:7360–7365

Mucci A (1983) The solubility of calcite and aragonite in sea water at various salinities, temperatures, and one atmospheric total pressure. Am J Sci 283:780–799

Naddafi R, Eklöv P, Pettersson K (2007) Non-lethal predator effects on the feeding rate and prey selection of the exotic zebra mussel *Dreissena polymorpha*. Oikos 116:1289–1298

Nelson EH, Matthews CE, Rosenheim JA (2004) Predators reduce prey population growth by inducing changes in prey behavior. Ecology 85:1853–1858

Neo ML, Todd PA (2011) Predator-induced changes in fluted giant clam (*Tridacna squamosa*) shell morphology. J Exp Mar Bio Ecol 397:21–26

Newell RIE, Cornwell JC, Owens MS (2002) Influence of simulated bivalve biodeposition and microphytobenthos on sediment nitrogen dynamics: a laboratory study. Limnol Oceanogr 47:1367–1379

Newell RIE, Fisher TR, Holyoke RR, Cornwell JC (2005) Influence of Eastern Oysters on Nitrogen and Phosphorus Regeneration in Chesapeake Bay, USA. In: Dame R, Olenin S (eds) The Comparative Roles of Suspension Feeders in Ecosystems. Springer, Netherlands, p 93 – 120

Newell RIE, Kemp WM, Hagy III JD, Cerco CF, Testa JM, Boynton WR (2007) Top-down control of phytoplankton by oysters in Chesapeake Bay, USA: Comment on Pomeroy et al. (2006). Mar Ecol Prog Ser 341:293–298

Newell RIE, Kennedy VS, Shaw KS (2007) Comparative vulnerability to predators, and induced defense responses, of eastern oysters *Crassostrea virginica* and non-native *Crassostrea ariakensis* oysters in Chesapeake Bay. Mar Biol 152:449–460

Newell RIE, Koch EW (2004) Modeling seagrass density and distribution in response to changes in turbidity stemming from bivalve filtration and seagrass sediment stabilization. Estuaries 27:793–806

Newell RIE, Langdon C (1996) Mechanisms and physiology of larval and adult feeding. In: Kennedy V, Newell R (eds) The eastern oyster Crassostrea virginica. Maryland Sea Grant College, University of Maryland System, College Park, MD, p 185–230

Nilsson GE, Dixson DL, Domenici P, McCormick MI, Sørensen C, Watson S-A, Munday PL

(2012) Near-future carbon dioxide levels alter fish behaviour by interfering with neurotransmitter function. Nat Clim Chang 2:201–204

Nowicki JP, Miller GM, Munday PL (2012) Interactive effects of elevated temperature and CO_2 on foraging behavior of juvenile coral reef fish. J Exp Mar Bio Ecol 412:46–51

O'Connor NE, Grabowski JH, Ladwig LM, Bruno JF (2008) Simulated predator extinctions: predator identity affects survival and recruitment of oysters. Ecology 89:428–438

Orr JC, Fabry VJ, Aumont O, Bopp L, Doney SC, Feely RA, Gnanadesikan A, Gruber N, Ishida A, Joos F, Key RM, Lindsay K, Maier-Reimer E, Matear R, Monfray P, Mouchet A, Najjar RG, Plattner G-K, Rodgers KB, Sabine CL, Sarmiento JL, Schlitzer R, Slater RD, Totterdell IJ, Weirig M-F, Yamanaka Y, Yool A (2005) Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. Nature 437:681–686

Paine RT (1966) Food web complexity and species diversity. Am Nat 100:65–75

Palmer RE (1980) Behavioral and rhythmic aspects of filtration in the bay scallop, Argopecten Irradians Concentricus (Say), and the oyster, Crassostrea Virginica (Gmelin). J Exp Mar Bio Ecol 45:273–295

Pane EF, Barry JP (2007) Extracellular acid-base regulation during short-term hypercapnia is effective in a shallow-water crab, but ineffective in a deep-sea crab. Mar Ecol Prog Ser 334:1–9

Parker LM, Ross PM, O'Connor WA, Pörtner HO, Scanes E, Wright JM (2013) Predicting the response of molluscs to the impact of ocean acidification. Biology (Basel) 2:651–692

Paynter KT, Burreson EM (1991) Effects of *Perkinsus marinus* infection in the eastern oyster, *Crassostrea virginica*: II. Disease development and impact on growth rate at different salinities. J Shellfish Res 10:425–431

Peckarsky BL, Cowan CA, Penton MA, Anderson C (1993) Sublethal consequences of stream-dwelling predatory stoneflies on mayfly growth and fecundity. Ecology 74:1836–1846

Peterson CH, Grabowski JH, Powers SP (2003) Estimated enhancement of fish production resulting from restoring oyster reef habitat: quantitative valuation. Mar Ecol Prog Ser 264:249–264

Piazza BP, Banks PD, Peyre MK La (2005) The potential for created oyster shell reefs as a sustainable shoreline protection strategy in Louisiana. Restor Ecol 13:499–506

Piehler MF, Smyth AR (2011) Habitat-specific distinctions in estuarine denitrification affect both ecosystem function and services. Ecosphere 2:art12

Plotnick RE, Baumiller T, Wetmore KL (1988) Fossilization potential of the mud crab,

Panopeus (brachyura: Xanthidae) and temporal variability in crustacean taphonomy. Palaeogeogr Palaeoclimatol Palaeoecol 63:27–43

Pörtner HO, Peck MA (2010) Climate change effects on fishes and fisheries: towards a cause-and-effect understanding. J Fish Biol 77:1745–1779

Powell EN, Klinck JM, Hofmann EE, Wilson-Ormond EA, Ellis MS (1995) Modeling oyster populations. V. Declining phytoplankton stocks and the population dynamics of American oyster (*Crassostrea virginica*) populations. Fish Res 24:199–222

Preisser EL, Bolnick DI, Benard ME (2005) Scared to death? The effects of intimidation and consumption in predator-prey interactions. Ecology 86:501–509

Pyke GH, Pulliam HR, Charnov EL (1977) Optimal foraging: a selective review of theory and tests. Q Rev Biol 52:137–154

Reimer O, Harms-Ringdahl S (2001) Predator-inducible changes in blue mussels from the predator-free Baltic Sea. Mar Biol 139:959–965

Ries JB, Cohen AL, McCorkle DC (2009) Marine calcifiers exhibit mixed responses to CO_2-induced ocean acidification. Geology 37:1131–1134

Riisgård HU (1988) Efficiency of particle retention and filtration rate in 6 species of Northeast American bivalves. Mar Ecol Prog Ser 45:217–223

Rindone RR, Eggleston DB (2011) Predator–prey dynamics between recently established stone crabs (*Menippe* spp.) and oyster prey (*Crassostrea virginica*). J Exp Mar Bio Ecol 407:216–225

Ringwood AH, Keppler CJ (2002) Water quality variation and clam growth: is pH really a non-issue in estuaries? Estuaries 25:901–907

Ripple WJ, Larsen EJ (2000) Historic aspen recruitment, elk, and wolves in northern Yellowstone National Park, USA. Biol Conserv 95:361–370

Rothschild BJ, Ault JS, Goulletquer P, Héral M (1994) Decline of the Chesapeake Bay oyster population: a century of habitat destruction and overfishing. Mar Ecol Prog Ser 111:29–39

Roy RN, Roy LN, Vogel KM, Porter-Moore C, Pearson T, Good CE, Millero FJ, Campbell DM (1993) The dissociation constants of carbonic acid in seawater at salinities 5 to 45 and temperatures 0 to 45°C. Mar Chem 44:249–267

Sabine CL, Feely RA, Gruber N, Key RM, Lee K, Bullister JL, Wanninkhof R, Wong CS, Wallace DWR, Tilbrook B, Millero FJ, Peng T-H, Kozyr A, Ono T, Rios AF (2004) The oceanic sink for anthropogenic CO_2. Science (80-) 305:367–371

Schluter D (1981) Does the theory of optimal diets apply in complex environments? Am Nat

118:139–147

Schmitz OJ (2008) Effects of predator hunting mode on grassland ecosystem function. Science (80-) 319:952–954

Schmitz OJ, Grabowski JH, Peckarsky BL, Preisser EL, Trussell GC, Vonesh JR (2008) From individuals to ecosystem function: toward an integration of evolutionary and ecosystem ecology. Ecology 89:2436–2445

Scyphers SB, Powers SP, Heck KL, Byron D (2011) Oyster reefs as natural breakwaters mitigate shoreline loss and facilitate fisheries (H Browman, Ed.). PLoS One 6:e22396

Shin PKS, Liu CC, Liu ZX, Cheung SG (2008) Marine mussels *Brachidontes variabilis* selected smaller places of refuge and enhanced byssus production upon exposure to conspecific and heterospecific cues. J Exp Mar Bio Ecol 361:16–20

Sih A, Crowley P, McPeek M, Petranka J, Strohmeier K (1985) Predation, competition, and prey communities: a review of field experiments. Annu Rev Ecol Syst 16:269–311

Sih A, Englund G, Wooster D (1998) Emergent impacts of multiple predators on prey. Trends Ecol Evol 13:350–355

Sinclair ME (1977) Agonistic behaviour of the stone crab, *Menippe mercenaria* (Say). Anim Behav 25:193–207

Small D, Calosi P, White D, Spicer JI, Widdicombe S (2010) Impact of medium-term exposure to CO2 enriched seawater on the physiological functions of the velvet swimming crab *Necora puber*. Aquat Biol 10:11–21

Smee DL, Weissburg MJ (2006) Hard clams (*Mercenaria mercenaria*) evaluate predation risk using chemical signals from predators and injured conspecifics. J Chem Ecol 32:605–619

Soluk DA (1993) Multiple predator effects: predicting combined functional response of stream fish and invertebrate predators. Ecology 74:219–225

Sorte CJB, Williams SL, Carlton JT (2010) Marine range shifts and species introductions: comparative spread rates and community impacts. Glob Ecol Biogeogr 19:303–316

Spicer JI, Raffo A, Widdicombe S (2006) Influence of CO_2-related seawater acidification on extracellular acid–base balance in the velvet swimming crab *Necora puber*. Mar Biol 151:1117–1125

Svanback R, Bolnick DI (2007) Intraspecific competition drives increased resource use diversity within a natural population. Proc R Soc B Biol Sci 274:839–844

Taylor JD, Layman M (1972) The mechanical properties of bivalve (Mollusca) shell structure. Paleontology 15:73–87

Taylor GM, Palmer AR, Barton AC (2000) Variation in safety factors of claws within and among six species of *Cancer* crabs (Decapoda: Brachyura). Biol J Linn Soc 70:37–62

Taylor RB, Sotka E, Hay ME (2002) Tissue-specific induction of herbivore resistance: seaweed response to amphipod grazing. Oecologia 132:68–76

Tierney AJ, Atema J (1986) Effects of acidification on the behavioral response of crayfishes (*Orconectes virilis* and *Procambarus acutus*) to chemical stimuli. Aquat Toxicol 9:1–11

Tolley SG, Volety AK (2005) The role of oysters in habitat use of oyster reefs by resident fishes and decapod crustaceans. J Shellfish Res 24:1007–1012

Trussell GC, Ewanchuk PJ, Matassa CM (2006) The fear of being eaten reduces energy transfer in a simple food chain. Ecology 87:2979–2984

Tsang S-Y, Ng S-K, Xu Z, Xue H (2007) The evolution of $GABA_A$ receptor-like genes. Mol Biol Evol 24:599–610

Tuomainen U, Candolin U (2011) Behavioural responses to human-induced environmental change. Biol Rev 86:640–657

Underwood AJ (1981) Techniques of analysis of variance in experimental marine biology and ecology. Oceanogr Mar Biol An Annu Rev 19:513–605

Uryu Y, Iwasaki K, Hinoue M (1996) Laboratory experiments on behavior and movement of a freshwater mussel, *Limnoperna fortunei* (Dunker). J Molluscan Stud 62:327–341

Vanderploeg HA, Scavia D (1979) Calculation and use of selectivity coefficients of feeding: zooplankton grazing. Ecol Modell 7:135–149

Vermeij GJ (1982) Unsuccessful predation and evolution. Am Nat 120:701–720

Vitousek PM, Mooney HA, Lubchenco J, Melillo JM (1997) Human domination of earth's ecosystems. Science (80-) 277:494–499

Waldbusser GG, Voigt EP, Bergschneider H, Green MA, Newell RIE (2011) Biocalcification in the eastern oyster (*Crassostrea virginica*) in relation to long-term trends in Chesapeake Bay pH. Estuaries and Coasts 34:221–231

Walther G-R, Post E, Convey P, Menzel A, Parmesan C, Beebee TJC, Fromentin J-M, Hoegh-Guldberg O, Bairlein F (2002) Ecological responses to recent climate change. Nature 416:389–395

Warner GF, Chapman D, Hawkey N, Waring DG (1982) Structure and function of the chelae and chela closer muscles of the shore crab *Carcinus maenas* (Crustacea: Brachyura). J Zool 196:431–438

Watson S-A, Lefevre S, McCormick MI, Domenici P, Nilsson GE, Munday PL (2014) Marine mollusc predator-escape behaviour altered by near-future carbon dioxide levels. Proc R Soc B Biol Sci 281:20132377

Welschmeyer NA (1994) Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll *b* and pheopigments. Limnol Oceanogr 39:1985–1992

Werner EE, Peacor SD (2008) A review of trait-mediated indirect interactions in ecological communities. Ecology 84:1083–1100

Wheatly MG, Henry RP (1992) Extracellular and intracellular acid-base regulation in crustaceans. J Exp Zool 263:127–142

Whetstone JM, Eversole AG (1981) Effects of size and temperature on mud crab, *Panopeus herbstii*, predation on hard clams, *Mercenaria mercenaria*. Estuaries 4:153–156

White ME, Powell EN, Ray SM (1988) Effect of parasitism by the pyramidellid gastropod *Boonea impressa* on the net productivity of oysters (*Crassostrea virginica*). Estuar Coast Shelf Sci 26:359–377

Wilberg MJ, Livings ME, Barkman JS, Morris BT, Robinson JM (2011) Overfishing, disease, habitat loss, and potential extirpation of oysters in upper Chesapeake Bay. Mar Ecol Prog Ser 436:131–144

Wilbur HM, Fauth JE (1990) Experimental aquatic food webs: interactions between two predators and two prey. Am Nat 135:176–204

Williams AB (1984) Shrimps, Lobsters, and Crabs of the Atlantic Coast of the Eastern United States, Maine to Florida. Smithsonian Institution Press, Washington, D.C., DC

Wong MC, Peterson CH, Kay J (2010) Prey size selection and bottom type influence multiple predator effects in a crab–bivalve system. Mar Ecol Prog Ser 409:143–156

Wood HL, Spicer JI, Widdicombe S (2008) Ocean acidification may increase calcification rates, but at a cost. Proc R Soc B Biol Sci 275:1767–1773

Yamada SB, Boulding EG (1998) Claw morphology, prey size selection and foraging efficiency in generalist and specialist shell-breaking crabs. J Exp Mar Bio Ecol 220:191–211

Zohar Y, Hines AH, Zmora O, Johnson EG, Lipcius RN, Seitz RD, Eggleston DB, Place AR, Schott EJ, Stubblefield JD, Chung JS (2008) The Chesapeake Bay blue crab (*Callinectes sapidus*): a multidisciplinary approach to responsible stock replenishment. Rev Fish Sci 16:24–34

zu Ermgassen PSE, Spalding MD, Blake B, Coen LD, Dumbauld B, Geiger S, Grabowski JH, Grizzle R, Luckenbach M, McGraw K, Rodney W, Ruesink JL, Powers SP, Brumbaugh R

(2012) Historical ecology with real numbers: past and present extent and biomass of an imperilled estuarine habitat. Proc R Soc London Ser B 279:3393–400

zu Ermgassen PSE, Spalding MD, Grizzle RE, Brumbaugh RD (2013) Quantifying the loss of a marine ecosystem service: filtration by the eastern oyster in US estuaries. Estuaries and Coasts 36:36–43