ABSTRACT

Title of Dissertation: GROWTH AND PHYSIOLOGY OF EASTERN AND SUMINOE OYSTERS AND THE IMPLICATIONS OF INCREASED HABITAT COMPLEXITY FOR ASSOCIATED OYSTER REEF FAUNA

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The introduction of a non-native oyster species (*Crassostrea ariakensis*) into Chesapeake Bay has been proposed as a way to help restore the oyster fishery and enhance the ecological services historically provided by eastern (*Crassostrea virginica*) oysters. A comparison of growth, mortality, and physiology between diploid *C. ariakensis* ("Oregon" strain) and diploid *C. virginica* was undertaken in quarantined mesocosms simulating mesohaline Chesapeake Bay. Growth of *C. ariakensis* was greatest during the late winter and early spring periods, with oyster condition substantially reduced during the summer due to low clearance rates and elevated respiration rates. Stunted growth and high mortality characterized the *C. virginica* treatment, although the reasons for this are unknown. Additional quarantined laboratory studies, conducted in Florida for both oyster species in conditions simulating a subtropical estuary examined the potential of *C. ariakensis* to expand southwards. While growth of *C. ariakensis* was comparable to that of *C. virginica*, mortality of *C. ariakensis* reached 100% by the end of the study, but

remained relatively low for *C. virginica*. Physiological studies under quarantined temperate euhaline conditions (Wachapreague, Virginia) confirmed that *C. ariakensis* is physiologically intolerant to warmer water (> 20ºC) because of low clearance rates. Oysters create reefs that provide refuge for prey species, and enhanced foraging opportunities for predatory fish species. Predator–prey interactions between organisms found on oyster reefs, such as grass shrimp (*Palaemontes pugio*), white perch (*Morone americana*), and striped bass (*Morone saxatilis*) were conducted on habitats of varying complexity. Habitats consisted of flat sand, and medium and high complexity structures constructed in mesocosms from PVC pipe. As structural complexity increased so did the attraction of grass shrimp and white perch to structure regardless of the provision of food resources or presence of striped bass. The attraction of grass shrimp to structure decreased when high densities of conspecifics were present. The presence of prey and/or predators enhanced white perch utilization of structure and increased complexity decreased their swimming and shoaling activity. Habitat complexity and the threat of predation interact to alter grass shrimp and white perch behavior under intermediate levels of structural complexity.

GROWTH AND PHYSIOLOGY OF EASTERN AND SUMINOE OYSTERS AND THE IMPLICATIONS OF INCREASED HABITAT COMPLEXITY FOR ASSOCIATED OYSTER REEF FAUNA

By

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Dissertation submitted to the Faculty of the Graduate School of the
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PREFACE

"The literature of science is filled with answers found when the question propounded had an entirely different direction and end."

- *John Steinbeck, "Log from the Sea of Cortez"*

DEDICATION

To my daughter, Paige:

All the mysteries of the world are for us to discover together.

May we explore tide pools, gaze at the stars,

and marvel at the beauty of nature

forever.

ACKNOWLEDGEMENTS

Way back in the winter of 2004, before this whole adventure began, I was just back from an assignment with the U.S. Peace Corps in Zambia, Africa where I was busy saving the world...one fish pond at a time. So naturally, I assumed that when I returned to the United States and applied for graduate school, potential advisors and/or graduate school selection committees would jump at the chance to work with me. There was this little issue of a sophomore grade slump during my undergraduate days, but I had rebounded, and besides, I had "Peace Corps" on my resume! I lived in a mud hut for two years, ate termites (regularly), and most importantly developed a sustainable fishery in Africa. I was golden.

I ended up getting a job selling body parts...not my own mind you...but for an organ donation company that collected parts to ship to researchers throughout the country. On about the same day they asked me if, since I was a biologist, I knew of any "sources of tissue" which they could access, I received an e-mail from one Dr. Roger Newell from the University of Maryland Center for Environmental Science. He wanted to know if I'd like to come down to Cambridge, Maryland and interview to work for him, with an eye towards getting a graduate degree. But how could this be? I never applied to him specifically, and I was already rejected from University of Maryland (along with two other very reputable graduate schools). During the interview he asked about my experience, time in the Peace Corps, research interests, all the normal stuff...and then he asked, "Was she worth it?". I was confused and didn't really know how to respond, or really what he meant, so he repeated the question once more "During your sophomore year, was she worth it". I thought to myself, to paraphrase a quote from "Casablanca", this is the beginning of a beautiful mentorship.

I'm not quite sure if it was my research experience or my knowledge of exactly how much an arm and a leg actually costs ($1,352 in 2004 dollars) but he hired me, and this dissertation is the direct result of that. For that, I am extremely grateful to Dr. Roger Newell for giving me the chance to prove myself. He has challenged me to think critically, be more analytical, and write more clearly. His constant pragmatism and advice propelled me through the darkest days of my research when a completed dissertation seemed unattainable. He's not going to like that I spent a page and a half in the acknowledgement section on him (most likely because I'm wasting a tree by doing so); however, he deserves it. Roger is the best mentor a graduate student can ask for. I wish him the best of luck in his retirement, and in his future sailing endeavors around the globe.

This dissertation would never have been possible without the love and support of my wife, Kari. She has helped support me both emotionally and financially through my graduate studies. Her advice has helped me remain optimistic through the low points, and her example has continuously spurred me to do my best. For those, and many more reasons too numerous to count, I am very grateful that she is the one I will spend the rest of my life with. On that note, I'd also like to thank my daughter, Paige, for being a ridiculously good baby girl. I appreciate that you waited to be born the day after I submitted my 1st chapter, that you decided sleeping through the night was a good thing, and that the problems associated with teething are really overrated. For this, I will give you a pass on that dent you'll accidentally put in my hover-car in 2027.

In addition to Dr. Roger Newell, I would like to express my appreciation and respect for the rest of my dissertation examining committee: Drs. Denise Breitburg, Mark Luckenbach, Tom Miller, Dave Secor, and Reginal Harrell. I've been constantly buoyed

by their recommendation to challenge assumptions and to better place my research into the context of the bigger environmental picture.

Blaise Brown, James “Bear” Kampmeyer, Paul Perunko, and Jack Seabreeze of the Horn Point maintenance department are superheroes. They appear miraculously when your experiment is threatened by clogged pipes, power outages, or...on the off chance...a million pound head tank providing water for your experiment breaks through its concrete base and (in addition to threatening to destroy the very building in which your experiment is housed) cuts off your water supply. This dissertation would not have been possible without their assistance. If I ever become a university dean I will confer an honorary doctorate to these guys…because I certainly owe mine to them.

Dr. Joan Manuel, Angela Padeletti, Kristi Shaw, and Stephanie Alexander from Horn Point Laboratory and Christopher Dungan, Carol McCollough, Judson Blazek, and Stuart Lehmann of the Maryland Department of Natural Resources – NOAA Cooperative Laboratory all were helpful in the implementation of the mesohaline *Crassostrea ariakensis* study. Drs. John Scarpa and Susan Laramore, my co-authors of the Florida *Crassostrea ariakensis* chapter, were gracious hosts during my time at Harbor Branch Oceanographic Institute at Florida Atlantic University (HBOI – FAU) and were highly instrumental in the development of this project. Krystal Baird, Eman El-Wazzan, Patrick Monaghan, James Webb, and David Wood at HBOI – FAU and Stephanie Boniwell, Reid Boniwell, and Ryan Carnegie from the Virginia Institute of Marine Science were also helpful with the execution of this study. Dr. Andy Lazur, Steven Lane, Erin Markin, Angela Hengst, and Christine Newell all assisted with various aspects of the two habitat chapters, from the building of PVC pipe structures to striped bass acquisition and holding.

Funding for these studies was provided by the Maryland Department of Natural Resources, NOAA project #NA04NMF4570414 and #NA06NMF4570245, and small grants, awards, and fellowships from the University of Maryland Center for Environmental Science - Horn Point Laboratory.

My friends Jeff Bierman, Dale Booth, Ben Fertig, Rebecca Fox, Maggie Sexton, Lee Frey, Angie Hengst, Ginger Jahn, Desmond Johns, Antti Koskelo, Erin Markin, Kevin Meyer, Joanna Worener, and Emily Vlahovich have helped me stay grounded over the past six years. They have provided me with necessary distractions such as daily lunch at the picnic tables, a triathlon addiction, altering the ecosystem of Death Valley, paving Chesapeake Bay, and the intricacies of cardboard boat building.

My family has continuously offered compassion, encouragement and support over the past 33 years, which has led me directly to this point. To that end, I'd like to thank my siblings Tim & Becky Kelly, my grandparents Livia & James Valentino, Mary & Frank Kelly, my "other" parents Bud & Kathy Lyon, my uncles and aunts James & Linda Valentino, Francis & Joan Kelly, and Margie Lyon & John Wright.

Finally I'd like to extend my deepest gratitude and thanks to my parents Michael & Denise Kelly. They encouraged my curiosity of the natural world and afforded me every opportunity in the universe to sustain it, usually with great sacrifice to themselves. I owe **EVERYTHING** to them, and all they ever ask in return is for me to try and be the best person I can be...

....well that, and also a phone call every now and then.

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CHAPTER 1:

Introduction and Synthesis

INTRODUCTION

Oysters are an ecological engineering species because they modify the environment by preferentially settling and growing on oyster shell, thereby creating structural habitat that provides hard substrate, refuge, and habitat for many epibenthic invertebrates and resident and facultative fish species (Jones et al. 1994). Oyster reefs also provide foraging opportunities for transient predatory fish species, which are attracted to these structures, in part, because of the enhanced productivity of prey species usually associated with these reefs (Harding & Mann 2001). Oysters void biodeposits that settle to the sediment surface. The organic carbon, nitrogen, and phosphorous in these biodeposits are consumed by benthic invertebrates that are then available as a food source for benthic and pelagic carnivores (Dame 1996, Newell & Ott 1999, Erbland & Ozbay 2008). However, not all oyster reefs will provide equal habitat value, with oysters reefs developing under optimum conditions providing larger and more diverse structure for the associated faunal community compared with oysters reefs developing under sub-optimum (i.e. hypoxia, disease, intense harvesting) conditions (Rodney & Paynter 2006).

Eastern oyster (*Crassostrea virginica*) populations and the reefs they create have declined throughout Chesapeake Bay over the past 150 years (Rothschild et al. 1994). Public perception is slowly changing from considering the decline of oyster biomass as solely the loss of a fisheries resource to recognizing that it is also an ecological problem. Over-harvesting (Kennedy & Breisch 1983, Rothschild et al. 1994), disease pressures from MSX (*Haplosporidium nelsoni*) and Dermo (*Perkinsus marinus*) (Ford & Tripp 1996), and lack of un-silted oyster shell habitat necessary for the settlement of oyster larvae (Smith et al. 2005) make it difficult for managers to adequately conserve large

abundances of oysters. These problems limit oyster reefs in Chesapeake Bay from reaching their full historical potential of ecosystem services (Newell 1988, Hargis & Haven 1999), which affects the carrying capacity of these reefs for a wide range of associated organisms. Natural trophic links are disrupted when an oyster reef is leveled through harvest techniques (Lenihan & Peterson 1998), or when the accretion of oyster shell is not able to outpace sedimentation rates, which results in the burial of an oyster reef (Smith et al. 2005, Mann & Powell 2007, Powell & Klinck 2007). Degraded oyster reefs do not provide either the production or the protection necessary to allow for an abundant and diverse associated community (Peterson et al. 2000, Rodney & Paynter 2006).

Oyster restoration in the upper Chesapeake Bay has generally focused on conservation of existing oyster stocks, and placing hatchery reared *Crassostrea virginica* spat-on-shell on existing degraded reefs. In the early 2000s the Maryland Department of Natural Resources proposed the introduction of a non-native oyster (*Crassostrea ariakensis*). This species was initially chosen because of its resistance to native disease pathogens and its relatively fast growth rate (NRC 2004). The combination of these attributes, it was hoped, would allow *C. ariakensis* to establish a breeding population and hence restore the once valuable public oyster fishery in Maryland. It was also hoped that *C. ariakensis* would provide potential habitat for associated fauna more rapidly than with restoration dependant upon *C. virginica* alone.

Both *Crassostrea ariakensis* and *Crassostrea virginica* create habitat for associated fauna equally well within Chesapeake Bay (Harwell et al. 2010). Therefore, regardless of which oyster species is used for restoration purposes, proper oyster reef

design must be made a priority when determining restoration strategies. Some general principles for oyster reef restoration include focusing on existing degraded oyster reefs in relatively shallow waters with high flow rates to prevent the occurrence of hypoxia or anoxia (Lenihan et al. 1999). Oyster reefs should extend vertically in the water column to prevent hypoxic condition as well as to outpace sedimentation rates (Soniat et al. 2004). A restored oyster reef should also consist of a central broodstock preservation and spawning area ringed by satellite reefs that may act as an area for further oyster production, and may be opened to harvesting when appropriate (Hargis & Haven 1999). However, while these studies address the most suitable ways of creating sustainable oyster reef habitat, very few address how structure and interstitial space affect the associated faunal community. This may be of interest to managers in deciding if an oyster reef is complex enough to withstand harvesting pressure without severely impacting the habitat value of those reefs.

Just how habitat complexity influences the attraction of organisms to structure is currently debated for not just oyster reefs (Breitburg 1999, Harding & Mann 2001), but for many other structurally complex communities, such as coral reefs and mangrove systems (Bohnsack 1989, Bohnsack et al. 1994, Levin et al. 1997, Cocheret de la Morinière 2004). Furthermore, how varying levels of habitat complexity influence interactions between predators and prey is also frequently questioned (Crowder & Cooper 1982, Savino & Stein 1982, Nelson & Bonsdorff 1990). Luckenbach et al. (2005) found that oyster reefs with higher levels of structural complexity had abundant and diverse faunal communities, and suggested that more research be done on specific predator–prey relationships to determine the extent to which varying levels of habitat complexity

influence interactions between them. Grabowski (2004) found that increased structural complexity on oyster reefs weakened trophic interactions between oyster toadfish (*Opsanus tau*), mud crabs (*Panopeus herbstii*), and juvenile oysters (*Crassostrea virginica*). On low complexity oyster reefs, oyster toadfish preyed upon mud crabs, thereby decreasing mud crab predation on juvenile oysters. On high complexity reefs predation on mud crabs by oyster toadfish was low; however, the mere presence of oyster toadfish was enough to induce defensive behavior in mud crabs, and reduce their feeding activity on juvenile oysters.

Similar studies investigating predator–prey interactions on structurally complex habitat have been done in other systems. Persson and Eklöv (1995) examined how levels of habitat complexity in ponds influenced interactions between piscivorous perch (*Perca fluviatilis*) and juvenile perch and roach (*Rutilus rutilus*). They found that the amount of juvenile perch and roach in the diet of piscivorous perch decreased when partial refuge was available, and was absent from their diets when complete refuge was available. More interestingly, the presence of piscivorous perch predators resulted in a shift in the diet of juvenile perch from feeding primarily on zooplankton in the absence of predators to feeding primarily on macroinvertebrates. The presence of the piscivorous perch resulted in an alteration of the species composition within the zooplankton community. Crowder and Cooper (1982), in their classic habitat complexity study, reported that bluegill (*Lepomis macrochirus*) inhabiting intermediate complexity habitat had faster growth rates and higher feeding rates than bluegill inhabiting low or high complexity structures. They postulated that predator feeding rates are maximized in intermediate structures

because of the adequate abundance and diversity of prey and easier access to prey because of reduced structural complexity.

A major difficulty in determining how structural complexity affects predator–prey interactions on habitat is that many studies use subjective metrics to determine differences between levels of structural complexity. It needs to be recognized that habitat complexity is a relative characteristic that depends partially on body size, population density, and behavior of an organism utilizing that habitat (Heck & Orth 1980, Ryer 1988).

An aquatic habitat that is structurally complex to one species may be recognized as structurally simple by another. I encountered this problem within my own research when I did preliminary habitat studies utilizing piled oyster shell as habitat for grass shrimp (*Palaemontes pugio*) and white perch (*Morone americana*) within a mesocosm. The shell pile protruded approximately 30 cm off the bottom of the tank and contained small pockets of interstitial space that grass shrimp utilized as a refuge. However, video analysis of white perch behavior showed that the shell pile was not complex enough to attract white perch due to the absence of protrusions and large interstitial spaces. Therefore, there was a disconnect between the location of the prey and predator communities within these preliminary studies. To standardize levels of structural complexity among experimental species, and to better study how physical attributes of a habitat (i.e. surface area, interstitial space) affect interactions between prey and predators I switched to using PVC pipe reefs constructed within mesocosms. The habitat studies described within this dissertation are based on experimental manipulations of habitat complexity, therefore there will be some difficulty in directly comparing the results from

these studies to field studies such as Crowder and Cooper (1982) or Persson and Eklöv (1995). The low levels of habitat complexity within those field studies may have surpassed my high levels of habitat complexity within my mesocosms in terms of bulk comparison of habitat metrics. However, the studies described within this dissertation illustrate the importance of surface area, interstitial space, and density of organisms in shaping community development and relations between predator and prey communities.

Results from this dissertation are bolstered by literature information from studies on artificial reef design that show the importance of creating habitats with large surface areas and abundant interstitial space (Bohnsack 1994 Charbonnel et al. 2002, Sherman et al. 2002, Warfe et al. 2008). The findings from my research are consistent with other studies that have investigated the importance of these physical parameters on natural systems; such as oyster reefs, corals, and mangrove systems (Heck & Crowder 1991, Hixon & Beets 1993, Forrester & Steele 2004, Gratwicke & Speight 2005, Luckenbach et al. 2005, Moore & Hovel 2010).

OBJECTIVES

My research was designed to elucidate the differences in growth, survival, reproductive development, and environmental tolerances between *Crassostrea ariakensis* and native *Crassostrea virginica* oysters in the mesohaline region of Chesapeake Bay. This research complements several field studies by others (Calvo et al. 2001, Paynter et al. 2008, Kingsley-Smith et al. 2009) who used reproductively sterile triploid oysters to determine similarities and differences between these two oyster species. Controlled laboratory studies of fertile diploid *C. ariakensis* were a necessary precursor to a

responsible decision about whether or not to introduce fertile *C. ariakensis* to Chesapeake Bay (NRC 2004).

If *Crassostrea ariakensis* were to be introduced into Chesapeake Bay the potential would exist for this oyster species to spread beyond its intended range into areas where native *Crassostrea virginica* populations are relatively robust, such as the southeastern coast of the United States (Grizzle 1990). Therefore, it is equally important to determine the growth, survival, reproductive development, and environmental tolerances between *C. ariakensis* and native *C. virginica* oysters within subtropical regions to further determine their potential for interspecific competition.

The fast growth rate of *Crassostrea ariakensis*, investigated in Chapters 2 and 3 of this dissertation and reported by many studies (Langdon & Robinson 1996, Calvo et al. 2001, Harding & Mann 2006), may enable this species to form a reef matrix more rapidly than reefs composed solely of *Crassostrea virginica*. An oyster reef that forms more rapidly may provide ecological services for associated fauna more quickly. However, the underlying issue of how habitat complexity influences predator–prey relationships in aquatic ecosystems, including oyster reefs, is still debated (Bohnsack et al. 1994, Harding & Mann 2001, Almany 2004, Luckenbach et al. 2005). The research presented in Chapters 4 and 5 examines how certain idealized attributes of a habitat (i.e., surface area, interstitial space, and density of organisms) interact to affect the behavior of fauna associated with oyster reefs such as grass shrimp (*Palaemontes pugio*), white perch (*Morone americana*), and striped bass (*Morone saxatilis*).

This dissertation research was initiated, in part, to help inform the U.S. Army Corps of Engineers Environmental Impact Statement associated with the introduction of

diploid *Crassostrea ariakensis* into Chesapeake Bay. The findings from the research described in Chapters 4 and 5, while prompted by the *C. ariakensis* introduction question, are intended to be more broadly applied to other complex aquatic environments. The following research questions and hypotheses were addressed throughout this dissertation.

RESEARCH QUESTIONS AND HYPOTHESES

- Are there differences in age specific growth rates and seasonal scope for growth between diploid *Crassostrea ariakensis* and diploid *Crassostrea virginica* in the mesohaline region of Chesapeake Bay?
    - **Hypothesis 1:** Growth of diploid *Crassostrea ariakensis* is comparable to that of diploid *Crassostrea virginica* within the mesohaline region of Chesapeake Bay.
    - **Hypothesis 2:** The scope for growth of *Crassostrea ariakensis* is minimal during the summer months within the mesohaline region of Chesapeake Bay when temperatures are at their maximum

- Is there a difference in the seasonal growth rate and physiology between diploid *Crassostrea ariakensis* and diploid *Crassostrea virginica* in a subtropical euhaline estuary (Indian River Lagoon, Florida)?
    - **Hypothesis 3:** Under subtropical conditions *Crassostrea ariakensis* will grow faster and larger than *Crassostrea virginica*.
    - **Hypothesis 4:** *Crassostrea ariakensis* will have less energy to allocate towards somatic growth and gamete production during the summer due to physiological stress induced by high water temperatures.

- How does habitat complexity influence predator – prey interactions under varying trophic complexity regimes?
    - **Hypothesis 5:** Aquatic organisms are attracted to increased levels of structurally complex habitat regardless of predatory threat or provision of food resources.
    - **Hypothesis 6:** The provision of food resources enhances the occurrence of fish species on structurally complex habitat.
    - **Hypothesis 7:** The occurrence of a predatory species on complex habitat is further enhanced when they themselves are subjected to a greater predatory threat.
    - **Hypothesis 8:** Swimming and shoaling activity of fish species will decrease with an increase in habitat complexity across trophic complexity levels.

- How do surface area, interstitial space, and prey and predator density interact to affect the utilization of complex habitats by associated fauna?
    - **Hypothesis 9:** When predator and prey densities are held constant, increased levels of structural surface area will enhance the attraction of prey species to structure and decrease predation.
    - **Hypothesis 10:** As the density of prey and predator were increased concomitant to structural complexity the attraction of the prey species will be limited by the amount of interstitial space available as a refuge.

SYNOPSIS OF DISSERTATION CHAPTERS

Chapter 2

The rapid growth rate of *Crassostrea ariakensis* has been well documented (Langdon & Robinson 1996, Calvo et al. 2001, Harding & Mann 2006). However, those studies chiefly examine triploid *C. ariakensis* individuals, do not look at seasonal variations in growth, and were performed in areas where annual mean salinity is greater than typically found in the mesohaline region of Chesapeake Bay. The goal of my study was to compare the growth, mortality, and reproductive capability of diploid *C. ariakensis* to that of diploid *Crassostrea virginica* over a 3.5 y period (June 2004 – January 2008) in quarantined mesocosms supplied with ambient flow-through water from the Choptank River.

There was a significant difference in the growth rate between the two oyster species. The average shell area of *Crassostrea ariakensis* was approximately 6 times greater than the average shell area of *Crassostrea virginica* by the end of the experiment in January 2008. This large size difference was likely due to the lack of growth of *C. virginica* oysters after the summer of 2005, rather than enhanced growth of *C. ariakensis*. Growth rates for *C. ariakensis* differed seasonally, with the highest rates recorded in the winter and spring periods. The specific growth rate of *C. virginica* did not differ by season. The cumulative mortality of *C. virginica* (90%) was significantly higher than *C. ariakensis* (35%). There were appreciable differences in the reproductive condition of these two species of oysters, with *Crassostrea ariakensis* becoming reproductively active at an earlier age and for a longer duration each summer than *Crassostrea virginica*.

Physiological responses of both oyster species were compared seasonally to better understand the effects of temperature on *Crassostrea ariakensis* growth, as well as to determine the cause of slow growth in *Crassostrea virginica*. For *C. ariakensis*, low clearance rates coupled with high respiration and ammonium excretion rates generated a negative scope for growth during the summer. This species was physiologically active and had a positive scope for growth during the winter, when water temperatures were cold (1.3 – 7.4ºC). Physiological investigation of *C. virginica* oysters did not yield the causes of the observed stunted growth within the mesocosms.

The only explanation for the slow growth of *Crassostrea virginica* was that there was an unknown stressor that was affecting these individuals, but having little effect on *Crassostrea ariakensis* individuals. These results indicate that *C. ariakensis* could grow moderately well in the mesohaline region of Chesapeake Bay, although they will likely not grow any faster than *C. virginica* already present within these regions (Kingsley-Smith et al. 2008, Paynter et al. 2008).

Chapter 3

The growth studies performed in Chesapeake Bay mesocosms (Chapter 2) revealed that there were distinct seasonal differences in growth between *Crassostrea ariakensis* and *Crassostrea virginica*. If *C. ariakensis* were to be introduced into Chesapeake Bay, either deliberately, or accidentally from research facilities holding diploid broodstock, it is considered likely that it would establish feral populations along the Atlantic coast of the United States (NRC 2004), including areas where *C. virginica* populations remain relatively robust.

The goal of this study was to compare the growth, mortality, and reproductive capability of diploid *Crassostrea ariakensis* to that of diploid *Crassostrea virginica* in conditions simulating a subtropical euhaline estuary in Florida. Oysters of both species were grown over a 8 mo period (December 2006 – August 2007) in quarantine mesocosms supplied with ambient flowing water from the Indian River Lagoon, Florida. Growth rates for *C. ariakensis* did not differ over time, but did for *C. virginica*. The growth rate of *C. virginica* was slowest in the winter and fastest in the spring. Mortality was extremely high for *C. ariakensis*, reaching 100% by the end of the study. By comparison, the cumulative mortality of *C. virginica* was relatively light (~40%). The mortality of both species of oysters was not directly associated with infections from any of the three well recognized oyster parasites present within this region, *Haplosporidium nelsoni*, *Perkinsus marinus*, or *Bonamia* sp. (Newell et al. 2009).

Physiological responses of both species of oyster were compared under seasonal temperate euhaline conditions to better understand how temperature affects these species without the confounding heavy mortality encountered within the subtropical mesocosms. These experiments were conducted under quarantine conditions at the Virginia Institute of Marine Science – Eastern Shore Laboratory in Wachapreague, Virginia. Clearance rates of *Crassostrea ariakensis* were half that of *Crassostrea virginica* during the summer, which resulted in a negative scope for growth during this season. During the winter, *C. ariakensis* remained physiologically active even when water temperatures were as low as 2ºC.

These results indicate that if *Crassostrea ariakensis* were to either be deliberately or accidentally introduced into Chesapeake Bay, their expansion into the subtropical

regions of the United States would be limited due to physiological stress caused by low clearance rates in year-round warm water temperatures.

Chapter 4

Structurally complex habitats are considered essential for fish (Coen et al. 1999) because they provide a refuge against predation (Heck & Crowder 1991, Hixon & Beets 1993), enhance foraging opportunities (Adams et al. 2004, Verweij et al. 2006), and serve as a place of refuge from adverse environmental conditions (Kelly & Bothwell 2002, Cocheret de la Morinière et al. 2004). The value of complex structure as essential habitat for transient predatory fish species is uncertain, because these species are considered opportunistic and may forage wherever prey densities are highest (Harding & Mann 2001).

The goal of this study was to determine trophic interactions on different levels of structural complexity under varying trophic complexity regimes. To determine these interactions, I used fauna that are associated with oyster reefs in Chesapeake Bay; grass shrimp (*Palaemontes pugio*), white perch (*Morone americana*), and striped bass (*Morone saxatilis*). Experiments were done in mesocosms simulating the mesohaline region of Chesapeake Bay, and structures were constructed from PVC pipe to standardize habitat attributes (surface area, interstitial volume) for differences in scale among grass shrimp, white perch, and striped bass. The levels of structural complexity used in this experiment were flat sand, medium, and high complexity habitats.

Grass shrimp were significantly attracted to the high complexity habitat in the absence of either fish predator. Attraction of grass shrimp to the high complexity habitat was further enhanced by the presence of white perch and striped bass, both separately and

together. The level of habitat complexity was the primary determinant of white perch attraction to habitat. The presence of grass shrimp significantly increased the amount of time white perch spent on the medium and high complexity habitats. Swimming and shoaling activity of white perch generally decreased with an increase in habitat complexity, except when striped bass were also present on the medium complexity treatment. Striped bass attraction to habitat was low across all levels of structural complexity, and was not influenced by the presence of either grass shrimp or white perch.

These results indicate that grass shrimp and white perch were attracted to structure regardless of prey availability or threat from predation. The provision of food resources will enhance the amount of white perch stay upon a structure, which is further enhanced when they themselves are subjected to predation. The level of structural complexity and predatory threat are the two main factors influencing fish behavior on structural habitat.

Chapter 5

When reef building species create extensive habitats that provide enlarged surface areas and greater interstitial volumes, the carrying capacity of those habitats can also increase due to a greater availability of structure on which associated fauna are able to colonize, grow, and eventually reproduce (Abelson & Shlesinger 2002, Luckenbach et al. 2005). As a result of increased attraction and enhanced secondary production, structurally complex habitats generally have high densities of prey and predator species. However, some studies investigating interactions between predators, prey, and habitat complexity have maintained predator and prey densities constant while increasing the level of habitat complexity (e.g. Savino & Stein 1982, Adams et al. 2004). This chapter builds on the

approach used by Mattila et al. (2008) and Canion and Heck (2009) who investigated how changes in prey and predator density influence interaction on structurally complex habitats.

The goal of this study was to examine how habitat complexity, prey and predator densities, and the combination of these factors influence habitat utilization and predation risk. To elucidate these interactions I used fauna which are associated with oyster reefs in Chesapeake Bay; grass shrimp (*Palaemontes pugio*) and juvenile striped bass (*Morone saxatilis*). These experiments were performed in mesocosms simulating the mesohaline region of Chesapeake Bay, and habitats were constructed from PVC pipe to standardize habitat attributes (i.e. surface area, interstitial volume) for differences in scale among grass shrimp and striped bass. Increased level of structural complexity was defined as an increase in surface area and interstitial space over the three complexity levels: flat sand, and medium, and high complexity structures.

In the presence of striped bass, grass shrimp were attracted to the visual refuge provided by the surface area of the medium complexity structure, and were attracted to the physical refuge provided by interstitial space within the high complexity structure. This attraction was reduced at the high complexity level when grass shrimp densities were high. In the absence of striped bass, attraction of grass shrimp to the two complex PVC pipe structures was similar because surface area was identical, and the utilization of interstitial space as a refuge was not needed.

Restoration and conservation efforts on structurally complex habitats should seek to weigh the necessity of increased structural surface area with the importance of

interstitial space. This will provide prey with an enhanced refuge which allows for increased productivity, while still providing ample foraging opportunities for predators.

CONCLUSIONS

In 2009 the states of Maryland and Virginia, along with the U.S. Army Corps of Engineers decided against the introduction of *Crassostrea ariakensis* into Chesapeake Bay. Their decision was primarily based on several key research findings. It was concluded that coincident spawning between *C. ariakensis* and native *Crassostrea virginica* is likely to occur. This may lead to a "gamete sink" where cross-fertilization between gametes from these two oyster species will produce non-viable zygotes, resulting in a reduction in larval production for both species of oysters (Bushek et al. 2008). It was also concluded that the shell of *C. ariakensis* is more fragile than *C. virginica*, which may lead to more intense predation upon this species, especially within the polyhaline regions of Chesapeake Bay where predation pressures are greatest (Newell et al. 2007a). Ultimately because of the factors described above, and the unknown consequences associated with an exotic species introduction, it was decided that *C. ariakensis* would not be a suitable means of enhancing oyster stocks. The current management emphasis has now shifted back to conservation and restoration of *Crassostrea virginica* stocks, and towards the development of disease resistant strains of this oyster species (Oliver et al. 2000, Calvo et al. 2003).

My findings indicate that if *Crassostrea ariakensis* were to be introduced into Chesapeake Bay, the propensity of this species to be physically active in cooler water temperatures would enable it to graze on the spring bloom. This bloom is currently under-utilized by the filter-feeding community of Chesapeake Bay because water

temperatures are too cold (< 5ºC) for most of the benthic filter feeder community to be physiologically active (Hagy et al. 2005, Fulford et al. 2007, Newell et al. 2007b, Fulford et al. 2010). Grazing pressure by abundant *C. ariakensis* populations on the spring bloom would likely result in a decrease in seston availability for copepod and menhaden populations (Nicholson 1978, White & Roman 1992), which, while stressing their populations, may return Chesapeake Bay back to the more benthic dominated system thought to prevail when eastern oyster stocks were abundant (Newell 1988, Cerco & Noel 2005, Fulford et al. 2007, Newell et al, 2007b, Fulford et al. 2010). Conversely, the intolerance of *Crassostrea ariakensis* to warmer water temperatures may negatively impact Chesapeake Bay fauna dependent on oyster reefs for habitat. Biodeposition rates of *C. ariakensis* are lower than that of *Crassostrea virginica* in the summer, which may decrease the transfer of nutrients to the benthos inhabiting the footprint of an oyster reef; resulting in a limitation of nutrients just when these communities are most physiologically active. Field studies comparing the density of associated reef fauna between *C. ariakensis* and *C. virginica* experimental reefs found that subtidal reefs comprised of only *C. ariakensis* or a combination of *C. ariakensis* and *C. virginica* oysters had lower densities of organisms associated with them per unit of oyster biomass than experimental reefs comprised of *C. virginica* individuals alone (Harwell et al. 2010). This finding was not attributed to differences in reef morphology between the two species. Differences in faunal abundance was most pronounced during the summer, where *C. virginica* reefs had a greater density of organisms than *C. ariakensis* or mixed species reefs (Harwell 2010). Oyster biodeposition rates were not measured; and so it is difficult to interpret that reduced nutrient availability had a role in limiting faunal abundances on

reefs containing *C. ariakensis* oysters. Although this does correspond with the decreased biodeposition rates that I found for *C. ariakensis* during the summer.

The physiological intolerance of *Crassostrea ariakensis* to warmer water temperatures, coupled with their inability to thrive in the intertidal zone (Kingsley-Smith & Luckenbach 2008, Wang et al. 2008), and susceptibility to the oyster disease *Bonamia* sp. (Bushek et al. 2008, Carnegie et al. 2008) reduces the likelihood that this species would extend its range southward if it were introduced into Chesapeake Bay; however, its northward expansion would be physiologically possible. In the coastal waters of the northeastern region of the United States, *C. ariakensis* would likely have the competitive advantage over *Crassostrea virginica* because cooler water temperature would promote its growth year-round. There is no difference in the type of habitat complexity created between *Crassostrea ariakensis* and *Crassostrea virginica* oysters when tested within Chesapeake Bay (Harwell 2010, Harwell et al. 2010). However, if *C. ariakensis* were to be introduced into Chesapeake Bay and their range were to expand northward, there is the possibility that oyster reefs formed by *C. ariakensis* in northeastern regions of the United States would likely grow more rapidly than reefs formed by *C. virginica*; because of an enhanced growing season. Scientists and managers in these areas would want to know what effects this will have on fauna associated with oyster reefs in the northeast region and how predator – prey relationships on these reefs would be affected.

My dissertation research was originally designed to be primarily a comparative study of growth and reef creation by the native eastern oyster *Crassostrea virginica* and the suminoe oyster *Crassostrea ariakensis*, a candidate species for introduction and naturalization in Chesapeake Bay. It soon became apparent, however, that when

considering potential differences in habitat created by these two oyster species, there were some fundamental ecological questions on how habitat complexity influenced predator–prey interactions which needed further clarification. I therefore designed a series of experiments to examine the various metrics of habitat complexity (i.e., surface area, interstitial space, density of organisms) and examine intra- and inter-specific interactions between oyster reef-associated species exposed to habitats of differing complexity.

These experiments showed that the attraction of benthic invertebrates (i.e., grass shrimp), and transient intermediate predatory fish species (i.e., white perch) was highly dependent upon the total surface area available on a structure, regardless of the presence of prey and predatory species. My research supports previous studies on seagrass beds where Orth et al. (1984) and Moore & Hovel (2010) found that the magnitude of grass blade surface area influenced the attraction of these habitats to grass shrimp and other benthic organisms, even when that attraction increased their vulnerability to predation over time (Stoner 1980). These fundamental ecological insights have important implications for the design of artificial reefs or restoring degraded habitats, such as oyster reefs.

My findings on the importance of structural surface area in attracting organisms to structure lends credence to the idea that increased structural complexity may serve only to aggregate fish species from a wider geographical area without necessarily enhancing their production (Bohnsack 1989). This concern is likely to only be in the initial stages of newly created artificial reefs, such as boats sunk to enhance sport fishing opportunities,

where a climax reef-associated faunal community may take an extended period of time to fully develop.

On reefs comprised of biogenic habitat, a complex associated faunal community forms in concert with the reef itself. A study by Norling and Kautsky (2007) reported that even just a few mussels clustered together could substantially increase nutrient concentrations through biodeposit production, which then results in an increase in the biomass of faunal organisms around the mussel cluster. This increase in faunal biomass will eventually lead to higher order trophic interactions. Although, even within natural systems, there are examples that increased structural surface area can aggregate fish species without increasing their productivity. Studies have shown that Atlantic menhaden (*Brevoortia tyrannus*) have a tendency to aggregate around oyster reefs (Lenihan et al. 2001, Coen & Grizzle 2007), with larger aggregations occurring on larger oyster reefs (Arve 1960), even though they seem to gain little benefit from the reef because they are exclusively a pelagic species (Munroe 2002).

I found that an increase in interstitial space without a concurrent increase in the surface area of the overall structure does not increase the attraction of fauna to structure, unless a predatory threat is present. The value of interstitial space is in providing a relatively safe area within a reef structure in which organisms can find refuge, which allows them to survive and eventually reproduce on the habitat. Many studies inadvertently increased the surface area of a structure concurrently with an increase in interstitial space and found an increase in organism density (Hixon & Beets 1993, Charbonnel et al. 2002). While these studies may be correct in identifying interstitial

space as an important attractant of individuals, it is likely that it was the increased surface area of the structure that resulted in an increased density of organisms on a structure.

The density of a species inhabiting a structure will limit the number of conspecifics that can utilize that structure. The threat of predation, coupled with an increase in prey population on a structurally complex habitat, may foster intraspecific competition for available refuge space within the structure (Holt 1987). When prey densities on a habitat are high, there is greater competition for space to utilize as a refuge. This may force individual prey to leave the structure to find refuge elsewhere. The predation rates within my experiments were low, so it is possible that if predation pressure was intense, grass shrimp may have chosen the risks associated with overcrowding of a refuge rater than the risks associated with increased predation. The density of predators should have no effect on the density of prey utilizing a structurally complex habitat and this has been reported in field studies (Kneib & Stiven 1982, Heck et al. 2000).

The availability of prey on a structurally complex habitat will increase the amount of time an organism utilizes a structure. The attraction of an intermediate predator to structure is further enhanced when they themselves are subject predation. The availability of prey and the need to seek refuge are also main factors which attract aquatic organisms to structure. Black sea bass (*Centropristis striata*), bluefish (*Pomatomus saltatrix*), and scup (*Stenotomus chrysops*) are fish species that spend time on oyster reefs primarily to forage on the abundant concentrations of mud crabs, polychaetes, and other benthic invertebrates found on those reefs (Coen & Grizzle 2007). In contrast, the utilization of structurally complex mangrove systems by grunts (*Haemulon* sp.) and snappers (*Ocyurus*

chrysurus) were more closely related to refuge than to food (Verweij et al. 2006). While a high level of structural complexity initially attracts organisms to structure, the reasons why they spend time on that structure will vary by species.

I found that intermediate structural complexity habitats were generally not advantageous to predators or prey because multi-level trophic interactions may force organisms into inadequate habitat which provides neither refuge nor foraging opportunities. This was evidenced in my medium complexity habitat, where the presence of striped bass forced white perch onto the structure. The medium structure was not complex enough to provide adequate refuge from the threat of predation for white perch, so they spent the majority of their time on the reef moving together. This behavior by white perch decreased the refuge value for grass shrimp on the medium complexity reef because of increased encounter rates between white perch and grass shrimp. Grass shrimp then sought an alternative refuge within the mesocosm such as the sides of the tank or surface of the water. These findings seem to run counter to the work of Crowder & Cooper (1982), who showed that intermediate habitat complexity can be beneficial to intermediate predators. It is important to recognize, however, that the term "Habitat Complexity" is relative in terms of faunal community density, individual body size, as well as the structural make-up of the habitat itself. A habitat which is considered "intermediate" by qualitative means within the context of a field study, may actually function ecologically as a highly complex habitat. This makes the comparison of field and mesocosm studies difficult because they are using fundamentally different measures of habitat complexity.

Studies in mesocosms generally investigate how individual aspects of habitat complexity influence species interactions with that habitat, while field studies typically investigate more complex interactions involving numerous trophic levels in an un-controlled system. I suggest that greater consideration should be given as to how these two types of studies may be better aligned. Field studies should start by quantifying the complexity of a particular habitat, and the body size and density of its inhabitants in relation to other habitats of a similar type to determine its complexity value (i.e. low, medium, high). The value of mesocosm studies is to consistently test which variables are important in shaping predator–prey interactions on complex habitat for a variety of species, which can then be used as a metric to quantify habitat complexity in the field.

CHAPTER 2:

Comparing Diploid *Crassostrea ariakensis* and *Crassostrea virginica* Growth, Reproduction, and Physiology in Mesocosms Simulating the Mesohaline Region of Chesapeake Bay

ABSTRACT

Shell growth, mortality, and physiology were compared between diploid suminoe (*Crassostrea ariakensis*) and eastern oysters (*Crassostrea virginica*) under conditions simulating the mesohaline region of Chesapeake Bay, USA. Oysters of both species were set and grown over a 3.5 y period (June 2004 – January 2008) in quarantine mesocosms (500 L), each supplied with ambient flowing ($\geq$ 20 L min^{-1}) water (annual temperature range 1 to 29ºC and salinity of 8.2 to 12.2). There was a significant difference in the absolute growth rate between the two oyster species, with *C. ariakensis* averaging a shell area of 3561 mm^2 and *C. virginica* averaging a shell area of 560 mm^2 at the end of the study. Specific growth rates for *C. ariakensis* differed seasonally, with the highest growth rates recorded in the winter and spring periods. The specific growth rate of *C. virginica* did not differ seasonally after its initial settlement period. Cumulative mortality of *C. ariakensis* from 3 months post-metamorphosis to age 3.5 yr was lower (35%) than that of *C. virginica* (90%), and seasonal absolute mortality of *C. virginica* was significantly higher than *C. ariakensis* for most seasons sampled. Physiological responses of both oyster species were compared seasonally to better understand the effects of temperature on *C. ariakensis* growth, as well as to determine the cause of slow growth in the *C. virginica* oysters. For *C. ariakensis*, low clearance rates (0.97 L g^{-1} h^{-1}) coupled with high respiration (1.12 mL O_2 g^{-1} h^{-1}) and ammonium excretion (36.27 mg NH_4-N g^{-1} h^{-1}) rates resulted in a negative scope for growth (-1 J g^{-1}) during summer. During the winter *C. ariakensis* remained physiologically active when water temperatures were as low at 4ºC. Physiological investigation of *C. virginica* oysters did not yield the causes of the observed stunted growth within the mesocosms, with positive scope for growth

measurements for all sampling periods except for February 2009 when these oysters were dormant and May 2009 (-2 J g^{-1}), which was likely caused by low absorption efficiency (6%). The reason for the stunted growth of *C. virginica* oysters within these mesocosms remains unknown. I conclude that *C. ariakensis* could grow moderately well in the mesohaline region of Chesapeake Bay, although they will not grow significantly faster than rates for *C. virginica* typically growing in the field. The year-round physiological activity of *C. ariakensis* may have a significant grazing affect on the spring bloom, as well as alter faunal communities associated with oyster reefs in Chesapeake Bay.

INTRODUCTION

The eastern oyster (*Crassostrea virginica* Gmelin 1791) is a keystone species within the Chesapeake Bay ecosystem. Individuals of this species grow in clusters to form large complex reefs that provide refuge and foraging habitat for a wide range of faunal organisms (Coen et al. 1999, Harding and Mann 1999, Grabowski 2004, Rodney and Paynter 2006). In addition to providing habitat, *C. virginica* also have the capacity to filter large quantities of water within relatively short time periods (Newell & Langdon 1996). The filtration capacity of pre-exploitation dense oyster populations in Chesapeake Bay was likely to have provided top-down control on phytoplankton (Newell 1988). Current populations of *C. virginica*, however, are but a fraction of their historical abundance due to habitat degradation (Smith et al. 2005, Mann & Powell 2007, Powell et al. 2007), increased epizootics of the protistan diseases Dermo (*Perkinsus marinus*) and MSX (*Haplosporidium nelsoni*) (Ford & Tripp 1996, Goedken et al. 2005), and persistent over-harvesting pressure (Rothschild et al. 1994). This reduction in oyster biomass over the past 150 years has led to a decrease in the availability of habitat for associated fauna

(Rothschild et al. 1994, Lenihan & Peterson 1998), and a loss of filtration capacity for the bay, which has had deleterious effects on water quality (Newell 1988). The decline in the eastern oyster population has transformed Chesapeake Bay from being a historically benthic-dominated system into the pelagic-dominated system we have today (Ulanowicz & Tuttle 1992).

The Maryland Department of Natural Resources proposed in the early 2000s that the non-native suminoe oyster (*Crassostrea ariakensis* Fujita 1913) be introduced into Chesapeake Bay as a self-recruiting diploid population to supplement native eastern oyster, *Crassostrea virginica*, populations for the public fishery. Secondary considerations were that such an introduction may serve to restore essential ecosystem services lost through the decline of native eastern oyster stocks. The introduction of *C. ariakensis* into Chesapeake Bay is controversial however, because such an introduction would possibly be irreversible and may have unintended consequences for native oyster stocks and their associated fauna; both within Chesapeake Bay and in the waters of the mid-Atlantic region (Kelly et al. 2011). This study is one of many that were commissioned to help make an informed decision about a potential introduction of *C. ariakensis*, and provide the scientific information necessary to develop a formal Ecological Impact Statement (U.S. Army Corps of Engineers 2009). Ultimately, the introduction of the non-native *C. ariakensis* was not considered to be a suitable means of enhancing oyster stocks. The current management emphasis has now shifted to conservation and restoration of *C. virginica* stocks.

The suminoe oyster was initially chosen because of its resistance to native disease pathogens and its relatively fast growth rate (NRC 2004). The combination of these

attributes, it was hoped, would allow *Crassostrea ariakensis* to establish a breeding population and hence restore the once valuable public oyster fishery (Kennedy & Breisch 1983, Rothschild et al. 1994). The suminoe oyster was hypothesized to do well within Chesapeake Bay because of similarities in seasonal temperature and salinity between this system and its native range within Asian coastal waters (Zhou & Allen 2003, Guo et al. 2008). Additionally, studies from the Pacific Northwest coast of the United States indicated that *C. ariakensis* could be easily bred and reared within existing hatchery systems (Breese & Malouf 1977), with some evidence indicating that this species may continue to grow well when subjected to cooler water temperatures (Langdon & Robinson 1996).

Field trials of *Crassostrea ariakensis* show that this species grew more rapidly than *Crassostrea virginica* within the higher salinity (30 – 35) regions of Chesapeake Bay, and had comparable growth to *C. virginica* under more moderate (6 – 20) salinities (Calvo et al. 2001, Paynter et al. 2008, Kingsley-Smith et al. 2009). However, these studies chiefly examine triploid *C. ariakensis* individuals; they do not look at the seasonal variations in growth, and were examined in areas where the annual mean salinity is higher than typically found in the mesohaline region of Chesapeake Bay.

There is also difficulty in comparing growth rates of triploid versus diploid individuals. Diploid adult oysters typically allocate ~ 50% of their total production towards gamete production and reproductive activity (Dame 1996). Triploids do not expend energy on reproduction, thereby allocating more energy towards somatic growth (Stanley et al. 1984, Allen & Downing 1990, Wang et al. 2002). This allows triploids to grow faster and attain larger sizes more rapidly than diploid individuals, making triploids

difficult to use in realistic comparison scenarios. This study is the first one to examine the growth potential of diploid *Crassostrea ariakensis* versus diploid *C. virginica* under Chesapeake Bay conditions.

I will compare the growth, mortality, and reproductive capability of *Crassostrea ariakensis* to that of *Crassostrea virginica* over a three and a half year period in quarantined mesocosms supplied with ambient flow-through water from the Choptank River. I hypothesize that the growth and condition of diploid *C. ariakensis* would be comparable to that of diploid *C. virginica* within the mesohaline region of Chesapeake Bay. Scope for growth was calculated to determine the seasonal allocation of energy for each species in order to better understand and compare differences in their physiology.

MATERIALS AND METHODS

Mesocosm Experiments

An oyster quarantined facility was constructed within a 170 m^2 secure indoor seawater room at University of Maryland's Horn Point Laboratory (HPL). This facility contained twelve 500 L rigid polythene mesocosms (1 m^2, 0.6 m high) which were each supplied with approximately 20 L min^{-1} of ambient flow-through water from the Choptank River. These high flows ensured that food was not limiting to oyster growth. The water in each mesocosm was vigorously bubbled to ensure mixing and aeration. Water temperature and salinity were measured weekly using a conductivity meter (YSI – model 85). Within each of the mesocosms I added large intact eastern oyster shells (= cultch) in a layer 10 cm deep and contained in four mesh wire baskets that were placed together to form a central rectangle with an overall area of 0.81 m^2. By containing the oyster cultch material in baskets, it facilitated handling as I could remove individual

baskets periodically as part of tank cleaning and when measuring and photographing oysters.

In July 2004, I obtained diploid *Crassostrea ariakensis* larvae from Taylor United Shellfish hatchery in Quilcene, Washington and diploid *Crassostrea virginica* larvae from the Virginia Institute of Marine Science – Eastern Shore Laboratory (VIMS-ESL). Eyed pediveliger larvae of each species were added to the mesocosms (6 randomly selected mesocosms for each species) so that larvae could metamorphose directly on the cultch material in the baskets. Larvae were added in sufficient numbers to produce a final adult density of ~ 100 oysters on the 0.81 m^2 of cultch in each mesocosm assuming that only 0.1% of the larvae added survived to adulthood. The flow-through Choptank River water was turned off until microscopic examination indicated that the larvae had metamorphosed, which took between 3 and 7 d. During this period of no-flow I added cultured microalgae (*Isochrysis galbana*, clone C-iso) to each mesocosm as a food source. In July 2006 an additional cohort of both larval diploid *Crassostrea ariakensis* and *Crassostrea virginica* reared at VIMS-ESL were added to each of the mesocosms in the same manner as described above to develop a multi-age population.

In order to prevent gametes or larvae produced by the diploid oysters from entering the Choptank River all effluent waste water was chlorinated. This effluent water passed through three underground 5,700 L sealed concrete septic tanks connected in series. The total capacity of the system was 17,000 L which provided a total residence time of 45 min at the maximum system flow of 250 L min^{-1}. By burying these tanks I ensured that the water did not freeze during winter operations. Water in these tanks was subject to chlorination using a free-chlorine analyzer (Foxcroft FX-1000p) and controller

(Foxcroft FX-8500) located in the first underground tanks that controlled a chlorine dosing pump that ensured absolute quarantine conditions. A second identical Foxcroft chlorination system sampled water from the second of the three septic tanks, which provided a system of redundancy and allowed extra chlorine to be added if necessary to maintain residual free chlorine at 2 ppm. Testing showed that free chlorine concentrations of 2 ppm caused 100% larval mortality within the 45 minute residence time of this treatment system.

Growth and Mortality

Three months after larval settlement I randomly selected 5 pieces of shell from the top 10 cm of cultch from each of the four mesh wire baskets in the 12 mesocosms. These shells were tagged with a marked aluminum label, resulting in 20 tagged pieces of cultch per mesocosm. I selected the top of the 10 cm deep cultch layer so that I would not disturb the developing reef structure every time I sampled oysters. Oysters attached to these cultch pieces were assessed at approximately three-month intervals using digital photographic and image analysis from the time they when they were first clearly measurable in October 2004 through the conclusion of the study in January 2008; a total of 372 *Crassostrea ariakensis* and 520 *Crassostrea virginica* were measured repeatedly over the duration of this study. Absolute shell growth was calculated by measuring the surface area (mm^2) of oysters at each sampling date using Image J software (Rasband 1997-2009). Measurements were calibrated using a ruler with 1 mm increments that was included in each digital image for size reference. Using absolute measurements to estimate growth may lead to an overestimation in the growth rate of larger oysters versus

smaller oysters; therefore in order to standardize growth rate relative to an oyster's size, I calculated growth as a daily specific growth rate (SPG):

$$SPG = \frac{\left(\ln A_2 - \ln A_1\right)}{t_2 - t_1}$$

Where A represents the area (mm^2) of the oyster shell at the beginning and end of each sampling period and $t_2 - t_1$ is the time (d) between each sampling date. Samples of oysters from both the 2006 cohort of *Crassostrea ariakensis* and *Crassostrea virginica* 2006 were measured only in January 2008 to determine their final size at the end of the study.

Photographs of the individually identified spat were also used to determine the mortality of individuals within each species 2004 cohort between successive sampling times. A percent absolute and cumulative mortality of each species was estimated by the number of missing and dead oysters at each sampling period as verified by digital analysis. If all spat on one of the tagged pieces of cultch died I selected another piece to ensure that I had sufficient oysters to assess growth over future time intervals. I could not assess mortality in this manner over the first time interval (August through October 2004) because individuals were not identified until October 2004 when the first detailed sampling was performed. Mortality of 2006 cohort oysters was not assessed.

Reproductive Condition

Samples of 24 oysters were chosen randomly for gametogenesis assays from *Crassostrea ariakensis* and *Crassostrea virginica* mesocosms in November 2004. At that time the spat had grown sufficiently for there to be sufficient tissue for reliable histological analysis. In subsequent years, oysters from were sampled in April, June, July, and November. In 2007, when oysters were approximately 3 years old, samples were

taken more frequently in the period between April and August to characterize the oyster's gametogenic condition at this critical time in the annual reproductive cycle. Oyster issues from samples of each species were wet-weighed, fixed, embedded, sectioned, stained with hemotoxylin and eosin and examined microscopically.

For oysters that were large enough to provide a histological cross section through the plane of their gonad I calculated a quantitative index described by Kennedy et al. (1995). Cross-sections from these individual oysters were photographed (Fisher Scientific MZD digital Microscope Head and Micron Basic Software) under a dissecting microscope at 10× ocular power, with a piece of graph paper positioned within the field of view for scale measurement. Digital images were processed using Image J software (Rasband 1997 – 2009), with calibration based on the 1 cm scale from the graph paper grid in each image. The area of somatic tissue was concurrently measured (mm) at four places. These widths were then averaged and divided by somatic area (mm^2) × 100 to produce the proportional reproductive index of imaged tissues. I also undertook detailed microscopic analysis of histological slides of *Crassostrea ariakensis* prepared in 2007 to assess the extent of their spawning activity. A six stage nominal scale (Table 2.1) was developed that lists levels of qualitative gamete maturity and mobilization within gonad follicles and gonoducts revealed by microscopic analyses.

Many oysters sampled were so small that there was insufficient material to allow histological sections to be made that included a full cross section across the gonad. For these oysters I enumerated individuals with either eggs or sperm visible. This total number of males and females was added to the number of each gender from oysters used to qualify the reproductive index; and allowed us to calculate the percentage of total

oysters sampled that were male and female. The number of individuals in which gametogenesis had been initiated, but had not resulted in the production of distinct eggs or sperm, was also counted to give an index "percentage showing any gonadal development". Oysters in this latter category had distinct follicles and the amount and pattern of stain adsorption was indicative of high concentrations of DNA, but the cells had not yet differentiated sufficiently to discern if they were going to develop into eggs or sperm.

Statistical Analysis

Many oysters measured over the duration of this growth study had missing data points at one period or another due to a poor photographic angle or a missing picture; therefore for statistical analysis the absolute and specific growth rates of individual oysters were averaged by mesocosm replicate. A repeated measures ANOVA was used to test for differences in individual oyster growth rates seasonally between and within oyster species. Post-hoc least significant difference (LSD) multiple mean comparison tests were used to determine significant periodic differences in growth rate within species.

Percent absolute mortality was arcsine-transformed to achieve approximate normality. A repeated measures ANOVA was performed on the transformed data and post-hoc LSD multiple means comparison tests were performed to determine significant monthly differences between species.

Physiological Experiments

Physiological studies were performed under ambient seasonal conditions at HPL in April, August, October 2008; February and May 2009. The *Crassostrea ariakensis* (shell height: 39 – 109 mm) and *Crassostrea virginica* (shell height: 22.6 – 52.6 mm)

individuals (n = 16) used for this study were chosen randomly from the 12 mesocosm tanks described above. I also collected *C. virginica* (shell height: 49.6 – 138.8 mm) from a natural oyster reef, Sandy Hill, in the Choptank River to use in the physiological studies to better compare the physiological differences between the two species and help determine the reasons for the unexpectedly lower growth of *C. virginica* within the mesocosms. These "wild" oysters were held in a separate mesocosm under ambient Choptank River flow-through conditions until they were used. Approximately 2 d before being used for experimental studies, oysters were scrubbed to remove fouling organisms (barnacles, mussels, etc.) and repeatedly soaked in a chlorine solution (0.1% V:V Chlorox bleach to tap water) to remove organisms that could not be scrubbed off, such as *Polydora* sp., a worm that borrows into the oyster's shell (Newell 1985). This process was repeated, usually 3 – 4 times, until *Polydora* sp. were no longer observed exiting the oyster's shell.

Clearance Rate and Absorption Efficiency

Ambient flow-through water was pumped from the Choptank River into two head tanks that supplied water via lengths of Tygon tubing (6 mm I.D.) to 18 rectangular plastic pans (36 cm long × 30 cm wide × 10 cm high). A PVC plug with a precisely drilled hole was inserted into each tube that allowed a flow rate of either 40 L h^{-1} (for oysters ≥ 5 cm shell height) or 20 L h^{-1} (for oysters < 5 cm shell height) to the bottom of each pan. Preliminary studies showed that these high flow rates ensured that oysters would not be able to appreciably reduce particle concentrations during the experiment. All pans drained at the water surface through a standpipe at the end farthest from the inflow tube. This setup ensured adequate water column mixing through each pan. Six

oysters from each species and *Crassostrea virginica* "wild" representatives were randomly assigned to 18 separate pans with an appropriate flow rate for their shell size. Controls, that did not contain oysters, were assigned to 3 pans with both flow rates, respectively. Each run of the experiment (3 runs per season, totaling n = 18 for each treatment) lasted 36 h, during which hourly water samples for seston analysis were taken using an ISCO water sampler (Model 3700 Sampler Controller). Each oyster was held in a shallow plastic container placed in each pan in order to retain biodeposits. Appropriate containers were also placed in the control pans of both flow rates. Oysters were briefly removed from the pans after 12 h to remove biodeposits produced from seston that had been filtered and ingested before the start of the experimental run.

Oysters were removed from pans at the end of the experiment. The shallow containers from oyster and control pans were carefully removed, sealed, and held at 5ºC for 12 h to allow suspended material to settle. Overlying water was aspirated off and the container filled with 200 mL of DI water to wash out salt from the deposits before holding at 5ºC for another 12 h to once again allow suspended material to settle. The majority of DI water was aspirated off and two one mL aliquots of biodeposits from the containers were removed for absorption efficiency determination. Each aliquot was placed onto two pre-weighed Whatman GF/C filters that had first been washed and heat treated at 450ºC for 1 h. The remainder of the biodeposit slurry was transferred into a pre-weighed aluminum pan and these placed into a 90ºC drying oven for 24 h, after which time dry weights were taken. The filters were also dried at 90ºC and weighed to determine total dry weight. The filters were then heat treated at 450 ºC for 6 h to determine the organic fraction of the biodeposits. Material from the control containers

was treated identically to the material from the oyster containers and was collected to determine the amount of material that naturally settled into the experimental containers independently of oyster feeding activity. To correct for this extra material in the oyster containers, the amount of organic and inorganic material from the containers was determined as described above, and then subtracted from the total material present in the oyster containers.

Known aliquots of water (300 – 500 mL) collected by the ISCO sampler were filtered through GF/C filters and treated in the same manner as biodeposits to estimate seston concentration. The total inorganic fraction of oyster biodeposits (filters + aluminum pans) was calculated by determining the ratio of organic to inorganic matter from the material on the filter, and applying that ratio to calculate the inorganic portion of the material in the aluminum pans. Inorganic material from the filter was then added to the inorganic material from the pan to total obtain a total inorganic matter value. Clearance rate (L g^{-1} h^{-1}) was calculated as: (mg inorganic matter egested both as feces and pseudofeces h^{-1}) / (mg inorganic matter available L^{-1} of seawater) (Hawkins et al. 1996). Absorption efficiency was calculated using the Conover ratio (Conover 1966, Bayne et al. 1985).

Ammonium Excretion

For nitrogen excretion assays ambient river water was filtered (Millipore 0.45 μm pore) and used to fill beakers (200 – 900 mL) into which individual oysters were submerged or assigned as controls (n = 18 per treatment). Beakers were covered with plastic food wrap and incubated at ambient Choptank River water temperature in a water bath for 2 h. Oysters were then removed from the beakers and two 10 mL aliquots of

water from each beaker was placed into labeled test tubes. The phenol-hypochlorite method (Solórzano 1969, Bayne et al. 1985) was used to determine ammonium concentration. Ammonium excretion rates (μg NH_4-N g^{-1} h^{-1}) were calculated as described by Bayne et al. (1985).

Respiration Rate

Rates of oxygen consumption were measured using the methods described by Bayne et al. (1985). Individual oysters (n = 18 per treatment) were placed into either a large (2.3 L) or small (0.3 L) glass respirometer chamber supplied with ambient flow-through river water. These chambers were maintained at ambient river temperature by submerging in a water bath. After a 1 h period of acclimatization the water flow was stopped and the decline in oxygen concentration measured with a calibrated oxygen electrode (Radiometer-Copenhagen Model E5047-0). Controls were run using the same methods described above but without an oyster in the respirometer chamber. Control respiration rates were subtracted from the oyster runs to eliminate background respiration. The calculation of oxygen consumption rates required the volume of oysters to be subtracted from the total volume of water within the respirometer chamber (Bayne et al. 1985). Therefore oyster volume was determined by the displacement of water within a graduated cylinder. Respiration rates (mL O_2 g^{-1} h^{-1}) were calculated as described by Bayne et al. (1985).

Statistical Analysis and Scope for Growth

Dry tissue weight (dw) of all experimental oysters was obtained by removing oyster tissue from its shell, placing it in a pre-weighed pan, and drying it at 90ºC for 24 h. Seasonal physiological rates of individual oysters were regressed against their dry tissue

weight for *Crassostrea virginica* and *Crassostrea ariakensis* separately. Atomic ratios of oxygen consumption to nitrogen excretion (O:N) were calculated as described by Bayne et al. (1985) from standardized 1 g dw seasonal rates for each species.

An Analysis of Covariance (ANCOVA) was performed to determine a species-specific common slope. Using this, slope intercepts were recalculated using the allometric equation: $Y=aX^b$. These intercepts represent the seasonal physiological rates of an oyster of 1 g dw. This weight was selected as it was close to the average weight of the oysters studied, and also this animal weight is commonly used in comparisons of physiological rate functions within and between species of bivalves (Bayne & Newell 1983). The standard deviation of each seasonal physiological rate was calculated from the standard error reported in the ANCOVA analysis. This test was also used to test for differences in the seasonal physiological rates within each species. Post-hoc LSD multiple mean comparison tests were performed to determine which seasons were significantly different from each other.

Percent absorption efficiency was arcsine-transformed to approximate normality. An ANOVA was performed to determine if there was a seasonal difference in absorption efficiency within species, and post-hoc LSD multiple means comparison tests were performed to determine significant seasonal differences.

The physiological rates described above were converted into energy equivalents ($J\ g^{-1}\ h^{-1}$; Bayne et al. 1985). Energy absorbed from the seston was determined using a POM value 23.5 mg-1 (Widdows et al. 1979). This value is representative of the energy value for food materials such as seston (Slobodkin & Richman 1961, Bayne et al. 1985). Metabolic energy demand ($J\ h^{-1}$) was determined by multiplying energy respired

(mL O_2 h^{-1}) by 20.33 (Bayne et al. 1985). Energy excretion (J h^{-1}) was determined by multiplying the ammonia excretion rate (μg NH_4-N h^{-1}) by 0.0249 (Bayne et al. 1985). Scope for growth (*P*) is the energy available for allocation to germinal and somatic tissue production and was calculated by the equation (Bayne et al. 1985):

$$P \text{ (J h}^{-1}\text{)} = A - (R+U)$$

Where *A* is energy absorbed from seston, *R* is energy respired, and *U* is energy excreted.

An estimate of variance for the seasonal scope for growth of each species was determined by calculating minimal and maximal physiological rates at one standard deviation above and below the mean rate value. These minimal and maximal physiological rates were then used in the scope for growth equation described above. They were then added or subtracted from the mean scope for growth value in order to determine a measure of variance.

RESULTS

Mesocosm Experiments

Environmental Conditions

Mesocosm water temperature and salinity were similar to the annual cycle observed in the Choptank River. Salinity values during this study ranged from 8.2 – 12.2 and were lowest during the spring and early summer seasons (Table 2.2). These salinities were well within the optimal range for *Crassostrea virginica* (Shumway 1996) and *Crassostrea ariakensis* (Calvo et al. 2001). Water temperatures during this study ranged from 1.0 – 7.4ºC in January and 21.3 – 28.5ºC in August (Table 2.2).

Total suspended solids (TSS) and particulate organic matter (POM) values were taken seasonally from April 2008 until May 2009 to determine food availability within

the mesocosms (Table 2.3). TSS was highest in early spring with a total of 14.3 mg L^{-1}, of which 2.1 mg L^{-1} as POM. The highest POM values occurred in the winter, averaging 4.5 mg L^{-1} out of a TSS load of 13.3 mg L^{-1}. These values are consistent with seston concentrations found in the Choptank River (Berg & Newell 1986, Jordan 1987)

Growth

A comparison of absolute growth for *Crassostrea ariakensis* and *Crassostrea virginica* in HPL mesocosms showed that there was a significant interaction between species growth and sampling period (Repeated Measures ANOVA; $F_{24,119} = 223$; $P < 0.0001$). For the first 9 months of the study there was no significant difference in growth between the two oyster species (Fig. 2.1A) as evidenced by measurements taken in October 2004 (LSD; $t_{119} = 0.02$; $P = 0.99$), January 2005 ($t_{119} = 0.74$; $P = 0.46$), and April 2005 ($t_{119} = 1.2$; $P = 0.23$). In October 2004 the average size of *C. ariakensis* was 9 mm^2, while their size in April 2005 was 233 mm^2. The average size of *C. virginica* in October 2005 was 6 mm^2 and was 29 mm^2 by April 2005. Both species experienced a growth spurt in the spring/summer of 2005 with *C. ariakensis* more than tripling its shell area to 732 mm^2, and *C. virginica* increasing its size tenfold to 276 mm^2. From the summer of 2005 until the end of the study in January 2008, *C. ariakensis* grew to a significantly larger shell size than *C. virginica* ($t_{119} = 2.69$; $P = 0.01$). The average shell area of *C. ariakensis* at the end of the study was 3561 mm^2. After the summer of 2005, much of this growth occurred during the winter and early spring periods (Fig. 2.1A). The *C. virginica* oysters did not grow well from the summer of 2005 until the end of the study in January 2008, a period during which their average shell area (560 mm^2) only doubled in size (Fig. 2.1A). This is not typical of the pattern of *C. virginica* growth in the Choptank

River and mesohaline regions of Chesapeake Bay (Paynter et al. 2008, Kingsley-Smith et al. 2009).

Growth of the 2006 cohort for each species from settlement in July 2006 through the end of the project in January 2008 was comparable to the growth of the 2004 cohort for each species from their settlement in July 2004 through to January 2006. The size of the 2006 *Crassostrea ariakensis* cohort (n = 66) after their first 17 months in HPL mesocosms was 725 ± 59 mm^2 compared to 930 ± 61 mm^2 for the 2004 cohort (n = 372). The size of the 2006 *Crassostrea virginica* cohort (n = 111) over the same period was 285 ± 11 mm^2 compared to 317 ± 14 mm^2 for the 2004 cohort (n = 520).

There was a significant interaction between sampling period and oyster species for the SPG of 2004 cohort oysters (Repeated Measures ANOVA; $F_{22,108} = 421$; $P < 0.0001$). The SPG of *Crassostrea ariakensis* was always significantly greater than *Crassostrea virginica* from settlement until spring of 2006 (Fig. 2.1B), with the exception of the combined spring and summer period of 2005 (LSD; $t_{108} = 17.25$; $P < 0.0001$). Over the remainder of the study the SPG of *C. ariakensis* was significantly greater than the SPG of *C. virginica* during the winter ($t_{108} = 4.64$; $P < 0.0001$) and spring ($t_{108} = 2.99$; $P = 0.004$) of 2007 (Fig. 2.1B). The SPG of each species was not significantly different in the summer ($t_{108} = 0.84$; $P = 0.40$) and autumn ($t_{108} = 0.14$; $P = 0.89$) of 2007; and the summer ($t_{108} = 0.63$; $P = 0.53$) and autumn ($t_{108} = 0.22$; $P = 0.82$) of 2008 (Fig. 2.1B).

Mortality

Cumulative mortality of the 2004 cohort of *Crassostrea ariakensis* was moderate with only 35% of the oyster on tagged shells dying before the end of the experiment (Fig. 2.2A). The cumulative mortality for *Crassostrea virginica* was more severe, with 90% of

the oysters on tagged shells dead by the end of the study (Fig. 2.2A). Qualitative observation of oyster baskets within the *C. virginica* treatment confirmed that this heavy mortality was not confined only to the tagged shells.

There was a significant interaction between oyster species and sampling period in the comparison of absolute mortalities of each species (Repeated Measures ANOVA; $F_{22,110} = 7.99$; $P < 0.0001$). The absolute mortality of *C. virginica* was usually significantly greater than *C. ariakensis* for all sampling periods, with the exception of winter (LSD; $t_{110} = 0.79$; $P = 0.43$), spring ($t_{110} = 0.04$; $P = 0.97$) and autumn ($t_{110} = 1.45$; $P = 0.15$) of 2006; and the winter ($t_{110} = 1.71$; $P = 0.09$) of 2007 (Fig. 2.2B). These periods corresponded with some of the lowest absolute mortality rates for both *C. ariakensis* and *C. virginica* during the study (Fig. 2.2B). Mortality rates of the 2006 cohorts of *C. ariakensis* and *C. virginica* were not calculated; however qualitative analysis suggests that mortality was similar to the 2004 cohorts over the same period.

Reproductive Condition

The 2004 cohort of *Crassostrea ariakensis* first started to become reproductively active in July 2005 when they were one year old (Fig. 2.3A). At that time, a large number of individuals (~75%) exhibited pronounced gametogenic activity and these gametes were mature as the sex of these individuals could be clearly distinguished (Fig. 2.3B). This was not the case for *Crassostrea virginica* sampled concurrently, which showed no evidence of reproductive activity until they were two years old during the summer of 2006. In 2006 and 2007 both species of oysters showed high levels of gonadal development, although the numbers of *C. ariakensis* showing such development were always greater than those of *C. virginica*. When the oysters were three years old in 2007,

gametes could be clearly identified in all of the *C. ariakensis* but only in ~60% of the *C. virginica* (Fig. 2.3A). Gametes were clearly visible in *C. ariakensis* from early June through mid-November, which was an appreciably longer period than for *C. virginica*, which only had distinguishable gametes present from early June through early August (Fig. 2.3B).

The quantitative gonad index analysis required larger oysters to provide the requisite amounts of tissue to be sectioned across the full diameter of the visceral mass, whereas the other methods of assessing reproductive activity required lesser amounts. When large oysters were available for analysis, *Crassostrea ariakensis* had percent gonad index values of between 10 and 14 for two sampling occasions in the summers when they were one year of age (Table 2.4). By the time they were three years old, in the summer of 2007 they exhibited values of between 1 to 8 for all sampling times between June and November. In contrast, for *Crassostrea virginica*, only for sampling times in early June and early July 2007 did oysters have reproductive index values of ~ 4 (Table 2.4).

Direct examination of gamete conditions among histological sections of gonad tissues from samples of the 2004 *Crassostrea ariakensis* cohort that were preserved during the seven month period from February through November 2007, provided a direct assessment of spawning activity that complemented the percent gonad index data. The percentage of the 25 individuals processed at each sampling time that were in one of the six qualitative gametogenic categories (Table 2.1) showed a distinct seasonal pattern (Fig. 2.4), with 4% of the oysters having ripe gametes present in expanded follicles and/or free in the gonoducts in June. This proportion increased to 100% of oysters with ripe gametes during July and August, and decreased to 79% in September. Following apparent

widespread spawning during July and August, experimental *C. ariakensis* oysters exhibited post-spawning condition, with proportions increasing from 17% in September to 83% in November (Fig. 2.4).

The 2006 cohort *Crassostrea ariakensis* first started to become reproductively active in November 2006, when ~ 45% exhibited pronounced gametogenic activity, but there was no mature gametes at this time for any individuals. In the summer of 2007, when this cohort was one year of age, there was evidence of near 100% reproductive activity in both species. In early June only 4% of both species of oyster had clearly distinguishable gametes. This increased to 42% of *C. ariakensis* in the early July sampling, but declined to zero for *Crassostrea virginica*. None of the oysters were sufficiently large enough to allow for the measurement of Gonadal Index.

Physiological Experiments

For oyster clearance rates there was no interaction between dw and season for either *Crassostrea ariakensis* (ANCOVA; $F_{4,66} = 1.08$; $P = 0.37$), mesocosm-reared *Crassostrea virginica* ($F_{3,30} = 1.24$; $P = 0.31$), or wild *C. virginica* ($F_{2,31} = 0.03$; $P = 0.97$). This allowed us to calculate a common slope for *C. ariakensis* ($b = 0.50$) and *C. virginica* ($b = 0.29$) to apply in the regression equation that I used to recalculate the intercept for each season. There were no significant differences in the clearance rate of mesocosm reared *C. virginica* (Table 2.5) between April, August, and October 2008; and May 2009 (ANCOVA; $F_{3,33} = 0.04$; $P = 0.99$), which all averaged ~ 1.01 L g^{-1} h^{-1}. The clearance rate of wild *C. virginica* was significantly lower in summer 2008 (Table 2.5) than in the autumn of 2008 (LSD; $t_{33} = 2.52$; $P = 0.02$); and the spring of 2009 ($t_{33} = 3.58$; $P = 0.001$). Winter data was not included for either *C. virginica* treatment as these oysters were not

observed feeding and did not produce biodeposits during this period. For *C. ariakensis*, the highest clearance rates occurred in spring of 2008 (1.9 L g^{-1} h^{-1}) and 2009 (2.71 L g^{-1} h^{-1}); and the difference between these two periods were significant (t_{70} = 2.05; P = 0.04). A significantly lower, but measurable, clearance rate was recoded during the winter of 2009 (Table 2.6) when water temperatures averaged 5ºC (Table 2.3).

Absorption efficiency of mesocosm reared *Crassostrea virginica* (ANOVA; $F_{4,74}$ = 10.7; P < 0.0001) and wild *C. virginica* ($F_{3,58}$ = 18.3; P < 0.0001) differed significantly among seasons. Since neither *C. virginica* treatment ingested material in the winter of 2009, there was no energy absorption during this period (Table 2.7). Mesocosm reared *C. virginica* had only a 6.5% absorption efficiency during the spring of 2009, which was significantly less than the 34.2% absorption efficiency recorded for this treatment in the spring of 2008 (LSD; t_{74} = 3.11; P = 0.003). Wild *C. virginica* oysters had similar absorption efficiencies in the summer and autumn of 2088; and the spring of 2009 (Table 2.7). There was a significant difference in the absorption efficiency of *Crassostrea ariakensis* among seasons (ANOVA; $F_{4,71}$ = 8.57; P < 0.0001). The highest absorption efficiencies *of C. ariakensis* occurred during the winter (52%) and summer (42%) periods; while the lowest was recorded in the autumn (22%) period (Table 2.7).

There was no interaction between dw and season for the respiration rate of mesocosm reared *Crassostrea virginica* (ANCOVA; $F_{3,36}$ = 0.18; P = 0.91), wild *C. virginica* ($F_{2,34}$ = 2.90; P = 0.07), and *Crassostrea ariakensis* ($F_{4,58}$ = 0.82; P = 0.52). This allowed us to calculate a common slope for *C. virginica* mesocosm (*b* = 0.47) and wild (*b* = 0.83) oysters (Table 2.5), and *C. ariakensis* (*b* = 0.53) oysters (Table 2.6) for the regression equation that was then used to recalculate the intercept for each season.

Respiration rate did not vary significantly by season for either mesocosm reared ($F_{3,39}$ = 1,78; P = 0.17) or wild ($F_{2,36}$ = 1.65; P = 0.21) *C. virginica* oysters (Table 2.5). Respiration rate of *C. ariakensis* (Table 2.6) was greatest during the summer (1.12 mL O_2 g^{-1} h^{-1}) and lowest during the winter (0.36 mL O_2 g^{-1} h^{-1}), and this difference was significant (LSD; t_{62} = 4.30; P < 0.0001).

There was no interaction between oyster dw and season for ammonium excretion for mesocosm reared *Crassostrea virginica* (ANCOVA; $F_{4,45}$ = 1.71; P = 0.17), wild *C. virginica* ($F_{3,42}$ = 1.61; P = 0.20), and *Crassostrea ariakensis* ($F_{4,58}$ = 0.91; P = 0.46). This allowed us to calculate a common slope for *C. virginica* mesocosm (*b* = 0.44) and wild (*b* = 0.95) oysters (Table 2.5), and *C. ariakensis* (*b* = 0.53) oysters (Table 2.6) for the regression equation that was then used to recalculate the intercept for each season. Ammonium excretion was significantly lower in the autumn than for all other seasons (P < 0.05) for both mesocosm-reared (1.06 μg NH_4-N g^{-1} h^{-1}) and wild (1.61 μg NH_4-N g^{-1} h^{-1}) *C. virginica* oysters. For *C. ariakensis* (Table 2.6), ammonium excretion was highest in the summer (36.27 μg NH_4-N g^{-1} h^{-1}) and lowest in the autumn (4.62 μg NH_4-N g^{-1} h^{-1}); and this difference was significantly different (LSD; t_{62} = 5.55; P < 0.0001).

Crassostrea ariakensis exhibited a seasonal variation in scope for growth (Fig. 2.5A) with the greatest amount of energy available for growth occurring in the spring (2008 = 8 J g^{-1} h; 2009 = 27 J g^{-1}). Interestingly, during the winter when temperatures were coldest, *C. ariakensis* continued to have a positive scope for growth (9 J g^{-1}). In the summer there was a negative scope for growth (-1 J g^{-1}) due to the high metabolic activity of *C. ariakensis* (Fig. 2.5A). The scope for growth of the two *Crassostrea virginica* treatments also varied seasonally, however they were dissimilar to

each other. Mesocosm-reared *C. virginica* had a positive scope for growth in the spring (11 J g^{-1}), summer (10 J g^{-1}), and autumn (4 J g^{-1}) of 2008, with a slight negative scope for growth (-2 J g^{-1}) in the spring of 2009 (Fig. 2.5B). Winter scope for growth could not be calculated because of the missing respiration data. Wild *C. virginica* had a positive scope for growth in the autumn (4 J g^{-1}) of 2008 and the spring (24 J g^{-1}) of 2009. These oysters exhibited a slight negative scope for growth in the winter (-0.3 J g^{-1}) when feeding activity ceased (Fig. 2.5B). Interestingly, scope for growth for these wild *C. virginica* was lowest in the summer (-15 J g^{-1}) when they were metabolically active, but clearance were very low (Table 2.5).

The O:N ratio for *Crassostrea ariakensis* was lowest during the summer (<50) and highest (>100) in the autumn (Fig. 2.6A). The O:N ratio for both *Crassostrea virginica* treatments remained relatively low (<100) throughout the year, with the exception of autumn were the ratio for mesocosm reared oysters exceeded 300, and for wild oysters was close to 500 (Fig. 2.6B).

DISCUSSION

Diploid *Crassostrea ariakensis* grew significantly faster and larger than dipolid *Crassostrea virginica* within conditions simulating the mesohaline region of Chesapeake Bay. At the conclusion of this study, and 3.5 y after initial settlement, the average shell surface area of *C. ariakensis* was six times larger than that of *C. virginica* oysters. This large difference in size was not necessarily due to the enhanced growth of *C. ariakensis*, but rather the lack of growth of *C. virginica* oysters after the summer of 2005. Over the first 10 months of the study, there was no difference in the absolute growth rate between the two oyster species.

A comparison of the specific growth rate between the two oyster species over the same time period show that newly settled *C. ariakensis* had significantly faster growth than *C. virginica* through the fall of 2004 and the winter of 2005. During the spring–summer sampling period, however, the specific growth rate of *C. virginica* was significantly greater than *C. ariakensis*. The enhanced growth of *C. ariakensis* during the winter and early spring, relative to the enhanced growth of *C. virginica* in the later spring and summer periods has been described in other studies (Calvo et al. 2001, Kelly et al. 2011). This finding indicates that *C. virginica* growth within the mesocosms was proceeding normally until after the summer of 2005. That autumn the growth of *C. virginica* slowed considerably, and three years later at the conclusion of the study, these oysters remained small and only doubled their absolute shell size from measurements taken in the summer of 2005. The specific growth rate of *C. virginica* after the summer of 2005 also remained low, and not significantly different from the remaining seasons. This is not typical growth for *C. virginica* in the mesohaline region of Chesapeake Bay, where oysters generally grow well in the late spring and early summer at a rate of approximately one inch per year. The depressed growth rate of 2004 *C. virginica* was not limited to this cohort only, as the 2006 cohort also exhibited similarly low growth rates over the duration of this study.

The reason for the lack of *Crassostrea virginica* growth from the summer of 2005 onwards is unknown. Oyster growth in mesocosms may not reflect the true growth potential of oysters in the field. DeBrosse and Allen (1996) found reduced growth and high mortality of both *C. virginica* and *Crassostrea gigas* reared within mesocosms when compared to field grown oysters. They were not able to determine the cause the stressor

that affected the oysters, but speculated that elevated densities of *Polydora* sp. brought into the mesocosms with the water flow-though system may have stressed their oysters. In my experiment, qualitative *Polydora* sp. density seemed relatively low on large oysters, and absent on most stunted *C. virginica* individuals. Oysters appeared morphologically normal and did not exhibit symptoms of any oyster diseases (Bricelj et al. 1992, Newell et al. 2009). Measured clearance rates were slightly lower than normal (Loosanoff & Nomejko 1946, Jordan 1987), but did not seem to negatively impact the scope for growth of this species. The meat of both oyster species periodically had a greenish tinge, indicating a potentially elevated copper concentration within the flow-through water; however copper concentration measurements were not taken. The Horn Point oyster hatchery, which also uses ambient Choptank flow-through water, also reported "green" *C. virginica* and *Crassostrea ariakensis* oysters (S. Alexander, personal communication); however, other than meat color, they did not observe any negative effects such as a reduction in growth or decline in reproductive capacity of these oysters. The retention of copper within *C. virginica* may be used to promote antimicrobial activity within the oyster, and even high concentrations of this metal may not be detrimental to oyster condition (Shuster and Pringle 1969, Fisher 2004). Stunted oysters concentrate heavy metals much more efficiently than oysters exhibiting a normal growth rate (Phelps & Hetzel 1987); therefore the presence of copper is unlikely the cause, and instead a symptom, of stunted growth within my *C. virginica* population.

What makes these growth results for *Crassostrea virginica* especially difficult to explain was that there was no corresponding decrease in *Crassostrea ariakensis* growth or condition after the summer of 2005, even though they were subjected to exactly the

same conditions as *C. virginica*. Each mesocosm was treated identically, received the same amount and quality of ambient flow-through water from a central system, and was randomized to minimize any experimental error due to location within the oyster research facility. The abnormal growth of *C. virginica* oysters within this study also makes it impossible to compare their growth rates to diploid *C. ariakensis* grown under mesohaline conditions within the same system, which was the original intent of this study.

Crassostrea ariakensis grew well within the mesocosms over the course of this study. Increases in shell area generally occurred in the winter and early spring, with a period of reduced shell growth during the summer. Bivalves generally promote shell growth during the spring and early summer periods, and increase body tissue growth during the summer (Hilbish 1986). In the present study, reduced shell growth of *C. ariakensis* was accompanied by a decrease in body tissue weight and loss of general condition (Newell et al. 2009). The shell height of diploid *C. ariakensis* within the mesocosms (~ 85 mm) after two years of growth was slightly smaller than the size of triploid *C. ariakensis* of the same age reported in studies from the field (~100 mm) (Calvo et al. 2001, Kingsley-Smith 2009). This slight difference in shell height is likely attributed to differences in the allocation of energy between diploid and triploid individuals. Seasonal shell growth for *C. ariakensis* was determined by calculating the specific growth rate at each period. The highest specific growth rates occurred in the winter and spring periods when cool water temperatures (6 – 19ºC) were predominate. Little growth occurred when water temperatures were warmer (19 – 27ºC) during the summer and fall periods, and the shell growth in these seasons was not significantly higher than the *Crassostrea virginica* oysters.

Crassostrea ariakensis have been reported within the subtropical region of China (Guo et al. 2008), its population there is divided into two distinct genetic strains with a "northern-type" strain primarily found in the temperate regions, and a "southern-type" primarily found in the subtropical regions (Zhang et al. 2005). Increased growth under cooler water temperatures is likely due to the fact that *Crassostrea ariakensis* individuals, which are used for research in the United States initially came from a small founder population of 7 males and 9 females (U.S. Army Corps of Engineers 2009) cultured and bred within the cooler waters of Yaquina Bay, Oregon (Breese & Malouf 1977).
Of the *C. ariakensis* oysters being actively cultured and studied within the United States, approximately 97% of them are derived from the "northern-type" strain (Zhang et al. 2005). Given the highly restricted genetic make-up of these "Oregon" stock introduced oysters, and the fact that they likely originated in the cooler temperate regions of Asia, it is not surprising that they tend to grow better under the cool water temperatures experienced in the late winter and early spring periods characteristic of mesohaline Chesapeake Bay.

A major impetus for this work was to compare growth of diploid *Crassostrea ariakensis* and diploid *Crassostrea virginica* within the mesohaline conditions found in the mid-Chesapeake Bay region where oyster populations had been historically abundant (Smith et al. 2003). The abnormal growth rate of the *C. virginica* treatment precluded us from making this comparison. Field trials that compared diploid *C. virginica* to triploid *C. ariakensis* within the mesohaline regions of Virginia (Calvo et al. 2001) and triploid *C. virginica* to triploid *C. ariakensis* within mesohaline regions of Maryland (Paynter et al. 2008, Kingsley-Smith et al. 2009) show that there is little difference in the comparative

absolute growth rate of each species. This differs from the statistically larger differences in absolute growth rate between the two species observed at higher salinity sites (Calvo et al. 2001, Paynter et al. 2008, Kingsley-Smith et al. 2009). While salinities are variable (2 – 34) within the native range of *C. ariakensis* (Yingya et al. 1992, Zhou & Allen 2003), they are found in greatest abundance where salinities average 20 – 28 (Guo et al. 2008).

The growth of *C. ariakensis* spat is optimal between salinities between 25 – 35 and a water temperature of 25ºC (Langdon & Robinson 1996). Based on the trajectory of shell sizes recorded for the mesocosm-reared *C. ariakensis* versus published normal growth rates of *C. virginica* from the field (Calvo et al. 2001), these findings would have likely shown no significant difference in growth between the two species under mesohaline conditions, had the *C. virginica* not been stunted.

The cumulative rates of mortality in *Crassostrea ariakensis* (~ 35%) was lower that that of *Crassostrea virginica* (~ 90%), even though environmental conditions, including the abundance of micropredators, were similar among all tanks. Results from concurrent laboratory predation studies (Newell et al. 2007a) indicate that *C. ariakensis* is just as vulnerable as *C. virginica* to the common polyclad-flat worm *Stylochus ellipticus*, and significantly more vulnerable to mud crab predators. No mud crabs were observed in mesocosm tanks, but based on qualitative observations high numbers of flat worms were present.

Pressures from oyster diseases were limited to low intensity *Perkinsus* sp. (Dermo) infections that occurred among oysters of both species at moderate infection prevalence similar to those detected regionally among wild *C. virginica* oysters (Newell et al. 2009). Neither *Haplosporidium nelsoni* (MSX) nor *Bonamia* sp. infections were

detected among sampled experimental cohorts of either oyster species exposed to ambient, mesohaline Choptank River water during the four years of the study (Newell et al. 2009). The low growth rate of *C. virginica* coupled with the high mortality rate of this species further indicate that an unknown stressor affected *C. virginica*, but had no affect on *C. ariakensis* was present within the mesocosms.

There were appreciable differences in the reproductive condition of the two species of oysters, with *Crassostrea ariakensis* becoming reproductively active at an earlier age and for a longer duration each summer than *Crassostrea virginica*. In July 2005, 75% of 1 y old *C. ariakensis* had distinct eggs and sperm present in the follicles, whereas, the similarly aged but much smaller, *C. virginica* showed no evidence of gametogenesis. In the summer of 2006, although the 2 y old *C. virginica* showed evidence of germinal activity, only ~ 5% of the oysters had eggs or sperm visible in the follicles, whereas ~ 45% of the *C. ariakensis* had gametes distinctly visible. By the time these oysters had reached 3 y of age in 2007, *C. virginica* had gametes distinctly visible in the follicles and were exhibiting Gonad Index values of ~ 4% in June and July. This value is comparable to literature values reported for reproductively active *C. virginica* from nearby locations in Chesapeake Bay (Kennedy et al. 1995). In comparison, *C. ariakensis* had gametes distinctly visible in the follicles for the entire five month period from early June through early November, which is a much more extended period than for *C. virginica*. The *C. ariakensis* were exhibiting Gonad Index values broadly comparable to the *C. virginica* but these were maintained over the entire five month reproductive season. This extended period of high gametogenic condition is in distinct contrast to comparably sized > 5 cm shell height *C. virginica* from nearby locations in Chesapeake

Bay (Kennedy et al. 1995). In these field locations the gonadal index of freshly collected *C. virginica* was at peak levels during June and July of 1991 and declined to low post-spawning values by the end of August. This is an important finding indicating that if released into the wild, *C. ariakensis* might be able to breed at an earlier age than native *C. virginica* oysters and for a longer period each season. It is important to recognize, however, that there are some limitations to my data such as the comparisons of size and age at which these two species of oysters became reproductively mature. As discussed earlier, the *C. virginica* in these mesocosms were exhibiting appreciably slower growth rates than expected compared to oysters within Chesapeake Bay. It is highly likely that this reduced vitality was a major contributing factor leading to delayed onset of gametogenic development.

The duration of the season when *Crassostrea ariakensis* has a large number of developed gametes, indicated by a high gonadal index, means that these oysters were continuously developing new gametes during the reproductive period. Microscopic examination of the condition of gonads during 2007 confirmed that these oysters were indeed spawning gametes actively during July and August, instead of simply maintaining gametes that ripened due to the absence of a specific spawning cue. Importantly, these data indicate that the spawning seasons of *C. ariakensis* and *Crassostrea virginica* in the Choptank River would overlap during July, which was when oysters of both species exhibited high reproductive condition. This suggests that coincident spawning is likely to occur, and hence that cross-fertilization between gametes from these two species of oysters will produce non-viable zygotes, which would lead to an overall loss of gametes for both species. These data support the "gamete sink" hypothesis proposed by Bushek et

al. (2008) that suggest an introduction of diploid *C. ariakensis* would lead to a reduction in larval production for both species of oyster. But the mesocosm data also indicate that oysters of both species held under identical environmental conditions have reproductive peaks (June for *C. virginica*; August-September for *C. ariakensis*) that are not coincident with each other. Because of the time of peak reproductive condition is not exactly coincident, this suggests that both species will have a period when spawning will result in normal fertilization and the production of viable larvae.

Physiological studies indicate that *C. ariakensis* are better adapted to cooler water temperatures than *C. virginica*, as evidenced by the high level of energy available to be allocated towards germinal and somatic production in the spring (27 J g^{-1} h^{-1}) and winter (9 J g^{-1} h^{-1}) periods. The increased scope for growth of *C. ariakensis* during the spring period was due primarily to an enhanced clearance rate (1.86 – 2.71 L g^{-1} h^{-1}) and absorption efficiency (22 – 38%). During the winter the increased scope for growth was driven by a relatively high clearance rate (0.29 L g^{-1} h^{-1}) and absorption efficiency (52%), coupled with a relatively low respiration rate (0.36 mL O_2 g^{-1} h^{-1}). During the summer, *C. ariakensis* had a negative scope for growth (-1 J g^{-1} h^{-1}) due to a relatively low clearance rate (0.97 L g^{-1} h^{-1}) and an enhanced respiration rate (1.12 mL O_2 g^{-1} h^{-1}).

The negative scope for growth of *Crassostrea ariakensis* during the summer within the mesohaline mesocosms was also reported for *C. ariakensis* oysters of the same stock in conditions simulating the high salinity (29 – 32) waters of coastal Virginia (Kelly et al. 2011). Oysters in Virginia were also found to have relatively low clearance rates without an equivalent decrease in other physiological rates such as respiration or ammonium excretion. Studies by Zhang et al. (1959, cited in Zhou & Allen 2003) have

shown that *C. ariakensis* (= *Ostrea rivularis*) feeds less actively when water temperatures were high (22 – 30ºC) and salinities were low and variable (2 – 26). Zhou and Allen (2003) suggest that the decrease in feeding activity was likely due to changes in salinity and not temperature induced; however the results from this study and that of Kelly et al. (2011) indicate that temperature intolerance to warmer waters was likely the reason for the lower feeding of *C. ariakensis* observed in Zhang et al. (1959).

There would be several potential ecosystem affects if *Crassostrea ariakensis* were to be introduced into Chesapeake Bay. The spring bloom is under utilized by the filter feeding community because water temperatures are too cold (< 5ºC) for most of them to be physiologically active (Hagy et al. 2005, Newell et al. 2007b, Fulford et al. 2007, Fulford et al. 2010). The spring bloom would be grazed on by *C. ariakensis*, unlike other native benthic filter-feeding species in Chesapeake Bay, because of its physiological tolerance to colder waters. Communities of *C. ariakensis* would shunt nutrients by way of biodeposits to associated fauna much quicker than *C. virginica* communities, which may favor associated oyster reef fauna, which are also more physiologically active during the colder seasons. Intense grazing pressure of large *C. ariakensis* communities within the Bay during the late winter and early spring may put pressure on organisms that are able to take advantage of the spring bloom, such as copepods and menhaden (Nicholson 1978, White & Roman 1992, Lynch et al. 2010). This pressure may decrease these populations that may reverberate throughout the entire food web of Chesapeake Bay, although perhaps returning the Bay back to a more benthic-dominated system thought to prevail when *C. virginica* stocks were abundant (Newell 1988, Cero & Noel 2005, Newell et al. 2007b, Fulford et al. 2007, Fulford et al. 2010).

Conversely, the intolerance of *Crassostrea ariakensis* to warmer water temperatures may negatively impact Chesapeake Bay fauna dependent on oyster reefs for habitat. Biodeposition rates of *C. ariakensis* are lower than that of *Crassostrea virginica* in the summer, which may decrease the shunting of nutrients just when these communities are most physiologically active. This suggestion is supported by field studies comparing the density of associated reef fauna between *C. ariakensis* and *C. virginica* experimental reefs. Harwell et al. (2010) found that subtidal reefs comprised of only *C. ariakensis* or a combination of *C. ariakensis* and *C. virginica* oysters had lower densities of organisms associated with them per unit biomass than experimental reefs comprised of *C. virginica* individuals alone; this finding was not attributed to differences in reef morphology between the two species. Differences in faunal abundance were most pronounced during the summer, where *C. virginica* reefs had a greater density of organisms that *C. ariakensis* or mixed species reefs (Harwell 2010). Oyster biodeposition rates were not measured, and so it is difficult to interpret that reduced nutrient availability had a role in limiting faunal abundances on reefs containing *C. ariakensis* oysters. Although this does correspond with the decreased biodeposition rates that I found for *C. ariakensis*.

Over the long-term *Crassostrea ariakensis*, if introduced into Chesapeake Bay, may adapt and evolve to be more physiologically tolerant of warmer water temperatures over time, as evidenced in other bivalve species (Thompson & Newell 1985, Wrange et al. 2010) and within the native range of *C. ariakensis* itself (Guo et al. 2008). This potential adaptation may neutralize the potential ecological impacts described above. The observed seasonal physiological differences suggest a potential influence of *C. ariakensis* on

enhanced benthic-pelagic coupling during the late autumn-winter-early spring period and decreased benthic-pelagic coupling when temperatures are maximal during the summer, which is completely opposite to the current paradigm within Chesapeake Bay.

While my scope for growth analysis correlates well with the growth patterns observed between the two oyster species within the mesocosms, it does not help in identifying the causes for slow growth of the *Crassostrea virginica* oysters. The physiological measurements reported here indicate that there should have been adequate energy available for germinal and somatic growth during the spring, summer, and fall periods to support *C. virginica* growth. This finding does not correlate with the growth data observed during this study, in which *C. virginica* grew extremely slowly. The scope for growth of *C. virginica* was calculated for two spring periods (2008 and 2009), during which they were expected to have similar values. However, scope for growth was dramatically different between spring 2008 (11 J g^{-1} h^{-1}) and spring 2009 (- 2 J g^{-1} h^{-1}) while temperature, salinity, and seston concentrations were similar between these two spring periods; the reason for this is unknown.

In order to more fully investigate the lower than expected growth of *C. virginica* in the mesocosms I also used wild *C. virginica* to act as a control in the scope for growth analysis. The wild *C. virginica* exhibited a negative scope for growth during the summer (- 15 J g^{-1} h^{-1}) due to a low clearance rate (0.42 L g^{-1} h^{-1}). The reasons for the low clearance rate during the summer are unclear as they were collected from the Sandy Point oyster bar in the Choptank River less than a mile from the HPL ambient flow-through water intake pump and were held in the same tanks as the mesocosm reared *C. virginica*. Remarkably, they had a much higher scope for growth (24 J g^{-1} h^{-1}) than mesocosm-

reared *C. virginica* (- 2 J g^{-1} h^{-1}) in the spring of 2009. The addition of the wild *C. virginica* oysters did not contribute to the understanding of what was occurring with *C. virginica* within the mesocosms. Overall, my physiological studies indicate that *C. virginica* in the mesocosms should have been exhibiting normal growth rates.

Analysis of the O:N ratio of bivalves can indicate if individuals were stressed, with high ratios signifying normal utilization of energy through lipid and carbohydrate catabolism, whereas low ratios are more indicative of nutritional stress related to energy utilization through protein catabolism (Bayne et al. 1985). Very low O:N ratios (< 20) indicate high nutritive stress in marine bivalves (Bayne et al. 1985, Huang & Newell 2002). While the O:N ratio for both species were low (< 100) during most seasons sampled, these values were not low enough to indicate that stress caused by energy production through protein catabolism was a factor in the stunted growth I recorded for *Crassostrea virginica.* Both species had very high O:N ratios (~ 150 for *Crassostrea ariakensis*; > 300 for *C. virginica*) in the autumn. During this period bivalves sequester stores of carbohydrates for use to sustain them through the winter and initiate next year's gametogenesis (Newell & Bayne 1980, Huang & Newell 2002).

The only explanation I currently have for the slow growth that was measured for *Crassostrea virginica* was that there was an unknown stressor that was affecting these individuals, but having little to no affect on *Crassostrea ariakensis*. The nature of this stress eludes us as both species of oysters were grown under the same conditions, disease and predation pressures in the tanks were extremely low (Newell et al. 2009), and Polydora sp. infestations were relatively light (DeBrosse & Allen 1996).

When compared to *Crassostrea virginica* in Chesapeake Bay, *Crassostrea ariakensis* have a longer growing season, grow at a faster rate, and have a longer period when ripe gametes are present. Greater annual growth suggests that *C. ariakensis* will build biomass at a faster rate than *C. virginica*, which could give *C. ariakensis* a long-term competitive advantage over *C. virginica*, especially combined with possibly earlier and more intense reproductive development. An introduction of *C. ariakensis* within Chesapeake Bay may also have unintended (positive and negative) ecological consequences. The greater physiological affinity of *C. ariakensis* for cooler water temperatures may alter benthic-pelagic coupling during the summer that would limit the availability of nutrients for associated fauna, and hence impact established food webs.

CHAPTER 2:

Tables

Table 2.1: Nominal scale for oyster reproductive condition

Six stage nominal scale for oyster gamete maturation and oyster spawning condition.

Stage name, Code	Stage characteristics
non-gametogenic	Indifferent inactive, resting: gonadal epithelia present, inactive, and reduced; follicles absent or vestigial.
gametogenesis I, GI	Oogonia or spermatogonia present; follicles developing.
gametogenesis II, GII	Oocytes or spermatocytes present; follicles expanded.
gametogenesis III, GIII	Spermatids, sperm, or ova present in expanded follicles.
spawn-ripe	Mature sperm or ova free in expanded follicles and gonads.
post-spawn	Resorbing gametes or gamete residulas present with hemocytes in gonoducts and/or follicles.

Table 2.2: HPL mesocosm water temperature and salinity

Average monthly water temperature and salinity (± SD) of ambient flow-through Choptank River water in mesocosms calculated from weekly measurement taken between August 2004 and January 2008.

Month	Temperature (ºC)	Salinity
January	6.3 ± 0.5	10.8 ± 3.0
February	4.9 ± 2.9	8.2 ± 0.3
March	8.6 ± 1.6	8.4 ± 0.1
April	12.3 ± 1.3	8.3 ± 0.7
May	19.1 ± 1.3	8.7 ± 1.8
June	23.8 ± 0.7	9.2 ± 1.4
July	26.7 ± 1.1	9.5 ± 0.8
August	26.8 ± 0.8	10.8 ± 1.2
September	23.7 ± 1.2	11.9 ± 1.7
October	19 ± 1.8	12.2 ± 2.2
November	13.1 ± 0.6	12.1 ± 1.9
December	7.1 ± 2.3	10.3 ± 0.6

Table 2.3: Water conditions for HPL physiology experiments

Mean (n=3) temperature, salinity, total suspended solids (TSS ± SD), and percent particulate organic matter (POM ± SD) of ambient flow-through Choptank River water during a 7 d period when seasonal physiological measurements were performed.

Season	Temperature (ºC)	Salinity	TSS (mg L^{-1})	POM (%)
April 2008	17.0	9.3	14.3 ± 4.2	14.9 ± 1.5
August 2008	25.0	11.2	12.2 ± 0.4	19.8 ± 0.3
October 2008	12.4	14.7	9.9 ± 3.1	17.7 ± 1.1
February 2009	5.0	12.3	13.3 ± 0.3	33.9 ± 2.3
May 2009	17.7	10.8	9.6 ± 1.9	17.5 ± 3.3

Table 2.4: Gonadal index of Crassostrea ariakensis and Crassostrea virginica

Mean (± SD) percent gonadal index for oysters large enough to provide sufficient tissue for analysis (see text for details) from *C. ariakensis* and *C. virginica* oysters at each sampling time

Species	Year	Month	N	% Gonadal Index
C. ariakensis	2005	June	3	10 ± 5
		July	17	13 ± 7
	2006	June	4	5 ± 2
		August	11	4 ± 2
	2007	June	19	1 ± 1
		July	24	4 ± 2
		August	24	4 ± 2
		September	23	8 ± 4
		November	9	2 ± 4
C. virginica	2007	June	14	4 ± 2
		July	7	4 ± 2

Table 2.5: Seasonal physiological rates of Crassostrea virginica in Maryland

Seasonal clearance rate, respiration, and ammonium excretion rates (*a*) for *Crassostrea virginica* reared in mesocosms or collected from the Choptank River (wild) and measured in ambient flow-through conditions. Rates are standardized to an oyster with a 1 g dry tissue weight by the allometric equation $Y = aX^b$ (see text for details). Oysters collected from the wild were not measured in spring (April) 2008. Common slope (*b*) for clearance and respiration rate (*) does not include winter (February) 2009, because these oysters did not feed, or have a measurable respiration rate for the duration of this experiment in this season. Common slope for the respiration of mesocosm reared oysters (†) was not calculated for winter 2009. Different letter indicate a significant difference in corrected *a* values for each oyster source among seasons, ns = no difference (LSD pairwise comparisons; $p \leq 0.05$). Mean (± SD) and range of oyster dry tissue weights are shown for each season. (see Table 2.5 next page)

Table 2.5 (con't): Seasonal physiological rates of Crassostrea virginica in Maryland

Rate	Source	*b*	Season	*n*	*a*	P ≤ 0.05	Tissue weight (g)	range (g)
Clearance Rate	Mesocosm	0.29*	Apr. 2008	10	0.97	ns	0.44 ± 0.52	0.08 - 1.45
(L g^{-1} h^{-1})			Aug. 2008	13	1.19	ns	0.05 ± 0.02	0.01 - 0.10
			Oct. 2008	13	0.89	ns	0.09 ± 0.04	0.04 - 0.16
			Feb. 2009	16	0.00		0.80 ± 0.27	0.31 - 1.32
			May 2009	6	0.98	ns	0.11 ± 0.03	0.07 - 0.15
	Wild	0.84*	Aug. 2008	13	0.42	b	0.87 ± 0.75	0.98 - 2.64
			Oct. 2008	15	1.32	a	0.92 ± 0.43	0.31 - 1.68
			Feb. 2009	14	0.00		0.80 ± 0.27	0.31 - 1.32
			May 2009	13	2.63	a	0.52 ± 0.21	0.32 - 0.98
Respiration	Mesocosm	0.47†	Apr. 2008	9	0.31	ns	0.49 ± 0.54	0.08 - 1.45
(mL O_2 g^{-1} h^{-1})			Aug. 2008	15	0.55	ns	0.05 ± 0.02	0.01 - 0.10
			Oct. 2008	14	0.25	ns	0.09 ± 0.04	0.3 - 0.16
			May 2009	6	0.25	ns	0.9 ± 0.06	0.02 - 0.19
	Wild	0.83*	Aug. 2008	14	1.00	ns	0.93 ± 0.71	0.10 - 2.64
			Oct. 2008	13	0.56	ns	0.84 ± 0.42	0.31 - 1.68
			Feb. 2009	14	0.00		0.80 ± 0.27	0.31 - 1.32
			May 2009	13	0.60	ns	0.73 ± 0.50	0.32 - 2.18
Ammonium excretion	Mesocosm	0.44	Apr. 2008	14	6.03	a	0.46 ± 0.50	0.08 - 1.45
(μg NH_4-N g^{-1} h^{-1})			Aug. 2008	7	11.21	a	0.05 ± 0.02	0.03 - 0.09
			Oct. 2008	6	1.06	b	0.14 ± 0.03	0.09 - 0.17
			Feb. 2009	16	7.08	a	0.10 ± 0.03	0.05 - 0.16
			May 2009	12	3.84	a	0.09 ± 0.04	0.02 - 0.19
	Wild	0.95	Aug. 2008	13	28.87	a	0.89 ± 0.75	0.10 - 2.64
			Oct. 2008	11	1.61	b	0.93 ± 0.48	0.34 - 1.59
			Feb. 2009	14	11.53	a	0.80 ± 0.27	0.31 - 1.32
			May 2009	12	16.14	a	0.56 ± 0.20	0.32 - 0.98

Table 2.6: Seasonal physiological rates of Crassostrea ariakensis in Maryland

Seasonal clearance, respiration, and ammonium excretion rates (*a*) for *Crassostrea ariakensis* measured in ambient flow-through conditions. Rates are standardized to an oyster with a 1 g dry tissue weight by the allometric equation $Y = aX^b$ (see text for details). Different letters indicate significant difference in corrected *a* values for each physiological rate among seasons (LSD pairwise comparisons; $p \leq 0.05$). Mean (± SD) and range of oyster dry tissue weights are shown.

Rate	*b*	Season	*n*	*a*	P ≤ 0.05	Tissue weight (g)	range (g)
Clearance Rate	0.50	Apr. 2008	16	1.86	b	1.48 ± 1.24	0.26 - 4.43
($L\ g^{-1}\ h^{-1}$)		Aug. 2008	16	0.97	c	0.54 ± 0.51	0.07 - 1.74
		Oct. 2008	15	1.66	b	0.50 ± 0.44	0.08 - 1.43
		Feb. 2009	15	0.29	d	1.52 ± 0.64	0.59 - 2.50
		May 2009	15	2.71	a	1.03 ± 0.61	0.25 - 2.10
Respiration	0.53	Apr. 2008	12	0.61	b	1.5 ± 1.0	0.34 - 3.22
($mL\ O_2\ g^{-1}\ h^{-1}$)		Aug. 2008	15	1.12	a	0.57 ± 0.52	0.07 - 1.74
		Oct. 2008	15	0.56	bc	0.50 ± 0.44	0.07 - 1.43
		Feb. 2009	13	0.36	c	1.63 ± 0.60	0.64 - 2.50
		May 2009	13	0.70	ab	0.94 ± 0.54	0.25 - 2.20
Ammonium excretion	0.53	Apr. 2008	16	9.82	b	1.48 ± 1.24	0.26 - 4.43
($\mu g\ NH_4\text{-}N\ g^{-1}\ h^{-1}$)		Aug. 2008	12	36.27	a	0.51 ± 0.44	0.73 - 1.44
		Oct. 2008	12	4.62	c	0.65 ± 0.42	0.19 - 1.22
		Feb. 2009	15	8.97	bc	1.52 ± 0.64	0.59 - 2.50
		May 2009	13	15.50	b	0.94 ± 0.54	0.25 - 2.20

Table 2.7: Seasonal absorption efficiency of oysters in Maryland

Back-transformed mean (± SE) seasonal percent absorption efficiency (*Ae*) of *Crassostrea ariakensis* and *Crassostrea virginica* reared in mesocosms and *Crassostrea virginica* collected from the Choptank River (wild) and measured in ambient flow-through conditions. Different letters indicate significance for each species among seasons (LSD pairwise comparisons; $p \leq 0.05$).

Species	**Season**	***n***	***Ae***	**+ SE**	**- SE**	**P ≤ 0.05**
C. ariakensis	April 2008	16	21.9	3.9	3.7	c
	August 2008	16	41.5	4.6	4.5	a
	October 2008	15	21.7	3.9	3.7	c
	February 2009	15	52.3	5.1	5.1	a
	May 2009	15	38.0	4.7	4.6	b
C. virginica (mesocosm)	April 2008	10	34.2	8.0	7.5	a
	August 2008	15	34.3	8.0	7.5	a
	October 2008	13	22.2	7.2	6.4	ab
	February 2009	16	0.0	0.0	0.0	c
	May 2009	15	6.5	4.8	3.5	b
C. virginica (wild)	August 2008	13	24.9	6.0	5.6	a
	October 2008	15	28.6	6.4	6.0	a
	February 2009	14	0.0	0.0	0.0	b
	May 2009	13	34.6	6.7	6.4	a

CHAPTER 2:

Figures

Figure 2.1: Growth of oysters in Maryland mesocosms

Mean (± SE) shell size (A) and specific (B) shell growth rate (mm^2) for *Crassostrea ariakensis* (solid line, white box) and *Crassostrea virginica* (dashed line, grey box) measured periodically between October 2004 and January 2008. Stars indicate significant difference in size between species (A) and different letters indicate significant differences among sampling periods and species (B) at $P < 0.05$. For specific growth rate (B), the y-axis is split so that values below 5×10^{-3} are at a different scale than those above.

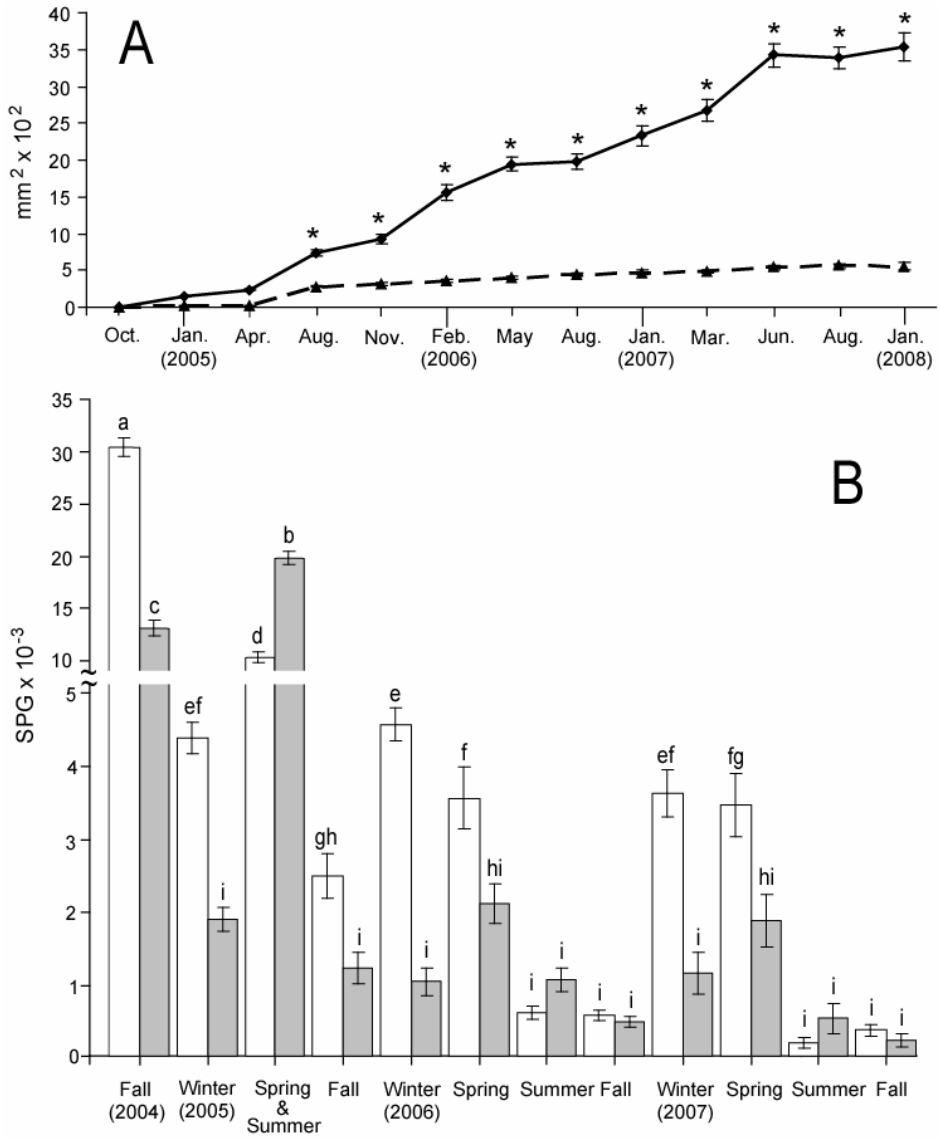


Figure 2.2: Mortality of oysters in Maryland mesocosms

Percent mean (± SE) cumulative (A) and absolute (B) mortality for *Crassostrea ariakensis* (solid line, white box) and *Crassostrea virginica* (dashed line, grey box) from October 2004 through January 2008. ns = no significant difference in mortality between species at $P < 0.05$.

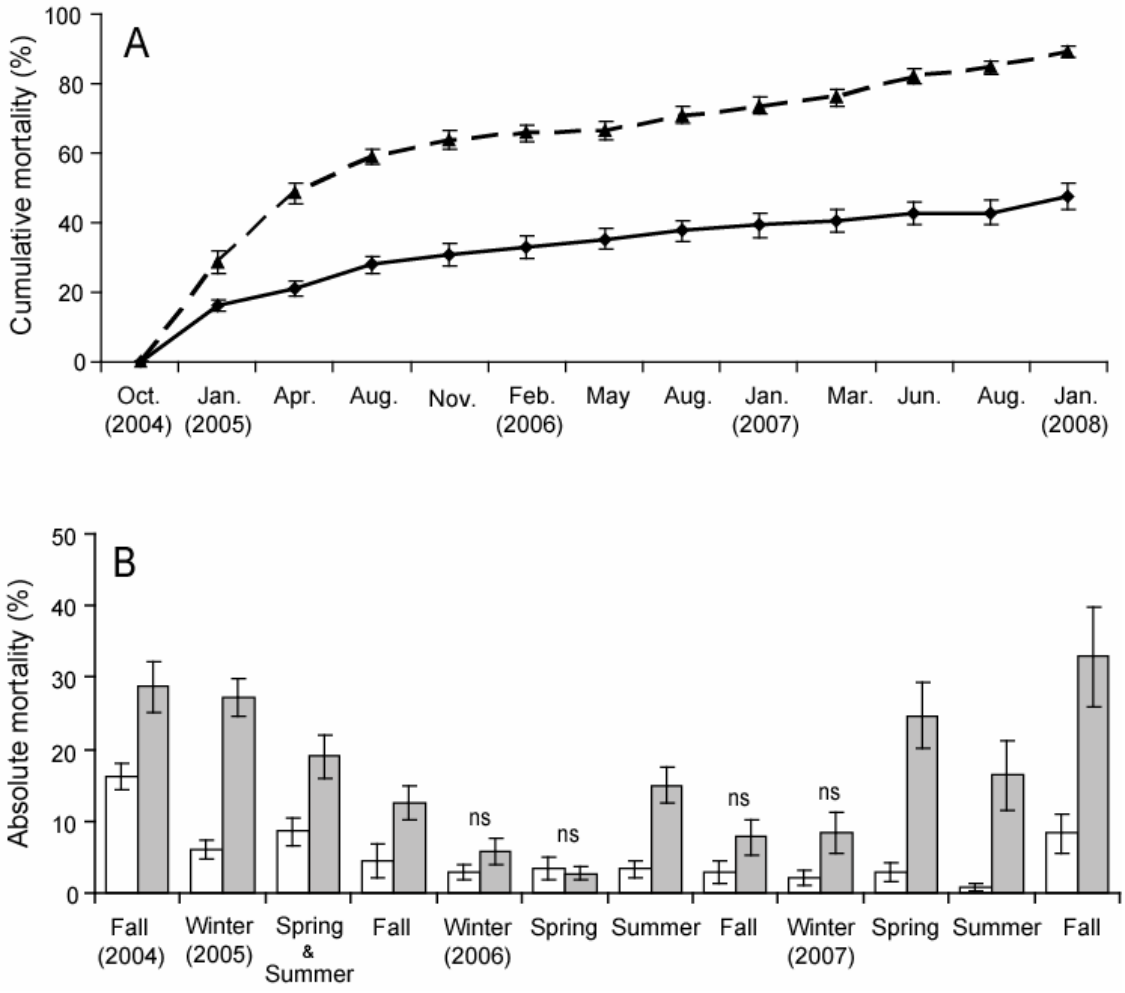


Figure 2.3: Gonadal development of oysters in Maryland mesocosms

Percentage of *Crassostrea ariakensis* (solid line) and *Crassostrea virginica* (dashed line) oysters (n = 24) showing evidence of vitellogenesis and spermatogenesis (A), and clearly distinguishable eggs or sperms (B).

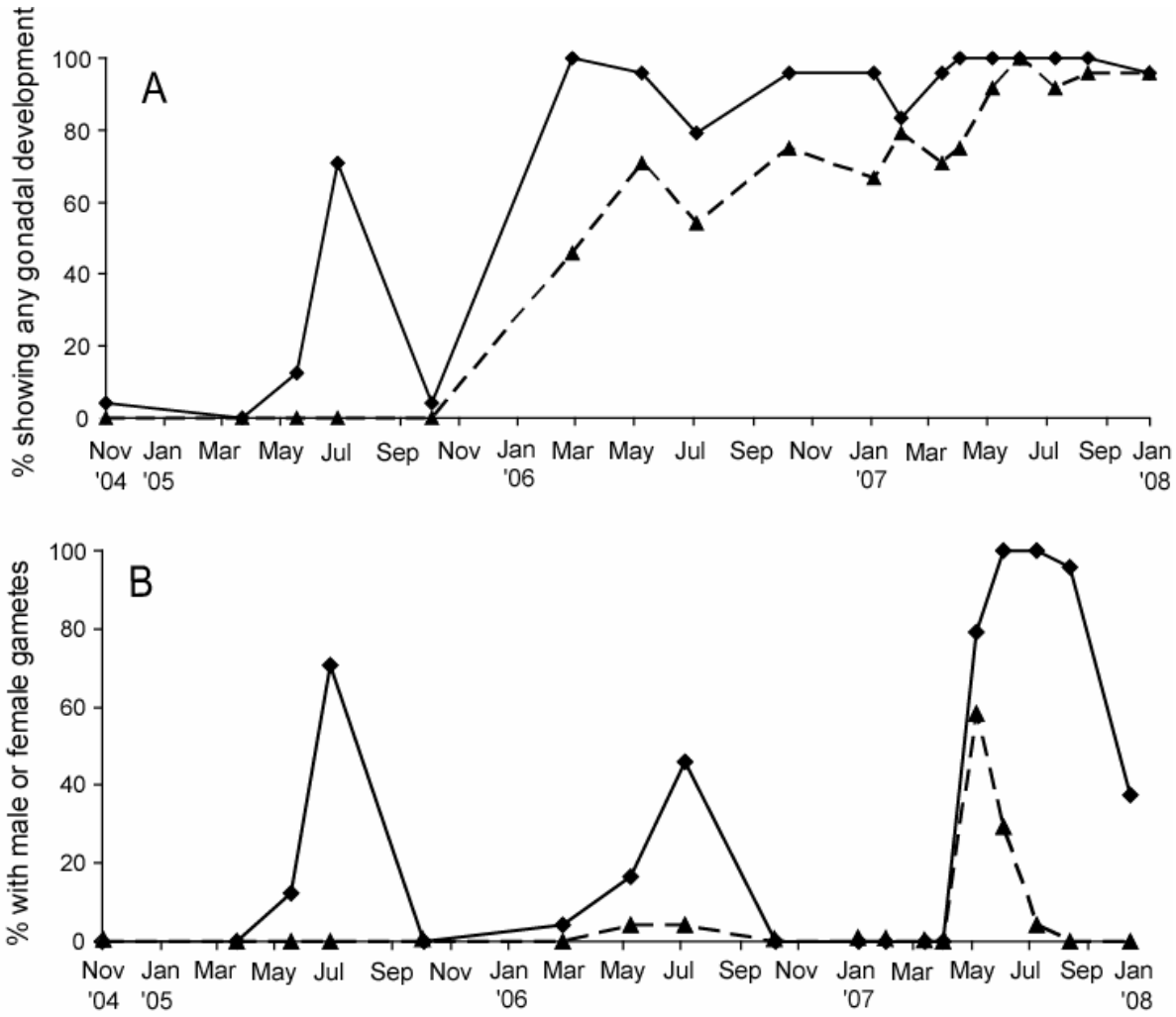


Figure 2.4: Reproductive condition of Crassostrea ariakensis in 2007

Reproductive condition of *Crassostrea ariakensis* at various months in 2007 (n = 24). Descriptions for each of the six categories of gamete condition are listed in Table 2.1. White box = non-gametogenic; light grey = G I; dark grey = G II; black with white stipple = G III; black = spawn ripe; white with black stipple = post spawn.

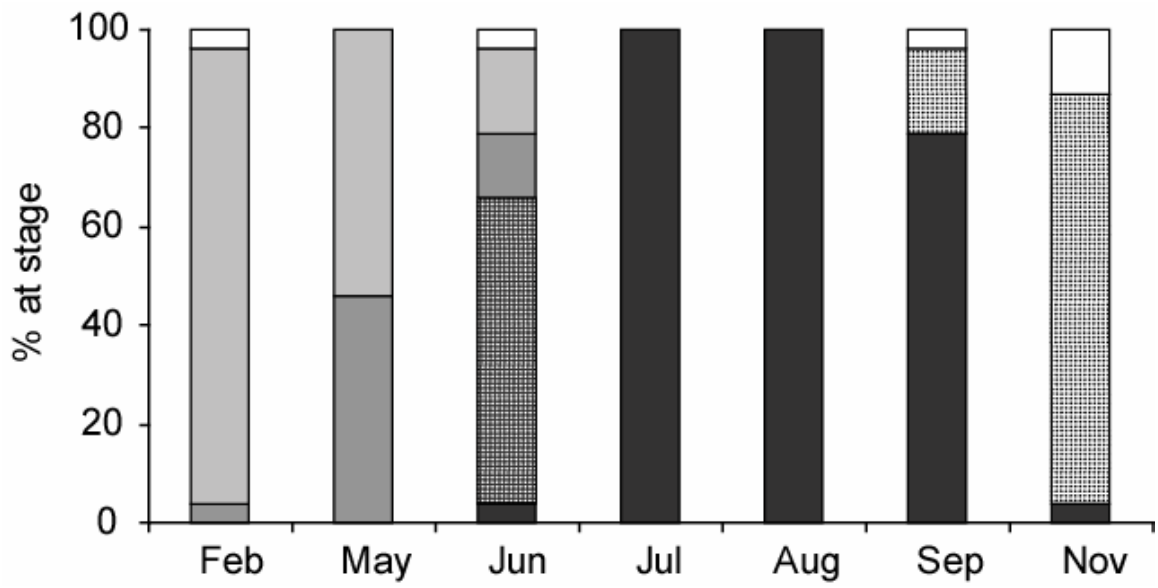


Figure 2.5: Seasonal scope for growth of oysters in Maryland

Seasonal scope for growth (J g^{-1} h^{-1}) of *Crassostrea ariakensis* (A) and *Crassostrea virginica* (B) in ambient flow-through conditions (± SD). Mesocosm reared *C. virginica* = grey, wild *C. virginica* = stippled. nd = no data. The scale of y-axis values is different between panels. The y-axis for *Crassostrea virginica* (B) is split so that values below 40 J g^{-1} h^{-1} are at a different scale than those above

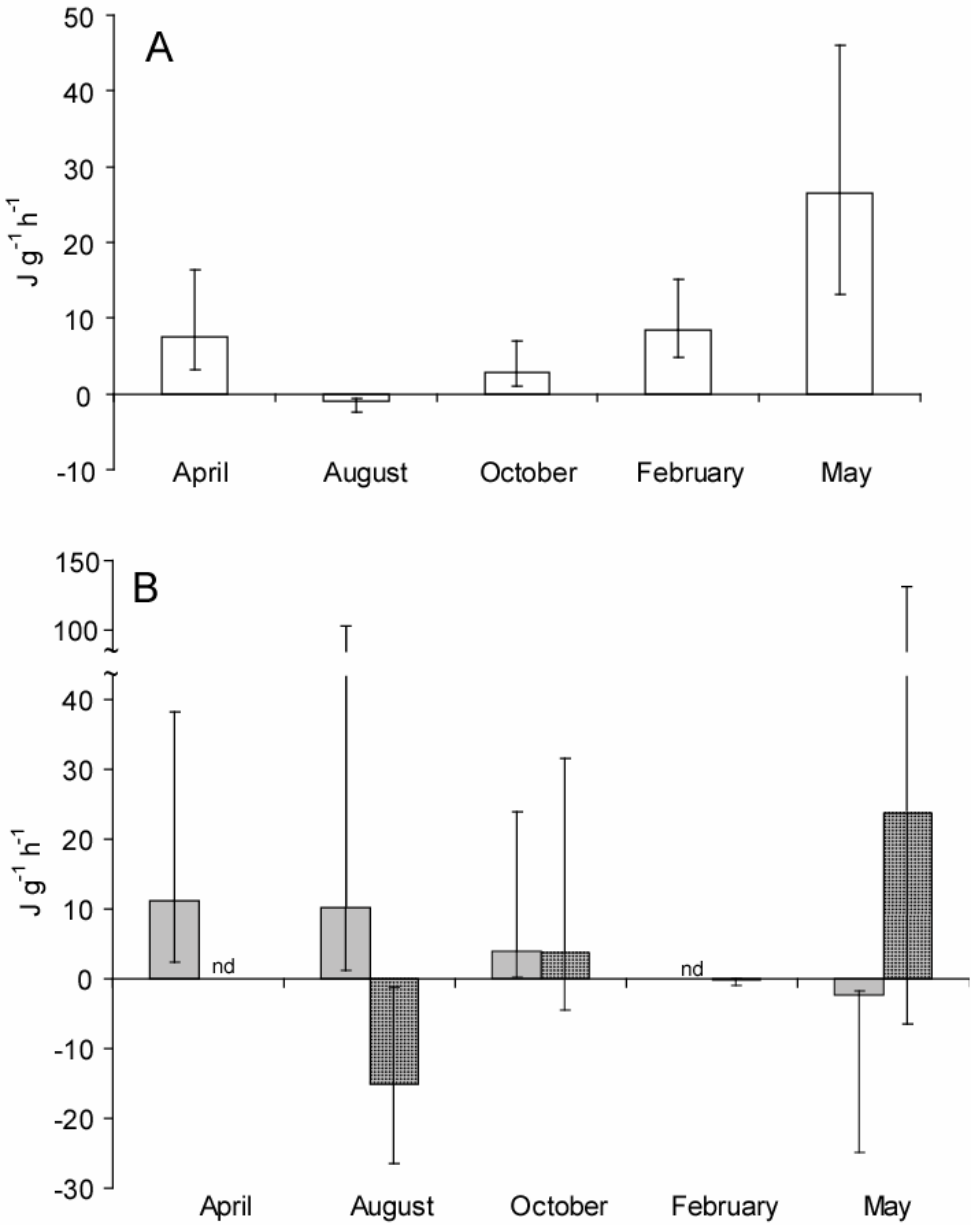
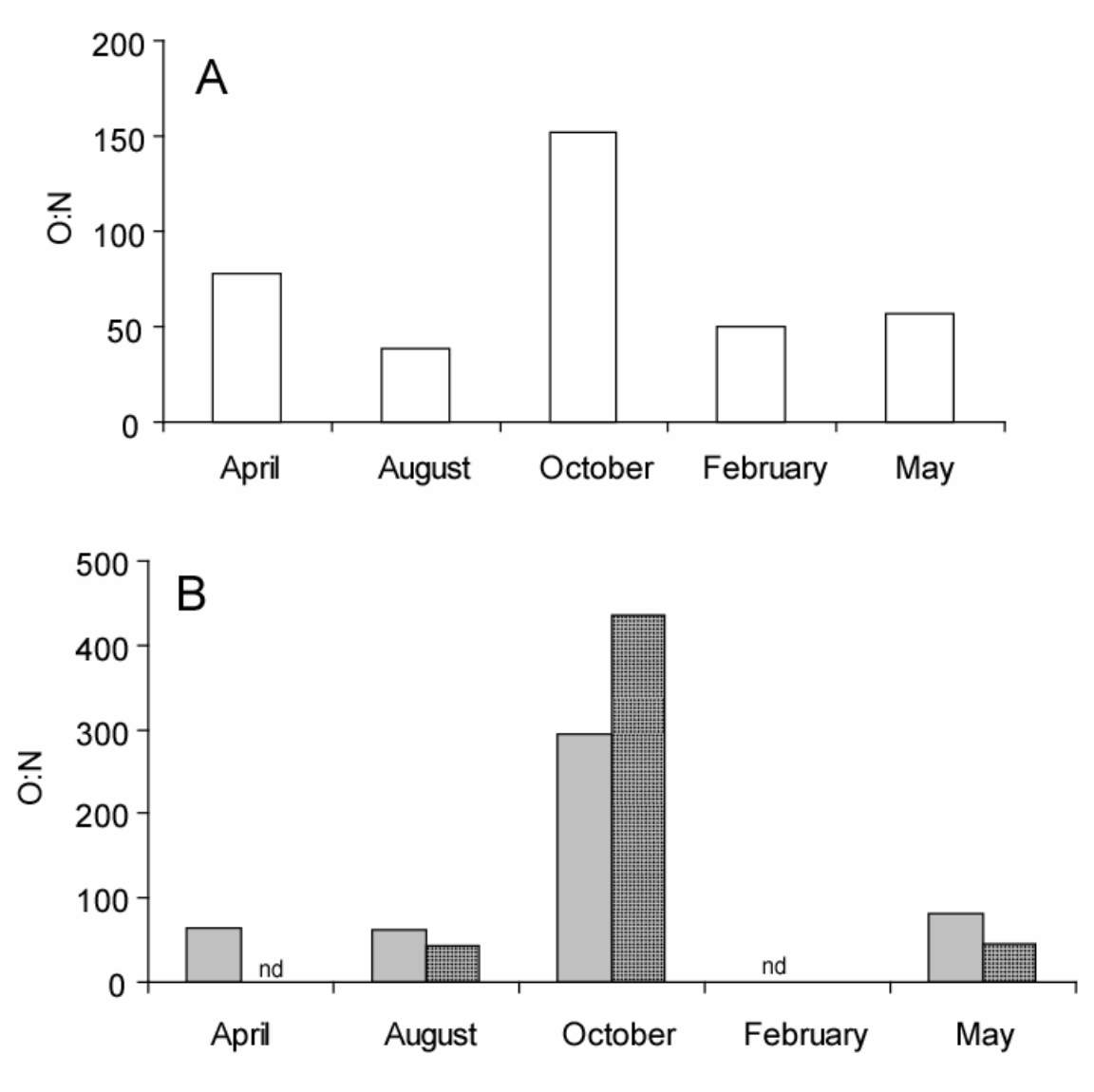


Figure 2.6: Seasonal O:N ratio of oysters in Maryland

Seasonal O:N ratio calculated from standardized 1 g dw population respiration and ammonium excretion rates (Tables 2.5 and 2.6) of *Crassostrea ariakensis* (A) and *Crassostrea virginica* (B) in ambient flow-through conditions. Mesocosm reared *C. virginica* = grey, wild *C. virginica* = grey stippled black. nd = no data. The scale of y-axis values is different between panels.



CHAPTER 3:

Seasonal Comparison of Physiological Adaptation and Growth of Suminoe (*Crassostrea ariakensis)* and Eastern (*Crassostrea virginica*) Oysters

CHAPTER CITATION:

Kelly, C., J., S. E. Laramore, J. Scarpa, & R. I. E. Newell. In Press. Seasonal comparison of physiological adaptation and growth of suminoe (*Crassostrea ariakensis*) and eastern (*Crassostrea virginica*) oysters. Journal of Shellfish Research

ABSTRACT

Shell growth, survival, and physiology were compared between diploid Suminoe (*Crassostrea ariakensis*) and eastern oysters (*Crassostrea virginica*) under conditions simulating a USA subtropical estuary. Two age groups (4-mo. and 28-mo.) of both oyster species were grown for a 9 mo. period (December 2006 through August 2007) in quarantine mesocosms (700 L) supplied with ambient flowing ($\geq$ 10 L min^{-1}) water (annual temperature range of 18.6 to 30.4ºC and salinity of 28 to 37.7). There was no difference in overall rates of shell growth between the two oyster species over the 8 mo. period. Specific growth rates for *C. ariakensis* did not differ over time, but did for *C. virginica.* The growth rate of *C. virginica* was slowest in the winter (8.9×10^{-4} mm^2 d^{-1}) and fastest in the spring (43.5×10^{-4} mm^2 d^{-1}). Mortality of both species rose abruptly in April 2007 and all (100%) remaining *C. ariakensis* were dead by the end of the study. Although 28% of the remaining *Crassostrea virginica* died in April 2007 there was little further mortality in this species before the study was terminated in August 2007. Physiological responses of both species of oysters were compared under seasonal temperate euhaline quarantine conditions to better understand how temperature affects these species without the confounding unexplained mortality encountered within the subtropical mesocosms. The clearance rate of *C. ariakensis* (1.2 L g h^{-1}) was half that of *C. virginica* (2.2 L g h^{-1}) during the summer (25ºC); however respiration rates for *C. ariakensis* (2.6 mL O_2 g h^{-1}) and *C. virginica* (2.5 mL O_2 g h^{-1}) were similar. The low clearance rate of *C. ariakensis* during the summer resulted in a negative scope for growth (-36.2 J g^{-1} h^{-1}) during this season. During the winter *C. ariakensis* remained physiologically active when water temperatures were as low as 2ºC; *C. virginica* were

quiescent during this time. I conclude that the "Oregon" strain of *C. ariakensis* tested will not thrive in the warm subtropical waters of the USA southeastern coast, but given its native range in Asia I do not discount the possibility of an adaptation to warmer temperatures over time.

INTRODUCTION

The eastern oyster (*Crassostrea virginica* Gmelin 1791) is an ecologically and economically important native species along the Atlantic and Gulf coasts of the United States (Newell 1988, MacKenzie 1996, Eggleston 1999, Posey et al. 1999, Coen et al. 2007). In Chesapeake Bay, populations of *C. virginica* have been in decline since the late 19th century due to a combination of habitat degradation, over-harvesting, and epizootics of the protistan diseases MSX (*Haplosporidium nelsoni*) and Dermo (*Perkinsus marinus*) (Rothschild et al. 1994, Ford & Tripp 1996). It was proposed by the Maryland Department of Natural Resources that the introduction of the non-native Suminoe oyster (*Crassostrea ariakensis* Fujita 1913) into Chesapeake Bay would help alleviate many of the problems associated with the loss of native *C. virginica* populations (NRC 2004). Because of the controversial nature of performing such an irreversible introduction, considerable research, including the work described here, was commissioned to help provide the scientific information necessary to inform such a decision. This body of research was used to develop a formal Ecological Impact Statement (U.S. Army Corps of Engineers 2009). Ultimately, the introduction of the non-native *C. ariakensis* was not considered to be a suitable means of enhancing oyster stocks at this time. The current management emphasis has now shifted to conservation and restoration of *C. virginica*.

The initial rationale for the proposed introduction of *Crassostrea ariakensis* was based on this species resistance to MSX and Dermo epizootic diseases that are major impediments to the restoration of *Crassostrea virginica* populations (Calvo et al. 2001, Paynter et al. 2008). If *C. ariakensis* were to be introduced into Chesapeake Bay, either deliberately, or accidentally from research facilities holding diploid broodstock, it was considered likely (NRC 2004) that it would establish feral populations along the USA Atlantic coast, including areas where *C. virginica* populations remain relatively robust.

The native range of *Crassostrea ariakensis* stretches from latitude 41ºN in Liaoning, China to latitude 20ºN in Vietnam (Guo et al. 2008). In the Americas, this is equivalent to the coastline between Connecticut, USA and the Yucatan Peninsula, Mexico (Guo et al. 2008). Most research in relation to the proposed introduction of *C. ariakensis* into Chesapeake Bay has been performed on triploid individuals grown under temperate estuarine conditions (Calvo et al. 2001, Grabowski et al. 2004, Hudson et al. 2005, McLean & Abbe 2008, Paynter et al. 2008, Kingsley-Smith et al. 2009) or on diploid *C. ariakensis* being assessed for aquaculture potential on the USA Pacific northwest coast (Breese & Malouf 1977, Perdue & Erickson 1984, Langdon & Robinson 1996). Considering that the natural range of *C. ariakensis* extends into subtropical regions (Zhou & Allen 2003, Guo et al. 2008) it is necessary to broaden past studies to include subtropical USA coastal environments. One such subtropical estuary is the Indian River Lagoon (IRL) on the Atlantic coast of Florida that is highly biologically diverse (Gilmore 1985, Duncan et al. 2004) due to its location near the boundary between the temperate and subtropical regions. Subtropical conditions within the IRL promote the enhanced growth of many species due to year-round warm water temperatures and high

phytoplankton availability. Populations of *Crassostrea virginica* in the IRL are relatively robust (Grizzle 1990, Boudreaux et al. 2006) in comparison to those in Chesapeake Bay.

In order to investigate the potential of *Crassostrea ariakensis* to form feral populations in sub-tropical conditions I examined their growth, mortality, and reproductive capability over a 9 mo. period in quarantined mesocosms supplied with seawater from the IRL. I hypothesized that under subtropical conditions *C. ariakensis* would perform better than or equal to *Crassostrea virginica*. My initial hypothesis was not supported; therefore in order to better understand *C. ariakensis* physiology under warm water temperatures, I then compared seasonal scope for growth in both species of oyster under salinities and summer water temperatures similar to that of the IRL. I hypothesized that *C. ariakensis* would have less energy to allocate towards somatic growth and gamete production during the summer due to physiological stress by high water temperatures.

MATERIALS AND METHODS

Mesocosm Experiments

Study System

Two age classes (4-mo. and 28-mo.) of both oyster species were used in this study. All *Crassostrea virginica* and the 4-month-old *Crassostrea ariakensis* were obtained as larvae from the Virginia Institute of Marine Science – Eastern Shore Laboratory (VIMS-ESL). The 28-month-old *C. ariakensis* were obtained as larvae in 2004 from Taylor United Shellfish hatchery in Quilcene, Washington. Larvae were allowed to metamorphose on large pieces of oyster shell and reared as described by Newell et al. (2007) in a quarantined ambient flow-through facility at Horn Point Laboratory (HPL) in

Maryland until use. All *C. ariakensis* larvae were progeny of adults originating from the "Oregon" stock (Newell et al. 2007)

Approximately 150 *Crassostrea ariakensis* and 150 *Crassostrea virginica* that were 28-months-old and 150 *C. virginica* that were 4-months-old were transferred in July 2006 to Harbor Branch Oceanographic Institute at Florida Atlantic University (HBOI-FAU) in Fort Pierce, Florida, USA. These oysters were placed in a quarantine tank with aerated static seawater (salinity = 30) that was changed twice a week. Oysters were fed the microalga I*sochrysis aff. galbana* (clone T-ISO), *Cyclotella sp.*, or both at concentrations of 50,000 – 100,000 cells mL^{-1} day^{-1} until the start of the experiment in September 2006. An additional group of 150 *C. ariakensis* of the same 4-month-old cohort as the *C. virginica* described above were transferred to HBOI in December 2006. These additional oysters were not transferred until this time in order to complete genetic analysis to confirm that these oysters were *C. ariakensis* (Newell et al. 2009).

Three circular (1.6 m diam, 0.6 m high) 700 L mesocosms at HBOI were supplied with ambient seawater from the IRL at a flow rate of 10 L min^{-1} and aerated to maintain high dissolved oxygen levels (Table 3.1) and keep seston in suspension. Effluent water was chlorinated to 2 ppm or greater free chlorine using a free chlorine analyzer (Foxcroft FX-1000p) and controller (Foxcroft FX-8500) in order to prevent any gametes or larvae produced by the diploid oysters from entering the IRL.

In September 2006, oysters of each species from the 28-month-old cohorts and the 4-month-old *Crassostrea virginica* cohort were separated into groups of approximately 50 individuals. Each group was placed into plastic trays (53 cm long × 38 cm wide × 14 cm high) with holes (2.5 cm diam) drilled into the bottom and sides of each

tray to allow for better water circulation. A tray for each oyster species was placed into the three mesocosms and suspended 5 cm above the bottom of the tank to avoid oysters becoming buried by particulate waste on the tank bottom. Tanks were periodically cleaned to remove accumulated sediment and organic matter. In December 2006 the additional 4-month-old *Crassostrea ariakensis* transferred from HPL were acclimated to ambient salinity and temperature conditions for 7 d. They were then distributed into plastic trays and to each of the three mesocosms as described above.

Mesocosm water temperature, salinity, and dissolved oxygen were measured daily using a conductivity meter (YSI - model 85). One water sample from each of the three mesocosm tanks, and concurrent triplicate samples from the seawater intake in the IRL, were collected once per month in July and August 2006 for chlorophyll *a* (Chl *a*) and seston analysis using the EPA 160.2 (USEPA 1999) method for seston and the EPA SM10200H method for Chl *a* (American Public Health Association 1998).

Growth and Mortality

Oyster shells (16 for each species) with attached live oysters (2 – 9 per shell) from the 4 and 28-month-old *Crassostrea ariakensis* and *Crassostrea virginica* cohorts were labeled with an aluminum tag. A total of 42 *C. ariakensis* and 56 *C. virginica* attached to these shells were used for the growth and mortality assessments. Each 4 and 28-month-old *C. virginica* oyster and 28-month-old *C. ariakensis* oyster on the tagged shells was digitally photographed in September 2006 at the start of the experiment to obtain an initial reference size. Each 4-month-old *C. ariakensis* oyster was digitally photographed in December 2006 to obtain an initial reference size. Each oyster was then photographed monthly from December 2006 until the study ended in August 2007. All digital

photographs included a scale with 1 mm increments within the field of view to provide calibration.

Absolute shell growth was calculated by measuring the surface area (mm^2) of oysters at each sampling date using Image J software (Rasband 1997-2009). Using absolute measurements to estimate growth may lead to an overestimation in the growth rate of larger oysters versus smaller oysters; therefore in order to standardize growth rate relative to an oyster's size, I calculated growth as a daily specific growth rate (*SPG*):

$$SPG = \frac{(\ln A_2 - \ln A_1)}{t_2 - t_1}$$

Where *A* represents the area (mm^2) of the oyster shell at the beginning and end of each sampling period and $t_2 - t_1$ is the time (d) between each sampling date. The percent cumulative mortality of each species was estimated by the number of missing and dead oysters at each sampling date as verified by digital image analysis.

Reproductive Condition

Reproductive assessment of mesocosm oysters on untagged shells was performed monthly between February and August 2007. Tissue samples from 12 – 21 oysters of each species (split between age classes), were wet-weighed, fixed, paraffin embedded, sectioned transversely, and stained with hematoxylin and eosin (Kennedy et al. 1995). In March and July several oysters from each cohort on untagged shells were also examined histologically for any types of cellular abnormalities. Tissue samples of 20 *Crassostrea virginica* (shell area: 22.9 ± 5.4 cm^2) from the nearby natural population in the IRL were collected in August 2007 in order to make additional comparison with mesocosm *C. virginica* at that same sampling date.

Gonadal tissue in histological sections for each oyster was examined microscopically and the number of oysters in which gametogenesis had been initiated was enumerated. Oysters in this category had distinct follicles, but the cells had not yet differentiated sufficiently to discern if they would develop as eggs or sperm. The number of those individuals in which gametogenesis had proceeded to the point that distinct eggs and sperm were visible in the follicles was separately enumerated.

Physiological Experiments

Physiological studies were performed under ambient seasonal temperate conditions at VIMS-ESL in July, October 2008; January, and April 2009. The *Crassostrea ariakensis* (shell height: 3.7 – 12 cm) used in this experiment were from the same cohorts used in the HBOI mesocosm study but reared at HPL. The size of *Crassostrea virginica* reared in HPL mesocosms (shell height: 2.5 – 5.3 cm) was smaller than *C. ariakensis* (Newell et al. 2009) so they were not used in this study. Instead, I collected *C. virginica* (shell height: 5.2 – 13.9 cm) directly from natural oyster reefs in the Choptank River, MD in order to study similarly sized individuals of both species.

All oysters were maintained in ambient, mesohaline (10 – 12), flow-through conditions at HPL. One month prior to each seasonal physiological study I transferred 16 oysters of each species (32 total oysters) to VIMS-ESL where they were gradually acclimatized to ambient conditions by increasing salinity by five salinity units every 4 to 5 d until ambient salinity (~30) was reached. During acclimatization oysters were maintained in static tanks at ambient water temperature and fed a maintenance diet of cultured microalgae *Isochrysis galbana* clone T. iso. Oysters of both species acclimatized to conditions at VIMS-ESL as indicated by the presence of biodeposits and growth of

new shell prior to the start of each seasonal study except for *Crassostrea virginica* in winter when all individuals were in a quiescent condition.

Ambient flow-through water was pumped from the adjacent Machipongo River into two head tanks that supplied water via lengths of Tygon tubing (6 mm i.d.) to 18 rectangular plastic pans (36 cm long × 30 cm wide × 10 cm high). A PVC plug with a precisely drilled hole was inserted into each tube that allowed a flow rate of either 40 L h^{-1} (for oysters ≥ 5 cm shell height) or 20 L h^{-1} (for oysters < 5cm shell height) to the bottom of each pan. Preliminary studies showed that these high flow rates ensured that oysters would not be able to appreciably reduce particle concentrations during the experiment. All pans drained at the water surface though a standpipe at the end farthest from the inflow tube. This setup ensured adequate water column mixing through each pan. Waste effluent was collected in a holding tank and was chlorinated to 2 ppm or greater free chlorine for 2 h. Six oysters from each species were randomly assigned to 12 separate pans with an appropriate flow rate for their shell size. Controls, which did not contain oysters, were assigned to 3 pans with the higher flow rate and 3 pans with the lower flow rate. Each run of the experiment (3 total for each season) lasted 36 h, during which hourly water samples for seston analysis were taken using an ISCO water sampler (Model 3700 Sampler Controller). Each oyster was held in a shallow plastic container placed in each pan in order to retain biodeposits. Appropriate containers were also placed in the control pans of both flow rates. Oysters were briefly removed from the pans after 12 h to remove biodeposits produced from seston that had been filtered and ingested before the start of the experimental run.

Oysters were removed from the pans at the end of the experiment. The shallow containers from oyster and control pans were carefully removed, sealed, and held at 5°C for 12 h to allow suspended material to settle. Overlying water was aspirated off and the container filled with 200 mL of DI water to wash out salt from the deposits before holding at 5°C for another 12 h to once again allow suspended material to settle. The majority of DI water was aspirated off and two one mL aliquots of biodeposits from the containers were removed for absorption efficiency determination. Each aliquot was placed onto two pre-weighed Whatman GF/C filters that had first been washed and heat treated at 450°C for 1 h. The remainder of the biodeposit slurry was transferred into a pre-weighed aluminum pan and these placed into a 90°C drying oven for 24 h, after which time dry weights were taken. The filters were also dried at 90°C and weighed to determine total dry weight. The filters were then heat treated at 450°C for 6 h to determine the organic fraction of the biodeposits. Material from the control containers was treated identically to the material from the oyster containers and was collected to determine the amount of material that naturally settled into the experimental containers independently of oyster feeding activity. To correct for this extra material in the oyster containers, the amount of organic and inorganic material from the control containers was determined as described above, and then subtracted from the total material present in the oyster containers.

Known aliquots of water (300 – 500 mL) collected by the ISCO sampler were filtered through GF/C filters and treated in the same manner as biodeposits to estimate seston concentration. Clearance rate (L g^{-1} hr^{-1}) was calculated as: (mg inorganic matter egested both as feces and pseudofeces h^{-1}) / (mg inorganic matter available L^{-1} of

seawater) (Hawkins et al. 1996). Absorption efficiency was calculated using the Conover ratio (Conover 1966, Bayne et al. 1985)

For nitrogen excretion assays ambient river water was filtered (Millipore 0.45 μm pore) and used to fill beakers (200 – 900 mL) into which individual oysters were submerged or assigned as controls. Beakers were covered with plastic food wrap and incubated at ambient seawater temperature in a water bath for 2 h. Oysters were then removed from the beakers and two 10 mL aliquots of water from each beaker was placed into labeled test tubes. The phenol-hypochlorite method (Solórzano 1969, Bayne et al. 1985) was used to determine ammonium concentration. Ammonium excretion rates (μg NH_4-N g^{-1} h^{-1}) were calculated as described by Bayne et al. (1985).

Rates of oxygen consumption were measured using the methods described by Bayne et al. (1985). Individual oysters were then placed into either a large (2.3 L) or small (0.3 L) glass respirometer chamber supplied with ambient flow-through river water. These chambers were maintained at ambient river temperature by submerging in a water bath. After a 1 h period of acclimatization the water flow was stopped and the decline in oxygen concentration measured with a calibrated oxygen electrode (Radiometer-Copenhagen Model E5047-0). Controls were run using the same methods described above but without an oyster in the respirometer chamber. Control respiration rates were subtracted from the oyster runs to eliminate background respiration. The calculation of oxygen consumption rates required the volume of the oyster to be subtracted from the total volume of water within the respirometer chamber (Bayne et al. 1985); therefore oyster volume was determined by the displacement of water within a graduated cylinder. Respiration rates (mL O_2 g^{-1} h^{-1}) were calculated as described by Bayne et al. (1985).

Dry tissue weight (dw) of all experimental oysters was obtained by removing oyster tissue from its shell, placing it in a pre-weighed pan, and drying it at 90ºC for 24 h. Seasonal physiological rates of individual oysters were regressed against their dry tissue weight for *C. virginica* and *C. ariakensis* separately. An Analysis of Covariance (ANCOVA) was then performed to determine a species-specific common slope. Using this slope intercepts were recalculated using the allometric equation: $Y = aX^b$. These intercepts represent the seasonal physiological rates of an oyster of 1 g dw. This weight was selected as it was close to the average weight of the oysters studied and also this animal weight is commonly used in comparisons of physiological rate functions within and between species of bivalves (Bayne & Newell 1983). The standard deviation of each seasonal physiological rate was calculated from the standard error reported in the ANCOVA analysis. Atomic ratios of oxygen consumption to nitrogen excretion (O:N) were calculated as described by Bayne et al. (1985) from standardized 1 g dw seasonal rates for each oyster species.

The physiological rates described above were converted into energy equivalents ($J\ g^{-1}\ h^{-1}$; Bayne et al. 1985). Energy absorbed from the seston was determined using a POM value 23.5 mg-1 (Widdows et al. 1979). This value is representative of the energy value for food materials such as seston (Slobodkin & Richman 1961, Bayne et al. 1985). Metabolic energy demand ($J\ h^{-1}$) was determined by multiplying energy respired ($mL\ O_2\ h^{-1}$) by 20.33 (Bayne et al. 1985). Energy excretion ($J\ h^{-1}$) was determined by multiplying the ammonia excretion rate ($\mu g\ NH_4\text{-}N\ h^{-1}$) by 0.0249 (Bayne et al. 1985). Scope for growth (*P*) is the energy available for allocation to germinal and somatic tissue production and was calculated by the equation (Bayne et al. 1985):

$$P\ (\text{J h}^{-1}) = A - (R+U)$$

Where A is energy absorbed from seston, R is energy respired, and U is energy excreted.

An estimate of variance for the seasonal scope for growth of each species was determined by calculating minimal and maximal physiological rates at one standard deviation above and below the mean rate value. These minimal and maximal physiological rates were then used in the scope for growth equation described above. They were then added or subtracted from the mean scope for growth value in order to determine a measure of variance.

Statistical Analysis

The distributions of the specific growth rate for *Crassostrea virginica* and *Crassostrea ariakensis* were not normal and could not be made to approximate normality through transformation. Therefore, a non-parametric Freidman's test (Zar 1999), which is similar to repeated measures ANOVA, was used to test for differences in growth rate between and within oyster species. Post-hoc non-parametric Wilcoxon Signed Rank Sum tests (Zar 1999) were used to determine significant monthly differences in growth rate within species.

Percent cumulative mortality was arcsine-transformed to achieve approximate normality. A repeated measures ANOVA was performed on the transformed data and post-hoc least significant difference (LSD) multiple mean comparison tests were conducted to determine significant monthly differences between species.

An ANCOVA was used to test for differences in the seasonal physiological rates within each species. Post-hoc LSD multiple mean comparison tests were performed to determine which seasons were significantly different from each other.

Percent absorption efficiency was arcsine transformed to approximate normality. An ANOVA was performed to determine if there was a seasonal difference in absorption efficiency within species, and post hoc LSD multiple mean comparison tests were performed to determine significant seasonal differences.

RESULTS

Mesocosm Experiments

Environmental Conditions

Mesocosm water temperature, salinity, and dissolved oxygen concentrations were similar to the annual cycle observed in the adjacent IRL. Salinity values during the experiment ranged from 28.0 – 37.7 and were highest during the spring and early summer seasons (Table 3.1). These salinities were well within the optimal range for *Crassostrea virginica* (Shumway 1996). Water temperatures during this study ranged from 18.6 – 30.4ºC and were highest in the late spring and summer seasons (Table 3.1). The percent dissolved oxygen saturation in mesocosms ranged from 74.1% − 91.2% (Table 3.1) and were similar to dissolved oxygen saturations found on natural oyster assemblages in the IRL (Wilson et al. 2005).

Seston and Chl *a* concentrations in mesocosms and the IRL were measured in the summer in order to compare food availability. Seston was higher in mesocosms (July, 9.5 ± 3.6 mg L^{-1}; August, 8.6 ± 2.7 mg L^{-1}) than in the IRL (July, 4.0 ± 1.6 mg L^{-1}; August, 5.4 ± 1.3 mg L^{-1}); however Chl *a* values were similar among mesocosms (July, 3.6 ± 0.9 μg L^{-1}; August, 4.4 ± 0.6 μg L^{-1}) and the IRL (July, 3.7 ± 0.4 μg L^{-1}; August, 6.8 ± 0.5 μg L^{-1}). These values are consistent with seston and Chl *a* concentrations found throughout the IRL (Christian & Sheng 2003).

Growth

Application of a Friedman's test showed that there was no significant difference in the *SPG* between *Crassostrea ariakensis* and *Crassostrea virginica* ($F_{r(3)} = 3.1$, $P > 0.05$) for the 4-month-old cohorts from January 2006 through April 2007. The absence of the 4-month-old cohort of *C. ariakensis* in the September – December 2006 sampling period and high mortality rates of *C. ariakensis* after April 2007 precluded these sampling periods from being used in the analysis. Differences in *SPG* between *C. ariakensis* and *C. virginica* from the 28-month-old cohorts were non-significant ($F_{r(3)} = 7.4$, $P_{\alpha < 0.05} = 0.06$) between September 2006 – March 2007. High mortality rates of *C. ariakensis* from the 28-month-old cohort after March precluded later sampling periods from being used in the analysis.

A Friedman's test performed on monthly *Crassostrea ariakensis* growth data (Table 3.2) showed that there was no significant difference in *SPG* for the 4 ($F_{r(6)} = 9.3$, $P > 0.05$) and 28-month-old cohorts ($F_{r(5)} = 1.8$, $P > 0.05$). The same test performed on monthly *Crassostrea virginica* growth data showed that there was a significant difference among months in *SPG* for the 4 ($F_{r(7)} = 52.10$, $P < 0.0001$) and 28-month-old cohort ($F_{r(7)} = 19.09$, $P_{\alpha < 0.05} = 0.008$) over the course of the experiment (Table 3.3).

The 4-month-old cohort of *Crassostrea virginica* had the fastest *SPG* between September – December 2006. Post-hoc comparisons using Wilcoxon Signed Rank Sum tests showed that *SPG* during this period was significantly greater than all other sampling periods (Table 3.3). An additional increase in SPG occurred between April and May, and May and June; however post-hoc comparisons showed that the SPG during these months

were only significantly greater than the SPG recorded between December and February (Table 3.3).

The 28-month-old cohort of *Crassostrea virginica* also had their fastest *SPG* between September – December 2006 (Table 3.3); however post-hoc comparisons using the Wilcoxon Signed Rank Sum test showed that *SPG* during this time was not significantly different from the rest of the study period, with the exception of January (S = -24.5. P > 0.0098), April (S = -17.5. P = 0.0391), and August (S = -10.5. P = 0.0313).

Mortality

A repeated measures ANOVA showed that there was a significant monthly interaction in the cumulative mortality for the 4-month-old *Crassostrea ariakensis* and *Crassostrea virginica* cohort ($F_{14,28} = 27.64$ $P_{\alpha<0.05} < 0.0001$) as well as the 28-month-old cohort ($F_{16,32} = 8.66$; $P_{\alpha<0.05} < 0.0001$). The 4-month-old cohort of *C. virginica* suffered high mortality between April and May 2007, during which cumulative mortality doubled. After this period and until experimental termination there was little mortality recorded. The 4-month-old cohort of *C. ariakensis* also had low cumulative mortality until between April and May 2007 when cumulative mortality increased four-fold (Fig. 3.1A). Unlike *C. virginica*, *C. ariakensis* continued to experience heavy mortality and all oysters were dead by August 2007 (Fig. 3.1A). Post-hoc LSD tests showed that mortality of 4-month-old *C. virginica* was significantly lower than C. *ariakensis* between the June sampling and the conclusion of the study in August 2007 (Fig. 3.1A).

The 28-month-old cohort of *Crassostrea virginica* also exhibited high mortality between April and May 2007 when 25% died; and additional mortality between July and August 2007, brought cumulative mortality to 52% (Fig. 3.1B). The 28-month-old cohort

of *Crassostrea ariakensis* experienced high cumulative mortality from February until July 2007 at which point 100% of the oysters in the mesocosms were dead (Fig. 3.1B). Post-hoc LSD tests showed that there was a significant difference between the cumulative mortality of *C. virginica* and *C. ariakensis* after March 2007.

Reproductive Condition

Some individuals from the 4-month-old cohorts of both species exhibited early stage gametogenesis for all sampling times between December and July although fewer *Crassostrea ariakensis* than *Crassostrea virginica* exhibited gametogenesis during March and April samplings (Table 3.4). The 28-month-old cohorts of *C. virginica* and *C. ariakensis* also exhibited high levels of early stage gametogenesis. Gametogenesis in *C. virginica* from the 28-month-old cohort proceeded to the point that eggs and sperm could clearly be identified in follicles of 30 to 60% of individuals from May through August `2007, but no developed gametes were visible from any *C. ariakensis* (Table 3.4). All *C. virginica* (n = 10) oysters sampled from the IRL in August 2007 had distinguishable male and female gametes, in contrast to the low to moderate percentage of those in the mesocosms. Both age classes from the two oyster species showed an abrupt decline in the number of individuals with evidence of gonadal development in April 2007, compared to March and May (Table 3.4). This sharp decline in reproductive activity coincided with the reduced growth and increased mortality of these oysters that occurred during this same period.

Physiological Experiments

Seasonal water temperatures at VIMS-ESL ranged from 27ºC in the summer to 5ºC in the winter and salinities remained euhaline (~30; Table 3.5). Seston loads at

VIMS-ESL were high (13.8 – 49.3 mg L^{-1}) for all seasons sampled (Table 3.5) and percent of organic matter (%POM) ranged from 9.3 % - 17.9%. Seston and %POM were similar among all seasons sampled, with the exception of summer (July) where the seston load of 49.3 mg L^{-1} was three times greater, but the 9.3% POM was only approximately half that of the other seasons (Table 3.5). The seawater intake pipes at VIMS-ESL are located in a muddy creek that is subject to high tidal currents which can resuspend bottom sediments thereby creating high seston concentrations and lower %POM.

There was no interaction between dw and season for clearance rate of *Crassostrea virginica* (ANCOVA; $F_{2,37} = 0.01$; $P > 0.05$) or *Crassostrea ariakensis* (ANCOVA; $F_{3,53} = 1.42$; $P > 0.05$). This allowed us to calculate a common slope for *C. ariakensis* (b = 0.62) and *C. virginica* (b = 0.44) for the regression equation that I then used to recalculate the intercept for each season, which equates to the clearance rate for a standardized oyster of 1 g tissue dw for each species over the four seasons. There were no significant differences in the clearance rate of *C. virginica* (Table 3.6) between summer, autumn, and spring (ANCOVA; $F_{2,39} = 1.39$; $P > 0.05$). Winter data was not included as these oysters were not observed feeding and did not produce biodeposits during this period.

There were significant differences in the seasonal clearance rate (Table 3.7) of *Crassostrea ariakensis* (ANCOVA; $F_{3,56} = 36.40$; $P_{\alpha<0.05} = 0.0001$) with significantly reduced rates during spring compared to autumn (LSD, $t_{56} = -2.38$; $P_{\alpha<0.05} = 0.0210$) and summer ($t_{56} = 3.52$; $P_{\alpha<0.05} = 0.0009$). Interestingly, *C. ariakensis* fed and voided biodeposits during the winter when temperatures were between 2 – 5ºC.

There was significant seasonal interaction in absorption efficiency for the two oyster species (ANOVA; $F_{3,95} = 49.04$, $P_{\alpha<0.05} < 0.0001$). The absorption efficiency of

Crassostrea virginica significantly differed among seasons (Table 3.8), with the highest efficiency occurring in spring (44.1%) and the lowest in winter (0%). The absorption efficiency of *Crassostrea ariakensis* also differed significantly among seasons (Table 3.8), with the highest efficiency occurring in winter (43.5%) and the lowest in summer (15.4%).

There was no interaction between dry tissue weight (dw) and season for the respiration rate of either *Crassostrea virginica* (ANCOVA; $F_{3,39} = 1.94$; $P > 0.05$) or *Crassostrea ariakensis* ($F_{3,43} = 1.35$; $P > 0.05$). This allowed us to calculate a common slope for *C. ariakensis* (b = 0.59) and *C. virginica* (b =1.07) for the regression equation that was then used to recalculate the intercept for each season (Table 3.7).

The respiration rate of *Crassostrea virginica* during the summer was significantly greater than in the autumn (LSD, $t_{42} = -4.15$; $P_{\alpha<0.05} = 0.0002$), winter ($t_{42} = 5.53$; $P_{\alpha<0.05} < 0.0001$), and spring ($t_{42} = -5.09$; $P_{\alpha<0.05} < 0.0001$) (Table 3.6). The respiration rate of *Crassostrea ariakensis* was also significantly greater in the summer than in the autumn (LSD, $t_{46} = -7.72$; $P_{\alpha<0.05} < 0.0001$), winter ($t_{46} = 10.90$; $P_{\alpha<0.05} < 0.0001$), and spring ($t_{46} = -8.03$; $P_{\alpha<0.05} < 0.0001$) (Table 3.7).

There was no interaction between oyster dry tissue weight and season for ammonium excretion for *Crassostrea virginica* (ANCOVA; $F_{3,42} = 0.41$; $P > 0.05$) or *Crassostrea ariakensis* ($F_{3,48} = 0.76$; $P > 0.05$). This allowed us to calculate a common slope for *C. ariakensis* (b = 0.44) and *C. virginica* (b = 0.57) for the regression equation that was then used to recalculate the intercept for each season (Table 3.6, 3.7).

The ammonium excretion rate of *Crassostrea virginica* was significantly higher in the summer than in the autumn (LSD, $t_{45} = -6.44$; $P_{\alpha<0.05} < 0.0001$), winter $t_{45} = -6.58$;

$P_{\alpha<0.05} < 0.0001$), and spring ($t_{45} = -4.63$; $P_{\alpha<0.05} < 0.0001$) (Table 3.6). The ammonium excretion rate of *Crassostrea ariakensis* differed significantly among all seasons sampled (Table 3.7), with the highest rates occurring in summer and the lowest occurring in winter.

Both oyster species exhibited a seasonal pattern in their scope for growth (Fig. 3.2). For a *Crassostrea virginica*, of 1 g tissue dw, the greatest amount of energy available for tissue growth (somatic and germinal) occurred in the spring (36 J g^{-1} h^{-1}); while in winter a negative scope for growth (-4.5 J g^{-1} h^{-1}) was calculated because *C. virginica* were not feeding. There was a negative scope for growth for 1 g tissue dw of *Crassostrea ariakensis* during the summer (-36.2 J g^{-1} h^{-1}) when this species had high metabolic activity, and in winter (-1.02 J g^{-1} h^{-1}) when this species was physiologically active but metabolic activity was low (Table 3.7); there was a positive scope for growth for the other seasons.

The O:N ratio for *Crassostrea virginica* was lowest during the summer (<50) and highest (>100) in the autumn and winter seasons (Fig. 3.3A). The O:N ratio for *Crassostrea ariakensis* remained relatively low (< 70) throughout the year, with the highest ratios (>50) during the winter and spring seasons (Fig. 3.3B).

DISCUSSION

Rates of shell growth did not differ significantly between *Crassostrea ariakensis* and *Crassostrea virginica* maintained under sub-tropical conditions. Although no statistical differences in growth rate were detected between the 28-month-old cohorts of each species, *C. ariakensis* grew at ~25% of the daily rate of *C. virginica* during the first 90 d (September to December) of the study. There was no significant difference in the monthly growth rate of *C. ariakensis* between age-cohorts; however the 4-month-old

cohort did exhibit a higher rate of growth from May until August. The lack of a detectable monthly significance in growth within this cohort may have been due to the severe mortality that reduced the sample size and hence the power of the statistical test. There was a significant difference in the monthly growth rate of *C. virginica*, where high rates of growth for the 4-month-old cohort occurred in May and June 2007. The enhanced growth of 4-month-old cohort oysters was not likely caused by changes in environmental factors such as increased temperature or increased food availability, as this growth spurt was not seen within the 28-month-old cohort of either species. The increased growth during this period may be due to 4-month-old oysters allocating more energy towards somatic growth than gamete development, an ontogenic shift in energy allocation that is typically seen in a long-lived invertebrate, such as oysters (Thompson et al. 1996).

Both *Crassostrea ariakensis* and *Crassostrea virginica* exhibited high levels of very early gametogenesis; however in very few individuals of either species or age class did gametogenesis proceed to the point that there were clearly distinguishable eggs or sperm within the follicles. Both oyster species showed a sharp, unexplained decline in the number of individuals with evidence of even early gametogensis in April 2007, compared with March and May. The only cohort to show any gamete differentiation by the end of the experiment in August was the 28-month-old *C. virginica* cohort (60%), whereas 100% of similarly sized *C. virginica* sampled from the IRL in the vicinity of the seawater intake in August 2007 had clearly distinguishable male and female gametes. This is in accordance with reports by Wilson et al. (2005) that oysters in south Florida waters have ripe gametes present from May to October. Furthermore, in mesocosms simulating the mesohaline region of Chesapeake Bay and containing individuals from the same 28-

months cohort oysters used in the present study, Newell et al. (2009) observed distinguishable gametes in 60% of *C. virginica* and 80% of *C. ariakensis* in June 2007, compared to 30% of *C. virginica* and 0% of *C. ariakensis* in the sub-tropical system described here.

Both age classes of *Crassostrea ariakensis* suffered greater mortality than *Crassostrea virginica* within the experimental mesocosms. The 28-month-old cohort of *C. ariakensis* began to die in mid-February with 100% mortality of all individuals by mid-June. The 4-month-old cohort of *C. ariakensis* began to die in mid-May and experienced total mortality by August. The 28-month-old cohort of *C. virginica* experienced a die-off in mid-April when ~20% of the oysters died, and in July a further ~20% died; however ~60% of *C. virginica* individuals from this cohort were still alive at the experimental termination in August 2007. The *C. virginica* from the 4-month-old cohort also experienced high mortality (~ 25%) in April, however little mortality was noted through the remainder of the study.

Oyster mortality within the mesocosms did not appear to be associated with any known adverse environmental conditions. Salinities in the experimental mesocosms were always fully euhaline (average salinity = 34) which is optimal for both *Crassostrea virginica* (Shumway 1996) and *Crassostrea ariakensis* (Calvo et al. 2001, Grabowski et al. 2004, Kingsley-Smith et al. 2009). Other environmental conditions in the mesocosms such as temperature, dissolved oxygen, and seston concentration also were typical of the annual cycle found in the IRL (Christian & Sheng 2003, Wilson et al. 2005). There is evidence that the warm temperatures encountered during the summer in Chesapeake Bay, and year-round in the IRL may reduce the growth rate of *C. ariakensis*. Calvo et al.

(2001) observed no growth of either triploid *C. ariakensis* or diploid *C. virginica* during the summer (22 – 29ºC) at high salinity sites within Chesapeake Bay. The lack of summer growth for *C. virginica* reported by Calvo et al. (2001) is likely attributable to an intense outbreak of *Perkinsus marinus*, which subsequently caused heavy *C. virginica* mortality. The lack of *C. ariakensis* summer growth could not be attributed to disease or any other stressors (Calvo et al. 2001). They noted that the majority of growth in this species occurred during the spring and fall periods when water temperatures were appreciably cooler. Conversely, other studies of triploid *C. ariakensis* in Chesapeake Bay (Paynter et al. 2008, Kingsley-Smith et al. 2009) and North Carolina estuaries (Grabowski et al. 2004) reported growth of *C. ariakensis* during the warm summer months. Langdon & Robinson (1996) reported that while *C. ariakensis* spat grew best at salinities of 25 to 35 at 20 − 25ºC during the summer, they also continued to grow equally well during the winter at several sites on the coast of Oregon. In its natural range *C. ariakensis* seems to flourish in waters with a wide annual temperature range of 3 to 28ºC (Kang et al. 2000, Harding & Mann 2006, Yoon et al. 2008). Most evidence seems to support that *C. ariakensis* should grow well in high salinity warm waters such as the IRL; however I found that diploid *C. ariakensis* grew poorly and suffered high mortality when maintained under such conditions during my study.

The mortality of both species of oysters was also not associated with infections of any of the three well recognized oyster parasites. Histological analysis did not reveal the presence of *Haplosporidium nelsoni* in either species of oyster, and *Perkinsus marinus* although present in both species of oysters was at low prevalence and intensities (Scarpa et al. 2009). *Bonamia* sp. was detected in February 2007; however the intensity of

infection was not high enough to cause mortality (Scarpa et al. 2009). Grabowski et al. (2004) found high mortality of small triploid *Crassostrea ariakensis* grown in subtidal estuaries of North Carolina during the summer. They suggest that high mortality may limit the growth advantage *C. ariakensis* seems to have over *Crassostrea virginica* in high salinity environments (Calvo et al. 2001, Paynter et al. 2008, Kingsley-Smith et al. 2009). Prevalence of *P. marinus* reported by Grabowski et al. (2004) in both species of oyster was light (0 – 16.7%) and not hypothesized to be the cause of the observed mortality; they did not test for the presence of *H. nelsoni* or *Bonamia* sp. Subsequent field trials in high salinity estuaries of North Carolina have shown that smaller (< 50mm shell height) *C. ariakensis* are particularly sensitive to *Bonamia* sp. infection, with mortality reaching 100% when temperatures exceed 20ºC during the summer and early fall (Carnegie et al. 2008). Audemard et al. (2008) confirmed in laboratory studies that high salinities (20 – 30) coupled with high temperatures (> 20ºC) resulted in high *Bonamia* sp. induced mortality of *C. ariakensis*. Prevalence of *Bonamia* sp. (60 – 100%) and intensity of infection reported by Carnegie et al. (2008) and Audemard et al. (2008) were much higher than the prevalence (0–40%) and intensity of infection in *C. ariakensis* from the mesocosms (Scarpa et al. 2009).

Concurrent with the onset of high mortality the physiological condition of both species declined. Oysters appeared emaciated and edematous, which may be an indication of lack of feeding. The digestive gland in oysters sampled as part of the routine histological samples in March and July 2007 were microscopically examined for evidence of feeding activity and nutrient assimilation. Both *Crassostrea ariakensis* and *Crassostrea virginica* showed equal evidence of feeding activity as indicated by the

presence of ingested food within the gut of 75 – 80% of the individuals examined; however by July *C. virginica* had a higher evidence of feeding activity (88%) than *C. ariakensis* (44%). Recent digestion of particles was indicated by columnar and cuboidal digestive gland epithelia which were at similar levels in both species of oyster in March (67 – 72%). By July recent particle digestion was inversely related to observed food ingestion frequencies, as 100% of *C. ariakensis* exhibited particle digestion compared to only 70% of *C. virginica*. A comparison of mesocosm and *C. virginica* freshly collected from location of the seawater intake in the IRL in August 2007 showed no difference in the feeding activity of mesocosm (78%) and wild oysters (77%), although recent particle digestion was lower for mesocosm oysters (65%) than for wild oysters (80%). Overall, there was no evidence of consistent deficiencies in feeding activity that may explain my gross observations of edematous emaciation in both species of oyster. It is possible that although I observed the ingestion and digestion of food particles that these particles were composed of phytoplankton species that could not support the oysters' nutritional requirements but this would not explain the observed rapid rise in mortality rates in *C. ariakensis* compared to *C. virginica*.

Taken together, these findings of reduced growth rate, increased mortality, and decline in the reproductive activity of *Crassostrea ariakensis* and to a much lesser extent in *Crassostrea virginica* between mid-February and mid-April indicate that oysters in the mesocosms were subjected to some unknown stress during this period. I postulate that the *C. ariakensis* may have experienced thermal stress in the prolonged period of >20ºC water temperatures that are characteristic of the sub-tropical IRL. *Bonamia* sp. was present in the mesocosms at this time; however prevalence and intensity of infection were

low (Scarpa et al. 2009), and do not fully explain the mortality and decreased reproductive activity observed during this period. It is possible that water pumped from the IRL contained a toxin that was inadvertently released during routine maintenance of a vessel in the channel. Alternatively there may have been a bloom of a toxic species of phytoplankton that was neither manifest (e.g., as a fish kill) nor readily detected by histology or measurement of environmental parameters (e.g., dissolved oxygen).

Physiological studies allowed us to examine if differences in physiological responses to temperature were responsible for the poor growth and survival of *Crassostrea ariakensis* compared to *Crassostrea virginica* of a similar range of dry tissue weights (Table 3.6, 3.7). These studies were performed in Virginia on oysters maintained under similar environmental conditions to those in the Florida mesocosms but without the confounding factor of *Bonamia* sp. presence. Summer water temperatures were similar between the two locations, but the Virginia study site was subjected to a wider range of water temperatures during the remaining seasons. The scope for growth for both species of oysters showed a distinct seasonality in the amount of energy available for somatic growth and gamete production.

During the summer, at temperatures >25ºC, *Crassostrea ariakensis* had a negative scope for growth (-36.2 J g^{-1} h^{-1}) which was due to a low clearance rate (1.16 L g^{-1} h^{-1}) without an equivalent decrease in the other physiological rates. This supported my hypothesis that mortality I observed of *C. ariakensis* in the Florida mesocosms was due to high water temperatures imposing an energetic stress. Zhang et al. (1959, cited in Zhou & Allen 2003) reported that *C. ariakensis* (= *Ostrea rivularis*) had a low feeding incidence (0 – 70%) during the summer when water temperatures were high (22 – 30ºC)

and salinities were low and variable (2 – 26); clearance rates were not reported. Zhou & Allen (2003) suggest that the decrease in feeding incidence may be more closely related to salinity than to temperature. Kelly (2011) reported clearance rates of 1.10 L g^{-1} h^{-1} for *C. ariakensis* from the same stock as I studied during the summer (Temperature 25ºC; salinity ~10). This is not different from the clearance rate of 1.2 L g^{-1} h^{-1} I measured from the high salinity location in Virginia, indicating that clearance rates in this species are apparently not affected by salinities in the range of 10 to 24. During the winter *C. ariakensis* remained active, with individuals observed to be feeding, producing biodeposits, and putting on new shell growth even when water temperatures dropped to 2ºC. Absorption efficiency was highest in the winter; therefore even with a reduced clearance rate, *C. ariakensis* was still benefiting from its continual activity by assimilating a greater portion of the food they were ingesting although the calculated scope for growth was negative (-1.02 J g^{-1} h^{-1}). From spring to summer *Crassostrea virginica* had a positive scope for growth which was primarily influenced by a relatively high clearance rate during the summer, and low respiration rates during the remaining seasons. The summer clearance rate for *C. virginica* (2.22 L g^{-1} h^{-1}) was 50% higher than *C. ariakensis* (1.16 L g^{-1} h^{-1}) in my study but lower than what has previously been reported in the literature (Loosanoff & Nomejko 1946, Jordan 1987, Newell & Langdon 1996). It is possible that the clearance rate of *C. virginica* was negatively impacted by the high seston (49.3 mg L^{-1}) present within the experimental system during the summer. High particle concentrations (> 25 mg L^{-1}) have been shown to decrease *C. virginica* clearance rates at temperatures above 20ºC (Newell & Langdon 1996). No feeding activity or biodeposit production was observed for *C. virginica* during the winter.

Continued respiration and ammonium excretion, albeit at low rates, resulted in a negative scope for growth for *C. virginica* during the winter.

The O:N ratio is a measure of the relative utilization of protein in energy metabolism (Corner & Cower 1968, Bayne et al. 1985). An O:N ratio below 20 indicates stress in marine bivalves (Bayne et al. 1985, Huang & Newell 2002). The O:N ratio for both species of oysters measured in Virginia during the summer is low, but above the level that would be indicative of nutritive stress in either species. This relatively low summer ratio may be due to an unobserved spawning event prior to or during the acclimation period. Post-spawning oysters are generally in a poor condition due to the need to reorganize or regenerate tissue (Bayne et al. 1985). Because the O:N ratios of *Crassostrea virginica* and *Crassostrea ariakensis* were almost identical during the summer, it is unlikely that spawning induced stress was the reason for differences in the scope for growth between the oyster species. The O:N ratio of *C. ariakensis* remained relatively low (<60) and consistent throughout the year, indicating a greater affinity for obtaining energy through protein degradation rather than lipid and/or carbohydrate catabolism compared to *C. virginica*; the reasons for this remains unclear.

These physiological studies indicate that high water temperatures impose a stress on *Crassostrea ariakensis* that results in highly reduced feeding activity, and a concomitant reduced scope of growth. In temperate locations, such as Chesapeake Bay, where high water temperature occurs only for two summer months, such stress may not be lethal. During the summer oysters can utilize nutrients accumulated in cooler months when the oysters are actively feeding. In the subtropical IRL where the mesocosm studies were performed, water temperatures were > 20 ºC for 11 mo. and > 25ºC for 6 mo. (Table

3.1). The possibility that heat stress alone was responsible for the high mortalities suffered by *C. ariakensis* in this study appears to be at odds with the species' geographical distribution in its native habitat where its range is reported to extend into subtropical regions in Asia (Zhou & Allen 2003, Guo et al. 2008).

There has been some confusion surrounding the identification of *Crassostrea ariakensis* within its native range (Zhou & Allen 2003). Zhang et al. (2005) compared genetic variation of *C. ariakensis* in USA hatchery stocks to wild Asian populations using polymerase chain reaction with restriction fragment length polymorphism to analyze the mitochondrial *COI* gene region and found genetic differentiation between "northern-type" and "southern-type" strains. Using similar genetic analysis Guo et al. (2008) found that *C. ariakensis* was a dominant member of mixed assemblages of oysters at only five spatially isolated sites within China and was present at low abundances throughout the rest of its range. Only one of those populations occurred in a subtropical climate; the remaining sites had annual temperature ranges similar to the temperate region of the east coast of the USA.

It is important to note that all *Crassostrea ariakensis* used in this and other studies of *C. ariakensis* in North America in the last decade descend from a small founder population consisting of 7 males and 9 females (U.S. Army Corps of Engineers 2009). Although the exact details concerning the introduction of *C. ariakensis* to Oregon, USA, from Asia are unknown, they were first isolated in the late 1960s among *Crassostrea gigas* oysters being cultured in Yaquina Bay, OR and then subsequently bred in Oregon (Breese & Malouf 1977, Malouf, Oregon Sea Grant, pers. comm.). Zhang et al. (2005)

reported that 97% of *C. ariakensis* within USA hatchery stocks are most genetically similar to the "northern-type" strain.

Given the highly restricted genetic make up of these "Oregon" stock introduced oysters and the fact that they likely originated in the cooler temperate regions of Asia it is perhaps not surprising that they exhibit low tolerance to sub-tropical warm waters. Potential future introduction of additional *Crassostrea ariakensis* from southern regions of Asia may result in a population of new oysters with higher temperature tolerance. It is also possible that higher temperature tolerance may evolve in the Oregon strain of *C. ariakensis*. The recent northward range extension of *Crassostrea gigas* in Europe has been attributed to increasing summer water temperatures sufficient to allow the species to reproduce in waters that were previously too cold (Wrange et al. 2010). But it is also plausible that sufficient time has lapsed since *C. gigas* was introduced into Europe in the early 1970's for adaptations to have evolved that allow this species to inhabit cooler waters. There is evidence that such physiological adaptations to temperature exist within latitudinally separated and reproductively isolated populations of the blue mussel along the east coast of North America (Thompson & Newell 1985).

In summary my results indicate that if *Crassostrea ariakensis* were to be either deliberately or accidentally introduced into Chesapeake Bay their expansion into U.S. subtropical regions may be limited. A depressed clearance rate resulting in a negative scope for growth under warm water conditions would result in reduced growth rates for *C. ariakensis* which may make them less competitive against the native *Crassostrea virginica*. Eastern oysters in the subtropical regions of the U.S. are primarily intertidal (Grizzle 1990, Coen et al. 2007) which provides them with a refuge against predation

(O'Beirn et al. 1996). The intertidal zone has been found to be inhospitable to *C. ariakensis* with mortality rates often reaching 100% largely due to physiological stresses possibly caused by some combination of thermal intolerance and desiccation stress (Kingsley-Smith & Luckenbach 2008, Kingsley-Smith et al. 2009). My observations of reduced clearance rates and a negative scope for growth for *C. ariakensis* in year-round subtropical waters would likely result in a growth rate much lower than native *C. virginica*. This would prevent juvenile *C. ariakensis* in the subtropical subtidal zone from rapidly reaching a size refuge against intense predation pressures; which when coupled with their relatively fragile shell (Newell et al. 2007) might serve to enhance predation rates on this species.

CHAPTER 3:

Tables

Table 3.1: Abiotic parameters within Florida mesocosms

Mean (n = 3) water temperature, salinity, dissolved oxygen concentration (DO), and percent dissolved oxygen saturation (%DO) of ambient flow-through water in Florida mesocosms from September 2006 through August 2007.

Month	Temperature (°C)	Salinity	DO (mg L^{-1})	%DO
September 2006	28.9	31.0	5.4	82.9
October 2006	25.8	32.0	6.0	87.1
November 2006	21.3	32.9	6.7	91.2
December 2006	21.8	33.2	6.4	87.9
January 2007	21.0	33.0	6.3	84.3
February 2007	18.6	32.8	6.9	89.5
March 2007	22.0	35.0	6.1	85.8
April 2007	22.9	36.2	6.1	87.1
May 2007	25.6	37.7	5.5	82.9
June 2007	28.3	35.7	5.1	79.0
July 2007	30.1	31.6	4.8	75.1
August 2007	30.4	28.0	4.8	74.1

Table 3.2: Growth rate of Crassostrea ariakensis within Florida mesocosms

Mean (± SE) absolute size and mean (± SE) specific growth rate (SPG) of 28 and 4-month-old *Crassostrea ariakensis* in Florida mesocosms between September 2006 and August 2007. Different letters denote significant differences in growth rate within cohort at $\alpha = 0.05$, ns = no difference (Wilcoxon Signed Rank Sum pairwise comparisons).

Cohort	Month	Absolute size (mm^2)	SPG [ln increase shell area ($mm^2\ d^{-1}$)	N	$P \leq 0.05$
28 mo	September	2205.9 ± 245.5		16	
	December	2262.7 ± 240.9	$8.2 \times 10^{-4} \pm 4.1 \times 10^{-4}$	16	ns
	January	2329.1 ± 272.7	$3.6 \times 10^{-4} \pm 1.6 \times 10^{-4}$	15	ns
	February	2318.1 ± 257.6	$3.9 \times 10^{-4} \pm 2.3 \times 10^{-4}$	15	ns
	March	2199.7 ± 317.2	$6.6 \times 10^{-4} \pm 4.0 \times 10^{-4}$	10	ns
	April	1450.9 ± 287.6	$7.3 \times 10^{-4} \pm 7.3 \times 10^{-4}$	4	ns
	May	1329.7 ± 267.2	0.0 ± 0.0	3	ns
	June	866.1 ± N/A	0.0 ± 0.0	1	ns
	July			0	
	August			0	
4 mo	September				
	December	249.5 ± 29.4		26	
	January	261.9 ± 30.3	$21.0 \times 10^{-4} \pm 7.0 \times 10^{-4}$	26	ns
	February	273.9 ± 30.9	$17.4 \times 10^{-4} \pm 12.7 \times 10^{-4}$	26	ns
	March	278.5 ± 31.9	$4.7 \times 10^{-4} \pm 2.4 \times 10^{-4}$	26	ns
	April	304.2 ± 34.6	$21 \times 10^{-4} \pm 9.4 \times 10^{-4}$	25	ns
	May	336.8 ± 37.9	$35.3 \times 10^{-4} \pm 15.9 \times 10^{-4}$	16	ns
	June	376.8 ± 39.1	$28.9 \times 10^{-4} \pm 13.3 \times 10^{-4}$	12	ns
	July	405.8 ± 36.0	$28.7 \times 10^{-4} \pm 12.3 \times 10^{-4}$	4	ns
	August			0	

Table 3.3: Growth rate of Crassostrea virginica within Florida mesocosms

Mean (± SE) absolute size and mean (± SE) specific growth rate (SPG) of 28 and 4-month-old *Crassostrea virginica* in Florida mesocosms between September 2006 and August 2007. Different letters denote significant differences in growth rate within cohort at $\alpha = 0.05$, (Wilcoxon Signed Rank Sum pairwise comparisons).

Cohort	Month	Absolute size (mm^2)	SPG [ln increase shell area ($mm^2\ d^{-1}$)	N	$P \leq 0.05$
28 mo	September	732.2 ± 32.9		14	
	December	894.4 ± 56.4	$35.6 \times 10^{-4} \pm 11.8 \times 10^{-4}$	14	a
	January	903.5 ± 55.2	$3.0 \times 10^{-4} \pm 2.1 \times 10^{-4}$	14	b
	February	934.5 ± 53.8	$11.0 \times 10^{-4} \pm 4.0 \times 10^{-4}$	14	ab
	March	971.8 ± 66.3	$13.7 \times 10^{-4} \pm 6.3 \times 10^{-4}$	13	ab
	April	996.6 ± 68.2	$6.1 \times 10^{-4} \pm 4.5 \times 10^{-4}$	12	b
	May	1073.3 ± 84.7	$12.0 \times 10^{-4} \pm 6.1 \times 10^{-4}$	9	ab
	June	1068.6 ± 100.7	$5.2 \times 10^{-4} \pm 5.2 \times 10^{-4}$	9	ab
	July	1117.2 ± 111.3	$5.3 \times 10^{-4} \pm 4.9 \times 10^{-4}$	8	ab
	August	1173.0 ± 108.5	0.0 ± 0.0	6	b
4 mo	September	97.1 ± 8.7		52	
	December	212.2 ± 16.8	$137.6 \times 10^{-4} \pm 19.8 \times 10^{-4}$	42	a
	January	230.8 ± 17.4	$12.8 \times 10^{-4} \pm 4.7 \times 10^{-4}$	46	d
	February	237.3 ± 18.2	$8.9 \times 10^{-4} \pm 3.7 \times 10^{-4}$	52	cd
	March	258.8 ± 18.1	$19.7 \times 10^{-4} \pm 5.0 \times 10^{-4}$	51	cbd
	April	285.9 ± 19.6	$16.4 \times 10^{-4} \pm 7.5 \times 10^{-4}$	41	cbd
	May	322.2 ± 26.9	$35.7 \times 10^{-4} \pm 11.2 \times 10^{-4}$	29	cb
	June	368.1 ± 34.6	$43.5 \times 10^{-4} \pm 14.6 \times 10^{-4}$	25	b
	July	376.5 ± 33.8	$17.4 \times 10^{-4} \pm 9.3 \times 10^{-4}$	28	cbd
	August	387.9 ± 42.3	$10.9 \times 10^{-4} \pm 10.9 \times 10^{-4}$	23	cbd

Table 3.4: Oyster reproductive condition within Florida mesocosms

Reproductive and gonadal index data for *Crassostrea ariakensis* and *Crassostrea virginica* reared in Florida mesocosms. Percent gonadal development indicates number of oysters which showed evidence of gametogenesis but no identifiable gender. Percent differentiated indicates number of oysters which had clearly distinguishable eggs or sperm.

Species	Age	Month	N	% Gonadal development	% Differentiated
C. virginica	4 mo	February	4	25	0
		March	4	100	0
		April	3	66	0
		May	9	89	0
		June	2	50	0
		July	11	91	0
		August	4	25	0
C. ariakensis	4 mo	February	9	78	0
		March	10	60	0
		April	14	21	0
		May	15	93	0
		June	16	81	0
		July	13	92	8
		August	0		
C. virginica	28 mo	February	8	63	0
		March	8	100	0
		April	7	43	0
		May	8	100	50
		June	10	80	30
		July	3	100	33
		August	5	80	60
C. ariakensis	28 mo	February	11	91	0
		March	9	100	0
		April	3	33	0
		May	5	80	0
		June	4	50	0
		July	1	100	0
		August	0		

Table 3.5: Seasonal seston concentration within Virginia flow-through system

Mean (n=3) temperature, salinity, total suspended solids (TSS ± SE), and percent particulate organic matter (POM ± SE) of ambient flow-through water at VIMS-ESL during a 7 d period when seasonal physiological measurements were performed.

Season	**Temperature (°C)**	**Salinity**	**TSS (mg L^{-1})**	**POM (%)**
July 2008	27	29.8	49.3 ± 7.2	9.3 ± 1.3
October 2008	14	29.4	13.8 ± 3.7	16 ± 3.9
January 2009	5	32	17.9 ± 0.9	17.1 ± 1.0
April 2009	14	30	18.7 ± 1.4	16.2 ± 1.1

Table 3.6: Seasonal physiological rates of C. virginica in Virginia

Seasonal clearance, respiration, and ammonium excretion rates (*a*) for *Crassostrea virginica* measured in ambient flow-through conditions in Virginia. Rates are standardized to an oyster with a 1 g dry tissue weight by the allometric equation $Y = aX^b$ (see text for details). Common slope (*b*) for clearance rate (*) does not include winter (January) 2009, because these oysters did not feed for the duration of the experiment in this season. Different letters denote significant difference in corrected *a* values among seasons, ns = no difference (LSD pairwise comparisons; $p \leq 0.05$). Mean (± SD) and range of oyster dry tissue weights are shown for each season.

Rate	*b*	Season	*n*	*a*	P ≤ 0.05	Tissue weight (g)	range (g)
Clearance Rate	0.44*	Jul. 2008	15	2.22	ns	0.93 ± 0.18	0.17 - 2.15
(L g^{-1} h^{-1})		Oct. 2008	14	1.86	ns	0.97 ± 0.15	0.34 - 2.03
		Jan. 2009	16	0		0.94 ± 0.12	0.23 - 1.50
		Apr. 2009	14	1.50	ns	0.92 ± 0.08	0.38 - 1.41
Respiration	1.07	Jul. 2008	16	2.52	a	0.88 ± 0.17	0.17 - 2.15
(mL O_2 g^{-1} h^{-1})		Oct. 2008	16	0.78	b	1.01 ± 0.13	0.34 - 2.03
		Jan. 2009	4	0.22	c	1.04 ± 0.29	0.23 - 1.50
		Apr. 2009	11	0.51	bc	0.95 ± 0.09	0.46 - 1.41
Ammonium excretion	0.57	Jul. 2008	16	77.30	a	0.88 ± 0.17	0.17 - 2.15
(μg NH_4-N g^{-1} h^{-1})		Oct. 2008	14	5.57	bc	1.04 ± 0.14	0.34 - 2.03
		Jan. 2009	6	2.14	c	1.00 ± 0.21	0.23 - 1.50
		Apr. 2009	14	11.65	bc	0.96 ± 0.07	0.38 - 1.41

Table 3.7: Seasonal physiological rates of C. ariakensis in Virginia

Seasonal clearance, respiration, and ammonium excretion rates (*a*) for *Crassostrea ariakensis* measured in ambient flow-through conditions in Virginia. Rates are standardized to an oyster with a 1 g dry tissue weight by the allometric equation $Y = aX^b$ (see text for details). Different letters denote significant difference in corrected *a* values among seasons, ns = no difference (LSD pairwise comparisons; $p \leq 0.05$). Mean (± SD) and range of oyster dry tissue weights are shown for each season.

Rate	*b*	Season	*n*	*a*	P ≤ 0.05	Tissue weight (g)	range (g)
Clearance Rate	0.62	Jul. 2008	14	1.16	a	1.06 ± 0.26	0.17 - 3.37
(L g^{-1} h^{-1})		Oct. 2008	16	1.54	a	0.71 ± 0.12	0.19 - 1.56
		Jan. 2009	15	0.18	c	1.08 ± 0.18	0.22 - 2.16
		Apr. 2009	16	0.67	b	1.68 ± 0.24	0.63 - 4.26
Respiration	0.59	Jul. 2008	13	2.60	a	1.13 ± 0.27	0.17 - 3.37
(mL O_2 g^{-1} h^{-1})		Oct. 2008	15	0.70	b	0.73 ± 0.13	0.19 - 1.56
		Jan. 2009	9	0.32	c	1.01 ± 0.22	0.22 - 2.12
		Apr. 2009	14	0.61	b	1.82 ± 0.25	0.68 - 4.26
Ammonium excretion	0.44	Jul. 2008	14	72.33	a	1.06 ± 0.26	0.17 - 3.37
(μg NH_4-N g^{-1} h^{-1})		Oct. 2008	16	25.19	b	0.71 ± 0.12	0.19 - 1.56
		Jan. 2009	11	6.18	d	1.13 ± 0.21	0.22 - 2.12
		Apr. 2009	15	11.36	c	1.74 ± 0.25	0.63 - 4.26

Table 3.8: Seasonal absorption efficiency of oysters in Virginia

Mean (± SE) seasonal percent absorption efficiency (A*e*) of *Crassostrea ariakensis* and *Crassostrea virginica* in ambient flow-through conditions at VIMS-ESL. Different letters denote significance of back-transformed means (± SE) for each species among seasons (LSD pairwise comparisons; $p \leq 0.05$).

Species	Season	*n*	A*e* (%) ± SE	Back-transformed avg. (%) ± SE			
				A*e*	+ SE	- SE	P ≤ 0.05
C. virginica	July 2008	11	25.53 ± 3.3	24.5	3.8	3.6	b
	October 2008	16	37.07 ± 2.8	36.5	3.5	3.4	a
	January 2009	15	0 ± 0	0	0	0	c
	April 2009	14	44.1 ± 4.6	43.5	3.8	3.8	a
C. ariakensis	July 2008	7	15.4 ± 2.8	14.9	4.1	3.6	c
	October 2008	14	29.4 ± 3.3	28.7	3.5	3.4	b
	January 2009	10	43.5 ± 3.8	43.4	4.5	4.4	a
	April 2009	16	36.5 ± 4.5	35.2	3.5	3.4	ab

CHAPTER 3:

Figures

Figure 3.1: Oyster mortality within Florida mesocosms

Cumulative percent mean mortality (± SE) of age 4 (A) and 28-month-old (B) *Crassostrea ariakensis* (solid line, black diamond) and *Crassostrea virginica* (dashed line, white diamond) in Florida mesocosms from December 2006 through August 2007. Stars denote a significant difference in the back-transformed cumulative percent mortality between oyster species (LSD pairwise comparisons; $p \leq 0.05$)

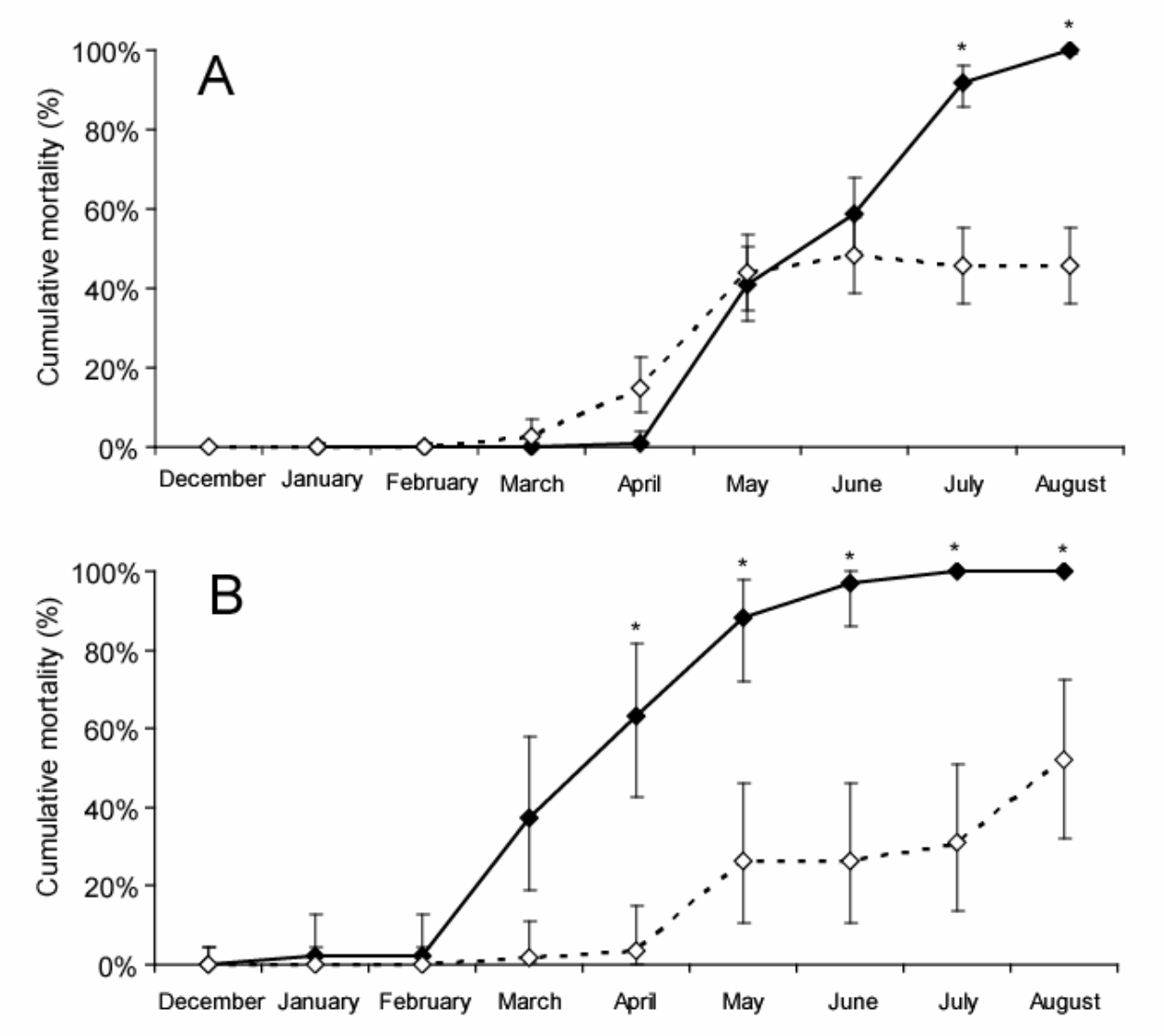


Figure 3.2: Seasonal scope for growth of oysters in Virginia

Seasonal scope for growth (J g^{-1} h^{-1}) of *Crassostrea virginica* (A) and *Crassostrea ariakensis* (B) in ambient flow-through conditions in Virginia (± SD). The scale of y-axis values is different between panels.

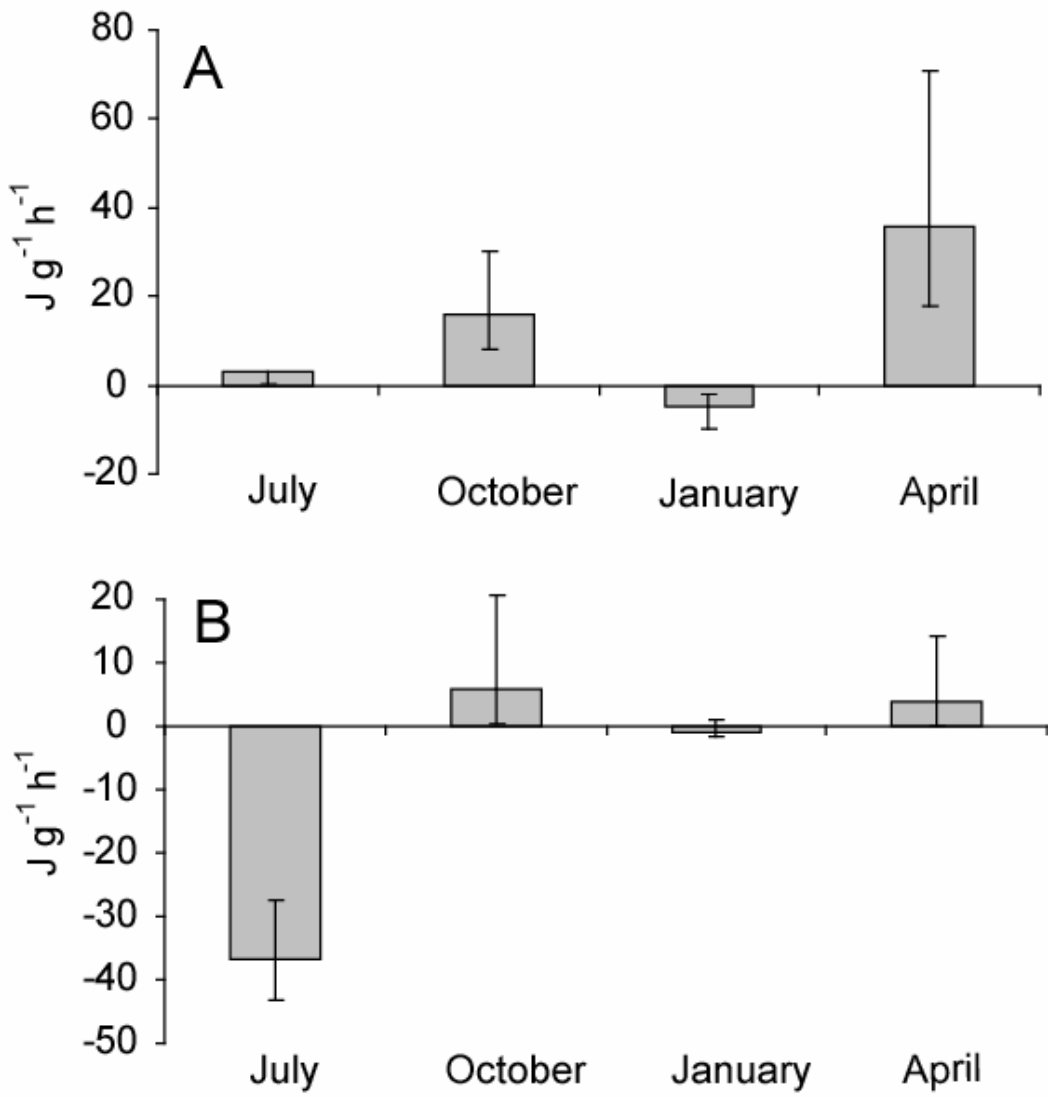
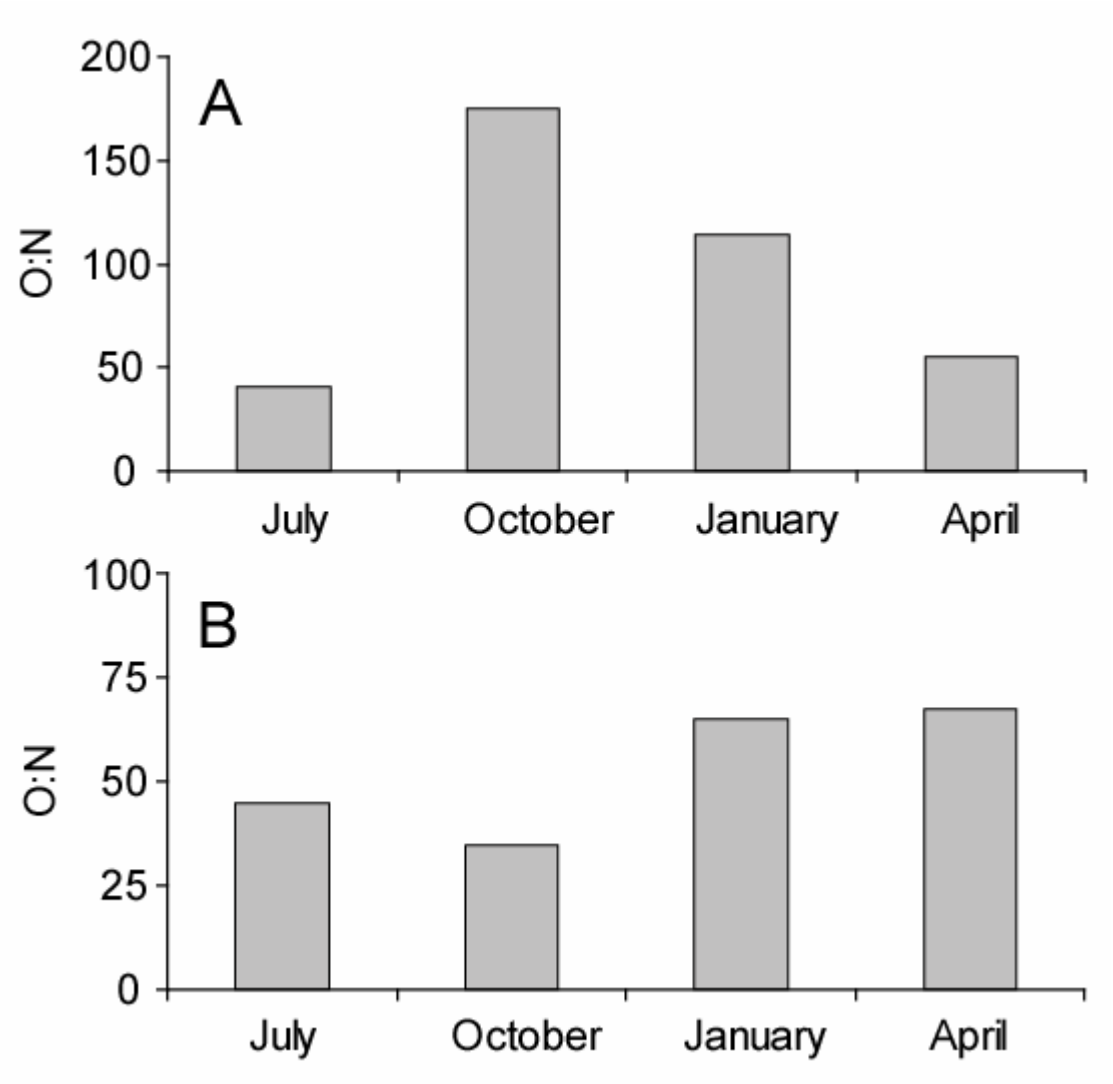


Figure 3.3: Seasonal O:N ratio of oysters in Virginia

Seasonal O:N ratio calculated from standardized 1 g dw population respiration and ammonium excretion rates of *Crassostrea virginica* (A) and *Crassostrea ariakensis* (B) in ambient flow-through conditions in Virginia. The scale of y-axis values is different between panels.



CHAPTER 4:

The Importance of Habitat Complexity, Refuge, and Prey Availability on the Attraction of Grass Shrimp (*Palaemontes pugio*), White Perch (*Morone americana*), and Striped Bass (*Morone saxatilis*) to Structure

ABSTRACT

I examined how differing levels of habitat complexity affect interactions among prey (grass shrimp), intermediate predator (white perch), and apex predator (striped bass) species. In laboratory mesocosms five predator–prey treatment combinations (shrimp only, white perch only, shrimp–white perch, shrimp–striped bass, and shrimp–white perch–striped bass) were paired with each of three habitat complexities (flat sand, medium, and high) and replicated five times. Grass shrimp were significantly attracted to the high complexity habitat in the absence of either fish predator. Attraction of grass shrimp to the high complexity habitat was further enhanced by the presence of each predator species both separately and together. The level of structural complexity was the primary determinant of white perch attraction to habitat. The presence of grass shrimp significantly increased the attraction of white perch to habitat, while the presence of striped bass enhanced the amount of time white perch spent on the medium and high complexity habitats. Swimming and shoaling activity of white perch generally decreased with an increase in habitat complexity, although white perch swimming and shoaling activity on the medium complexity habitat was enhanced by the presence of striped bass. I attribute this to the limited refuge offered by the medium complexity habitat coupled with the increased threat of predation by striped bass. Striped bass attraction to structure was low across all levels of structural complexity, and was not influenced by the presence of either grass shrimp or white perch. I conclude that intermediate predatory fish species are attracted to structure for food resources; however when subjected to a predatory threat themselves, they spend increased time within structurally complex habitat.

INTRODUCTION

Structurally complex habitats are considered essential for fish (Coen et al. 1999) because they provide a refuge against predation (Heck & Crowder 1991, Hixon & Beets 1993, Steele 1999), enhanced foraging opportunities (Adams et al. 2004, Verweij et al. 2006), and a place of refuge from adverse environmental conditions (Kelly & Bothwell 2002, Cocheret de la Morinière et al. 2004). Complex habitat helps boost production of resident and visiting fishes because it attracts and sustains their prey populations while also providing a refuge against predation on themselves (Polovina & Sakai 1989, Ebeling & Hixon 1991). The value of complex structure as essential habitat for transient predatory fish species is uncertain, however, because these species are considered opportunistic and may forage wherever prey densities are highest (Harding & Mann 2001). There is evidence from studies on artificial reefs that complex habitat may serve to aggregate transient predatory fish species from a wider geographical area without enhancing their production (Bohnsack 1989), which in turn makes them more vulnerable to fishing pressure (Samples & Sproul 1985).

The density and diversity of organisms found on highly complex habitats has frequently been found to be an order of magnitude higher than on structurally simple habitats (Kohn & Leviten 1976, Russ 1991, Diehl 1992, Bohnsack et al. 1994, Adams et al. 2004, Rodney & Paynter 2006). Large structurally complex biogenic habitats may seed other environmentally suitable areas with constituent foundation species (Baums et al. 2006, North et al. 2008). The creation of these new habitats will then attract associated fauna through emigration and production.

The importance of increased structural complexity of aquatic habitats in attracting organisms (Gotceitas & Colgan 1989, Steele 1999, Peterson et al. 2003, Verweij et al. 2006) and influencing their predator–prey relationships (Savino & Stein 1982, Orth et al. 1984, Johnson and Heck 2006) have been well documented. There is, however, some question on which characteristics of habitat complexity are driving this attraction. Surface area (Orth et al. 1984, Stoner 1984, Moore & Hovel 2010), interstitial space (Hacker & Steneck 1990, Hixon & Beets 1993, Charbonnel et al. 2002, Adams et al. 2004), and presence of conspecifics (Lecchini et al. 2007, Hay 2009) have all been put forth as important factors to consider when developing structurally complex habitat for restoration purposes. When interpreting results from such studies, one should recognize that habitat complexity is a relative characteristic that depends partially on body size, population density, and behavior of an organism utilizing that habitat (Heck & Orth 1980, Ryer 1988). An aquatic habitat that is structurally complex to one species may be recognized as structurally simple by another.

The behavior of fish and invertebrate species is also influenced by the level of structural complexity present within an aquatic habitat. Increased levels of structural complexity can make it more difficult for predators to maneuver around physical obstacles and barriers, and decrease the line of sight for both predator and prey species. Many fish species switch to an ambush predatory style within highly complex habitats (Savino & Stein 1982, Verweij et al. 2006) because it is energetically beneficial to sit and wait for prey, rather than to search within a spatially difficult terrain. Increased complexity has been shown to decrease the territory and territorial aggression of many

fishes due to reduced visibility (Basquill & Grant 1998, Breau & Grant 2002), which may lead to greater fish densities on more complex habitats.

The affect of habitat complexity on shoaling behavior of fishes is complicated. On low and intermediate complexity habitat fish shoaling activity is increased because it provides informational cues on prey location which enhance foraging efficiency of individuals, while also providing increased protection from being subjected to predation themselves (Clark & Mangle 1986). High complexity habitat decreases shoaling activity of fish species because the high density and diversity of prey species decreases the need for informational cues to find prey, and the surrounding structure provides a higher degree of refuge (Butler 1988, Eklöv 1997, Orpwood et al. 2008).

Crowder and Cooper (1982) postulated that intermediate habitat complexities can benefit both prey and predator communities because these habitats provide limited refuge, while also providing easier access for predators to encounter and capture prey within the refuge. More recent studies have questioned this assertion on the basis that increased complexity also leads to the increased density of prey species through production and immigration to the habitat (Mattila et al. 2008, Canion & Heck 2009, Chapter 5), resulting in more highly productive habitats. High predator diversity, habitat overlap between predatory species, and predator behavior may also interact to influence prey habitat selection because of the risk of predation associated with that habitat (Schmitz 2007), which may be more pronounced on intermediate complexity habitats than on highly complex ones.

Grass shrimp (*Palaemontes pugio*), white perch (*Morone americana*), and striped bass (*Morone saxatilis*) are three species found in abundance around structurally complex

habitats within Chesapeake Bay and the Atlantic coast of the United States (Posey et al. 1999, Coen et al. 1999, Clark et al. 2003, Davis et al. 2003, Peterson et al. 2003, Rodney & Paynter 2006, McGrath & Austin 2009). White perch and striped bass are both voracious predators on grass shrimp (Clark et al. 2003), while grass shrimp have been shown to alter their habitat preferences in the presence of these two fish predator species (Clark et al. 2003, Davis et al. 2003, Chapter 5). These attributes make these three species excellent organisms to investigate general predator–prey interactions on different complexity habitats. The results of which can then be used to make generalizations about interactions among other predator and prey species inhabiting a variety of structurally complex habitats.

The goal of this study was to ask how structural complexity, trophic complexity, and their interaction affect predation rates and the behavior of predators and prey. I hypothesized that aquatic organisms are attracted to increased levels of structurally complex habitat regardless of predatory threat or provision of food resources; that the provision of food resources will enhance the occurrence of fish species on structurally complex habitat; and that a species' occurrence would be further enhanced when they are subject themselves to a greater predatory threat. I also hypothesized that swimming and shoaling activity of each fish species will decrease with an increase in habitat complexity across most trophic complexity levels.

MATERIALS AND METHODS

This study investigated how increases in levels of structural complexity affect the attraction of grass shrimp, white perch, and striped bass to structure. Interactions among each species on structurally complex habitat and alterations in their behavior were also

examined. Increased structural complexity was defined as a change in structural surface area over three complexity levels; flat sand, medium, and high. This study was primarily conducted as a two-factorial Repeated Measures design with the two factors being structural and trophic complexity. The location of grass shrimp, and the location and behavior of white perch and striped bass were response variables that were repeatedly measured over time (day, morning, and night periods). Utilization of each level of structural complexity by grass shrimp was determined by the percentage of grass shrimp on each structure, and at the surface of the water at the end of each experimental trial. The effectiveness of each level of structural complexity as a refuge was determined by the percentage of grass shrimp surviving at the end of each experimental trial. Utilization of each level of structural complexity by white perch and striped bass was determined by video analysis of fish attraction to and behavior on structural habitat.

Trophic complexity

Experimental trails were conducted at Horn Point Laboratory between July and October 2009 within three 4164 L circular fiberglass mesocosms (diameter 2.5 m) filled with 2 μm filtered ambient Choptank River water to a depth of 0.6 m. Water temperature ranged from 21.7 – 26.7ºC and salinity ranged from 9.8 – 12.2. Each treatment consisted of one habitat complexity treatment (flat sand, medium, high) paired with one trophic complexity treatment (grass shrimp only, white perch only, grass shrimp/white perch, grass shrimp/striped bass, grass shrimp/white perch/striped bass) and was replicated in random succession five times over the duration of the experiment.

Grass shrimp (body length, 1.5 – 2.5 cm) were collected from the Choptank River and placed into a holding tank supplied with flow-through raw ambient Choptank River

water and fed fish flake food (Wardley: Goldfish flake food) *ad libidum*. Grass shrimp surviving predation were reused because the high number of individuals (n = 15,000) needed; however this supply was supplemented with new individuals on a weekly basis. Individual grass shrimp were never reused in consecutive trials.

White perch (14 – 18 cm; fork length) were collected from the Choptank River by use of an un-baited fish trap (FTFC Oval Fish Trap) and placed into a holding tank supplied with raw ambient Choptank River water for acclimatization to experimental conditions 5–7 d before the start of the experimental run in which they were used. White perch were fed *ad libidum* on grass shrimp during this acclimatization period. White perch were transferred to a separate flow-through holding tank and starved for 24 h before the start of an experimental run. Individual white perch (n = 90) were used only once during the study to prevent adaptation and learned responses of the experimental conditions beyond that of the initial acclimatization period.

Twenty-five striped bass (38 – 43 cm; fork length) reared at Horn Point Laboratory's fish hatchery were kept in an ambient, flow-through holding tank and fed a maintenance ration of pellet food between experimental runs. Pre-trials showed that hatchery-reared striped bass on a pellet food diet still fed voraciously on grass shrimp. These trials also showed that hatchery-reared striped bass recognized juvenile white perch as a prey species, and successfully captured white perch within 34 h after they were introduced into the holding tank. Striped bass were transferred to a separate holding tank before the start of an experimental run and starved for 24 h. Striped bass were reused over the course of the experiment because of the limited availability of new stock.

However once a striped bass was used, it was not reused until all other striped bass had been utilized. Individual striped bass were never reused in consecutive trails.

Habitat complexity

The flat sand treatment consisted of a 0.95 m^2 area of fine aquarium sand that rose 1 cm above the bottom of the tank. This complexity level was designed to not provide any structural refuge for grass shrimp or white perch to utilize for protection against predation.

The medium complexity structure (Fig. 4.1A) had a surface area of 2.2 m^2 and protruded 20 cm from the bottom of the tank. This complexity was comprised of split 10 cm diameter PVC pipes capped at each end so that the internal space of the pipe could not be used as a refuge. Affixed vertically to each split pipe were three or four flat PVC baffles. This assembly comprised one pipe unit. Each pipe unit was placed parallel to each other with a 5 cm gap and pressed gently into a 1 m^2 sand-bed constructed in the same way as the flat sand treatment. The end pipe units had three flat PVC pipe baffles that extended horizontally perpendicular from their base (Fig. 4.1A, oval). This complexity was designed to provide a visual refuge against predation, but did not provide interstitial space for use as a physical refuge. All three species were physically capable of accessing the entirety of this structure's surface.

The high complexity structure (Fig. 4.1B) had a surface area of 3.4 m^2 and protruded 40 cm from the bottom of the tank. This complexity was comprised of the same pipe units in the same configuration placed on a1 m^2 sand-bed as described above, however affixed vertically to each pipe unit were two capped 1.3 cm diameter PVC pipes. Additionally, two horizontal 10 cm diameter open PVC pipes were laid horizontally

across the structure. These pipes had two horizontally perpendicular PVC baffles extending from their sides. This complexity was designed to provide an enhanced visual refuge against predation of grass shrimp by white perch, but did not provide interstitial space for grass shrimp to use as a physical refuge from white perch. The two horizontal 10 cm diameter open PVC pipes excluded striped bass predators, and thus provided interstitial space for grass shrimp and white perch to utilize as a physical refuge against striped bass predation.

Experimental trials

Grass shrimp (n = 250) were added into each mesocosm 15 – 18 h before the addition of fish predators to allow them to acclimatize to the structure within each mesocosm without the threat of predation. Experimental trials were run for 34 h after the addition of 2 white perch and/or 2 striped bass into the mesocosm. The experimental photoperiod consisted of a 12 h light : 12 hr dark period. Digital photographs of the surface of the water were taken at just before 9 h, 23 h, 24 h, and 32 h time periods to determine the percentage of grass shrimp within the top 5 cm of the water column. These four times corresponded to the first day, night, morning, and the second day of the experiment, respectively.

Video of white perch and striped bass within each mesocosm was captured with a high resolution CCD camera (Pulnix TM-200NIR) suspended 2 m above each tank, which provided an overhead view of the activity within the mesocosm. Grass shrimp activity and location were not always clearly visible in the recordings. Video was taken in 1 h segments at 9 h, 23 h, 24 h, and 32 h after the start of the experiment. Each video segment represented the first day, night, morning, and the second day of the experiment,

respectively. A red lamp provided illumination for video recording during the night period without disturbing the organisms within the mesocosm.

At the end of the experimental trial a 1 m^3 wire cage covered with mesh fabric was placed over the structure to prevent grass shrimp from moving into and out of the zone of structural complexity. Fish predators were removed from the mesocosm, the water was drained, and the number of shrimp on and off the structure was enumerated.

Analysis

The percentage of shrimp attracted to the zone of structural complexity was calculated by dividing the number of shrimp found inside the mesh wire cage by the total number of remaining shrimp within the mesocosm at the end of each experimental run. To calculate the percentage of shrimp eaten by fish predators, the average number of shrimp missing from the no predator treatments was subtracted from the number of shrimp missing from each predator treatment and divided by the total number of shrimp released into the mesocosm at the beginning of the experimental run. The average number of shrimp missing when no predators were present was 2.1 individuals, indicating a 99% recovery rate. Percentages were arcsine transformed to normalize data and two-way Analysis of Variance (ANOVA) tests were performed to determine if structural and/or trophic complexity factors influenced grass shrimp utilization of, or predation on tested habitats. Post-hoc Least Significant Difference (LSD) multiple means comparisons tests were used to determine differences among treatments. Arcsine values were back-transformed to mean percentages (average ± SE) hence generating SE values that were not symmetrical about the mean.

Shrimp utilization of the surface of the water was estimated by determining the percentage of shrimp at the surface of the water during the four time periods (first day, night, morning, second day). The percentage of shrimp at the surface of the water during the first day period was calculated by dividing the number of shrimp at the surface of the water during the first day period by the total number of shrimp introduced into the mesocosm at the beginning of the experimental run. The percentage of shrimp at the surface of the water during both the night and morning periods were calculated by dividing number of shrimp at the surface of the water during these periods by the average between the number of shrimp introduced into the mesocosm at the beginning and the number of shrimp remaining at the end of the experimental trial. The percentage of shrimp at the surface of the water during the second day period was calculated by dividing the number of shrimp at the surface of the water during the second day period by the number of remaining shrimp at the end of the experimental trial. Percentages were arcsine transformed to normalize data and a two-way Repeated Measures ANOVA was performed to determine if level of structural and/or trophic complexity influenced grass shrimp utilization of the water's surface over the various time periods sampled. Post-hoc Least Significant Difference (LSD) multiple means comparisons tests were used to determine differences among treatments. Arcsine values were back-transformed to mean percentages (average ± SE).

The attraction of white perch and striped bass to the zone of structural complexity within each mesocosm was obtained by analysis of the video recording. The number of white perch and/or striped bass within the zone of structural complexity was noted every 30 sec, for a total of 121 observational points over the course of each hour. Observations

of white perch and striped bass were made separately from each other. Each observational point was given a score of 0, 0.5, or 1 depending on the usage of that zone by each fish predator species. For example, if no white perch were in the zone of structural complexity then that observational point was scored as zero. If only one white perch was present within the structural complexity zone, the observational point was scored as 0.5. If both white perch were present within the structural complexity zone, the observational point would be scored as 1. The same process was repeated for striped bass. The scores were totaled and then divided by the total number of observations within that hour to obtain a percentage of observed utilization of the structural complexity zone for each treatment and fish predator species. Percentages were arcsine transformed to normalize data and a two-way Repeated Measures ANOVA was performed to determine if level of structural and/or trophic complexity influenced the attraction of white perch or striped bass to structure over the various time periods sampled. Post-hoc Least Significant Difference (LSD) multiple means comparisons tests were used to determine differences among treatments. Arcsine values were back-transformed to mean percentages (average ± SE).

The movement of fish predators whilst on each zone of structural complexity was determined by video analysis. A fish was categorized as "in motion" if there was observable propulsive movement in any plane, while a fish was categorized as "stationary" if there was no observable propulsive movement. The movement of white perch and/or striped bass within the structural complexity zone was noted every 30 sec over the course of each hour. Determination of movement was made by observing a fish for 5 sec before and after each 30 sec observational point. Observations of white perch

and striped bass were made separately from each other. Analysis of fish behavior was done exactly as for the attraction data described in the previous paragraph.

The number of observations in which either white perch or striped bass were within one-half body length of its conspecific was noted every 30 sec for a total of 121 observations over the course of each sampled hour. The total number of observational points during which fish were together was divided by the total number of observational points to obtain a percentage. Percentages were arcsine transformed to normalize data and a two-way Repeated Measures ANOVA was performed to determine if level of structural and/or trophic complexity influenced the amount of time each fish species spends together with its conspecific over the various time periods sampled. Post-hoc Least Significant Difference (LSD) multiple means comparisons tests were used to determine differences among treatments. Arcsine values were back-transformed to mean percentages (average ± SE).

A Canonical Discriminant Analysis (CDA; *CANDISC* Procedure, SAS 9.1) was performed to more closely examine the relationships governing the attraction of these three species to each zone of structural complexity when all three species interacted with each other. All structural complexity treatments involving only the highest trophic complexity level (grass shrimp + white perch + striped bass) were examined. The variables used for CDA were the percentage of grass shrimp on the habitat structure at the end of the experimental trial, and percentage of time white perch and striped bass were observed on the structure within each habitat complexity treatment.

RESULTS

Grass shrimp

Grass shrimp attraction to the zone of structural complexity was influenced by an interaction among structural and trophic complexity levels (Two-way ANOVA; Table 4.1A). An increase in the level of structural complexity led to a significant increase in the percentage of grass shrimp attracted to the zone of structural complexity when neither fish predator was present within the mesocosm (Fig. 4.2A). The high complexity structure attracted significantly more grass shrimp (72%) than the structure within the medium complexity (36%) treatment (LSD, $t_{46} = 2.04$; $P = 0.047$), which itself attracted significantly more grass shrimp ($t_{46} = 2.18$; $P = 0.034$) than the flat sand treatment (23%). The same attraction to increased levels of structural complexity occurred when striped bass was the only fish predator present (Fig 2A). A significantly greater percentage of grass shrimp ($t_{46} = 4.97$; $P < 0.0001$) was attracted to the structure within the high complexity treatment (86%) than the structure within the medium complexity treatment (33%). The structure within the medium complexity treatment attracted more shrimp than the flat sand treatment (86%), and this attraction was also significant ($t_{46} = 3.90$; $P = 0.0003$).

The attraction of grass shrimp to structure was influenced by an interaction between structural and trophic complexity levels when white perch was the only fish predator present, and when both white perch and striped bass were present (Fig. 4.2A). When white perch was the only fish predator present, there was no difference ($t_{46} = 0.83$; $P = 0.41$) in the attraction of grass shrimp to the structure within the medium complexity treatment (44%) and the structural complexity zone within the flat sand treatment (34%).

There was, however, a significant difference in the attraction of grass shrimp to the zone of structural complexity within the flat sand treatment and the structure within the high complexity (72%) treatment (t_{46} =2.19; P = 0.034), as well as between the structures located within the medium and high complexity treatments (t_{46} =2.48; P = 0.017). When both fish predators were present there was also no difference in the attraction of grass shrimp (t_{46} = 0.34; P = 0.73) between the flat sand (12%) and medium (9%) complexity treatments. Although, there was a significant difference (t_{46} = 5.29; P < 0.0001) between the flat sand and high (72%) complexity treatments, and between the structures within the medium and high complexity treatments (t_{46} = 5.61; P < 0.0001).

The attraction of grass shrimp to the zone of structural complexity within the flat sand treatment differed across trophic complexity levels (Fig 2A). Significantly more grass shrimp were attracted to the zone of structural complexity when white perch was the only fish predator species than when there were no fish predators were present (t_{46} = 2.05; P = 0.047), when striped bass were the only fish predator present (t_{46} = 4.07; P = 0.0002), and when both white perch and striped bass were present (t_{46} = 2.31; P = 0.026). The attraction of grass shrimp to the structure within the medium complexity treatment also differed across trophic complexity levels (Fig. 4.2A). The presence of both white perch and striped bass significantly decreased grass shrimp attraction to this structure compared to when there were no fish predators present (t_{46} = 2.80; P = 0.008), when only white perch predators were present (t_{46} = 3.48; P = 0.001), and when there was only striped bass predators present (t_{46} = 2.48; P = 0.02). On the structure within the high complexity treatment the presence of fish predators tended to increase the attraction of grass shrimp to this structure (Fig. 4.2A), although, the only significant difference

occurred when no fish predator were present and when both fish predators were present ($t_{46} = 2.61$; P = 0.012).

The percentage of grass shrimp found in the top 5 cm of the water column was influenced by an interaction among structural and trophic complexity levels (Two-way Repeated Measures ANOVA; Table 4.1B). There was no time of day interaction (Table 4.1B, 4.2A). Increased levels of structural complexity generally decreased the percentage of grass shrimp attracted to the water's surface (Fig. 4.2B), except when there were no fish predators were present and grass shrimp attraction to the water's surface was low (0.2% – 1.8%) regardless of the level of structural complexity. Another exception occurred when both fish predators were present and there was no difference in the attraction of grass shrimp to the water's surface between the medium (18%) and high (17%) complexity treatments (LSD, $t_{48} = 0.14$; P = 0.9).

The percentage of grass shrimp eaten by fish predators was influenced by an interaction among structural and trophic complexity levels (Two-way ANOVA, $F_{2,36} = 2.65$; P = 0.049). When only white perch were present there was no significant difference in predation on grass shrimp regardless of the level of structural complexity (Fig. 4.3). When only striped bass were present, the greatest percentage of predation occurred on the medium complexity treatment (42%) which was higher than the percentage of shrimp eaten on the flat sand treatment (29%). This difference, however, was not significant ($t_{36} =1.54$; P = 0.13). Predation of grass shrimp on the high complexity treatment (16%) was significantly less than the medium complexity treatment ($t_{36} = 3.37$; P = 0.002). When both white perch and striped bass were present, predation on grass shrimp decreased as the level of structural complexity increased (Fig. 4.3).

White Perch

Increased levels of structural complexity significantly increased the occurrence of white perch within the zone of structural complexity regardless of level of trophic complexity (Table 4.1C, Fig. 4.4A). The high complexity structure had a significantly greater occurrence of white perch than the medium complexity structure ($t_{40} = 2.37$; $P = 0.02$), which was significantly greater than the occurrence on the flat sand treatment ($t_{40} = 5.67$; $P < 0.0001$). There was no interaction among structural complexity levels, trophic complexity levels, and time of day (Two-way Repeated Measures ANOVA; Table 4.1C).

Increased trophic complexity increased the occurrence of white perch within the zone of structural complexity regardless of the level of structural complexity (Table 4.1C, Fig. 4.4B). The presence of grass shrimp increased the occurrence of white perch within the zone of structural complexity compared to when no grass shrimp were present, however this difference was not significant ($t_{40} = 1.79$; $P = 0.08$). The presence of both grass shrimp and striped bass further increased the occurrence of white perch within the zone of structural complexity; however this difference was also not significant ($t_{40} = 1.47$; $P = 0.15$). There was a significant difference in white perch occurrence within the zone of structural complexity when white perch were alone compared to when they were in the presence of grass shrimp and striped bass ($t_{40} = 1.47$; $P = 0.015$). There was no affect of time on the occurrence of white perch within the zone of structural complexity (Table 4.1C, 4.2B).

The movement of white perch within the zone of structural complexity was not influenced by an interaction among structural complexity levels, trophic complexity levels, and time of day (Two-way Repeated Measures ANOVA; Table 4.3A). Increased

levels of structural complexity significantly decreased the movement of white perch regardless of trophic complexity (Fig. 4.5). White perch were observed to be in near constant motion on the flat sand treatment (99%), which was significantly greater than its motion on the medium complexity structure (57%) ($t_{40} = 7.68$; $P < 0.0001$). White perch barely moved on the high complexity structure (12%), which was significantly less movement than on the medium complexity structure ($t_{40} = 6.18$; $P < 0.0001$). Both level of trophic complexity and time of day did not affect the motion of white perch within the zone of structural complexity (Table 4.3A).

The occurrence of white perch within one-half body length of each other was influenced by an interaction among structural and trophic complexity levels (Two-way Repeated Measures ANOVA; Table 4.3B). This behavioral interaction occurred on the medium complexity treatment where white perch were observed in close proximity to each other when both grass shrimp and striped bass were present (91%). This finding was significant compared to when neither grass shrimp nor striped bass were present ($t_{36} = 3.33$; $P = 0.002$) and when only grass shrimp were present ($t_{36} = 3.85$; $P = 0.001$). Time of day did not affect the occurrence of white perch found together (Table 4.3B).

Striped bass

The level of structural complexity influenced the attraction of striped bass to structure (Fig. 4.6A). Striped bass occurred on the medium complexity structure (10%) significantly less often than on the flat sand (25%) treatment ($t_{26} = 2.25$; $P = 0.03$) and high complexity (28%) structure ($t_{26} = 2.68$; $P = 0.01$). Time of day also affected the occurrence of striped bass within the zone of structural complexity (Table 4.1D, 4.2C). Striped bass were less attracted to structural complexity levels at night than during either

of the daytime periods (Day 1 - $t_{83} = 2.03$; P = 0.045 / Day 2 - $t_{83} = 3.15$; P = 0.002). The level of trophic complexity did not influence the occurrence of striped bass within the zone of structural complexity (Table 4.1D). There was no interaction among structural complexity levels, trophic complexity levels, and time of day (Two-way Repeated Measures ANOVA; Table 4.1D).

Striped bass were in near constant motion while on the flat sand treatment (99%) and medium complexity (99%) structures (Fig. 4.6B). On the high complexity structure striped bass were in motion for a significantly less period of time (65%) than the flat sand treatment ($t_{26} = 4.55$; P = 0.0001) and medium complexity ($t_{26} = 4.35$; P = 0.0002) structures. The level of trophic complexity and time of day did not influence the movement of striped bass while on the zone of structural complexity (Table 4.3C). There was also no interaction among structural complexity levels, trophic complexity levels, and time of day on the movement of striped bass within the zone of structural complexity (Table 4.3C). Striped bass were generally found greater than one-half body length from each other within this experiment regardless of structural complexity levels, trophic complexity levels, or time of day (Table 4.3D).

Grass shrimp, white perch, and striped bass

At the highest level of trophic complexity there was enough data to determine the most important factors affecting the attraction of each organism to the various levels of structural complexity using CDA analysis. Canonical coefficients showed heavy loading on the attraction of grass shrimp to level of structural complexity as the factor that explained 79% of the total variance. This analysis indicates that there was little attraction of grass shrimp to either the flat sand and medium complexity treatment, and increased

attraction of grass shrimp to the high complexity structure when both fish predator species were present (Fig. 4.7). The second canonical structure explained the remaining 21% of the total variance and incorporated the attraction of white perch that was the only species attracted to the various levels of structural complexity. This analysis indicates that both grass shrimp and white perch avoided the flat sand treatment in the presence of striped bass, and that they were attracted to the high complexity structure when subjected to the same conditions. This structure also indicates that white perch alone were attracted to the medium complexity treatment when the three species were present within the same trophic complexity level (Fig. 4.7).

DISCUSSION

Results from this study indicate that increasingly complex physical structures have the capacity to attract organisms and influence their behavior regardless of the proximate provision of food resources or the threat from predation. Structures of low physical complexity may lack both an adequate visual and physical refuge for prey species against the threat from predation. This potentially reduces the attraction of fauna to structure while altering their behavior such that they become more aware of potential predation threats. A decrease in the attraction of prey species to structure may be especially detrimental to intermediate predator species that depend on habitat for foraging opportunities as well as refuge.

Attraction of grass shrimp to structure

Increased levels of structural complexity generally amplified the attraction of grass shrimp and white perch to structured habitat, while there was little enhancement of attraction for striped bass. Grass shrimp were significantly attracted to increased levels of

structural complexity even in the absence of predation or being supplied with food resources. An increase in surface area alone was sufficient to increase grass shrimp utilization of structurally complex habitat provided in these structures when no predators were present. This affinity to more structurally complex habitat is likely due to innate behavioral preferences in which greater levels of structural complexity generally provide greater refuge or foraging potential for grass shrimp; even when no predation threat or food resources actually exist. Grass shrimp are regularly associated with structurally complex habitats, such as seagrass beds, oyster reefs, and coarse woody debris (Welsh 1975, Posey et al. 1999, Clark et al. 2003), while their presence on unstructured habitats such as sand or structurally simple habitats such as moribund oyster reefs is low (Rodney & Paynter 2006), even when there is no apparent predation threat.

Infochemicals associated with predatory species in aquatic environments have been shown to trigger protective behavioral responses by prey organisms (Dicke & Grostal 2001). I used filtered ambient water from the Choptank River within this study. Therefore it is possible that grass shrimp were subjected to predator chemical cues that influenced their behavior, even within the no predator treatments. However, if present, chemicals in ambient river water did not mask responses to predators within treatments; there were strong behavioral responses to predators present in experiments. For example, shrimp moved towards the surface of the water in the presence of predators, especially at low levels of habitat complexity. This finding indicates that the responses observed for grass shrimp and white perch were due to planned experimental predatory conditions within the mesocosms.

White perch has little effect on the attraction of grass shrimp to structure. However, white perch presence increased grass shrimp utilization of near-surface water in low and medium complexity treatments. This difference may indicate that grass shrimp had exceeded the carrying capacity of the medium complexity structure regardless of the presence of white perch, which forced the remaining portion of the population to seek habitat elsewhere within the mesocosm because of interspecific competition for available refuge space on the structure (Holt 1987, Chapter 5). It is also possible that the presence of white perch upon the medium complexity structure may have prevented a portion of the grass shrimp population from seeking refuge there. However, if this were the case, I would not expect to see a similar density of grass shrimp attracted to this structure in both the presence and absence of white perch. The lack of grass shrimp at the surface of the water within the high complexity treatment when white perch were present indicates that this level of structural complexity provided enough refuge potential for grass shrimp; even though there was strong attraction of white perch to this structure, and no interstitial space for grass shrimp to use for protection.

The percentage of grass shrimp utilizing the flat sand treatment when white perch were present was significantly greater than the percentage of shrimp utilizing the flat sand treatment when no fish predators were present. This variation was possibly due to complex interactions between the behavior of white perch and grass shrimp. White perch were observed swimming around the perimeter of the mesocosm within the flat sand treatment, while mostly staying away from sand zone. Grass shrimp utilized this sand zone as a spatial refuge from the circling white perch. Interestingly, white perch did not venture onto this sand zone even though there was a high percentage of grass shrimp

attracted to it. The presence of an aggregated food resource was not enough to draw white perch to the unstructured area without the presence of structurally complex habitat. That the white perch were continuously swimming around the periphery of the tank indicates that these fish were not utilizing every bit of the tank equally. I interpreted this repetitive behavior as white perch searching for a patch of structural complexity, and never finding it. An alternative interpretation may be that white perch may have been attracted to the tank wall as structure within this treatment; however the decreased swimming behavior of white perch on structure observed in higher complexity treatments makes this senario less likely, as the tank wall did not reduce their swimming behavior in the flat sand treatment. In either case, this finding lends credence to white perch being more attracted to structure (i.e., habitat/tank walls) than the presence of prey in the middle of an unstructured tank.

The presence of striped bass significantly influenced the attraction of grass shrimp to each level of structural complexity. The movement of striped bass on the flat sand treatment was more haphazard than that of white perch, with striped bass regularly crossing the zone of structural complexity, which forced grass shrimp into the surface of the water. Grass shrimp were attracted to the medium complexity treatment similarly to the no fish predator and white perch treatments even though predation rates were much greater, which further lends credence to the possibility that this medium complexity structure may have been close to its carrying capacity for grass shrimp. The high complexity structure attracted the highest proportion of the grass shrimp among treatments. The potential refuge value of this structure for grass shrimp was not diminished by striped bass swimming through and exhibiting ambush behavior upon this structure. This finding seems to contrast with Davis et al. (2003) who found that grass

shrimp did not significantly utilize complex structure in the presence of striped bass. They report that grass shrimp used shallower water depths as a refuge against striped bass predation, which may indicate that their experimental levels of complexity did not afford grass shrimp with enough perceived refuge. Their findings were consistent with my findings in that when striped bass were present, grass shrimp within the medium complexity treatment chose to stay within the structure or the surface of the water in similar densities, possibly indicating that this structure level was not complex enough to be perceived as refuge by a majority of the shrimp population. In this study once the level of structural complexity was increased, grass shrimp significantly utilized the high complexity structure in the presence of striped bass.

When both white perch and striped bass were present with grass shrimp there was a behavioral interaction between these two fish predators that decreased grass shrimp utilization of the medium complexity structure. The constant motion of white perch evading striped bass on this structure most likely decreased the refuge potential for grass shrimp, forcing them to seek elsewhere within the mesocosm. Davis et al. (2003) found that in the presence of two fish predators, mummichogs and striped bass, grass shrimp were found higher in the water column away from structure, which more closely approximated the distribution in my studies when only striped bass were present. The distribution of grass shrimp under a dual predation threat within the medium complexity treatment of this study did not mimic either of the two individual predator treatments. In this study, grass shrimp chose neither the structure nor the surface of the water and instead were found throughout the tank away from the zone of structural complexity. The reason why so few grass shrimp chose the surface of the water as a refuge within this

trophic complexity level is unclear, as both fish predators did not seem to utilize the surface water any differently from the other treatments.

Attraction of fish to structure

The attraction of white perch to structure was highly dependent on the level of structural complexity, regardless of the presence of prey or predator species. White perch rarely entered the flat sand zone during this study, even when a large percentage of grass shrimp were aggregated there, while white perch utilized the medium and high complexity structures fairly frequently even in the absence of grass shrimp. White perch travel between brackish and freshwater habitats (Mansuetti 1961, Kraus & Secor 2004). However their utilization of structured habitat within those environments is not entirely understood. There have been several studies that have described white perch habitat preference to be open areas covered in sand, mud, or clay (Stanley & Danie 1983; Setzler-Hamilton 1991), although Setzler-Hamilton (1991) noted that white perch were common around structures and were even attracted to bubble curtains used to try and prevent fish incursion into power plant intake channels. Other, more recent studies have identified white perch as being a species that is more closely associated with complex structures such as oyster reefs, marsh grasses, and dock pilings (Peterson et al. 2003, McGrath & Austin 2009). These results also indicate that white perch are likely more closely connected to complex habitat than to open muddy or sandy bottoms.

The role that habitat complexity has in attracting transient intermediate predatory fish species is currently debated for all systems that afford some type of structural complexity such as oyster reefs, mangroves, coral reefs, and man-made structures such as piers and artificial reefs (Alevizon & Gorham 1989, Bohnsack 1989, Harding & Mann

2001). Many of these transient predatory fish are opportunistic and will feed wherever there is high habitat productivity. In a study investigating striped bass use of oyster reefs within Chesapeake Bay, however, Harding and Mann (2003) concluded that striped bass presence was positively correlated to the structural complexity afforded by oyster reefs. Studies on oyster reefs have shown that increased habitat complexity results in more abundant and diverse fish and benthic communities (Luckenbach et al. 2005, Rodney & Paynter 2006). Manipulative studies by Savino and Stein (1982) and Turner and Middlebach (1990) indicate that one of the main factors in attracting bluegill (*Lepomis macrochirus*) to habitat is the level of habitat complexity encountered. A study looking at the effects of habitat selection, food supply, and predation on pinfish (*Lagodon rhomboides*) recruitment in estuarine areas found that habitat complexity, and not predation, is the primary factor in determining fish distribution around aquatic habitat (Levin et al. 1997).

The level of trophic complexity also influenced the attraction of white perch to the zone of structural complexity across all habitat levels within my study. This result was are primarily influenced by the occurrence of white perch on the medium and high complexity structures, as they spent very little time within the flat sand zone (5%). White perch were least attracted to structure when there were no prey or predators present. Without the provision of food resources or the threat from predation white perch would have little reason for staying on a structure. On a potential food patch an organism will maximize its net energy intake over time before moving on to another patch (Stephens & Krebs 1986), and I may have seen a reflection of this within my study. When no grass shrimp were present I observed white perch either actively or passively searching for

grass shrimp over the structure, and when none were found white perch would leave the structure and swim around the perimeter of the tank several times before returning to the zone of structural complexity to repeat the search process. This was observed for all structural complexity levels. I interpret this behavior as an analogue to white perch moving between patches in the field foraging for food.

White perch attraction to the zone of structural complexity was enhanced with the presence of prey. Grass shrimp were present in high numbers on both medium and high complexity treatments when white perch were present, allowing for greater foraging opportunities for white perch and the possibility of a net energy gain by swimming slowly over the structure or adopting an ambush predatory behavior. This contrasts with the findings from the no prey treatments in which white perch likely simulated movement between structural complexity patches.

White perch attraction to structure was further enhanced by the presence of predatory striped bass. I did not have a trophic complexity level containing only the two fish predator species without prey, so it is difficult to differentiate between the effects of grass shrimp and striped bass on white perch utilization of structure. However, I can infer from these results that the presence of a predator likely had a greater influence than food availability on the attraction of white perch to complex structure due to this species' utilization of the medium complexity treatment when both grass shrimp and striped bass were present. As stated previously, there were very few grass shrimp present on the medium complexity treatment when both white perch and striped bass were present. While there were few grass shrimp upon this complexity (9%), the occurrence of white perch averaged approximately 85%, which indicates the white perch offset the need to

forage with the more immediate need of seeking refuge. This is difficult to discern from treatments conducted on the high complexity structure because of the high attraction of both grass shrimp and white perch regardless of predatory threat.

The provision of food resources and the need to seek refuge have been shown to be important factors that attract aquatic organisms to structure in other systems. In structurally complex mangrove systems, utilization of complex habitat by grunts (*Haemulon* sp.) and snappers (*Ocyurus chrysurus*) were more closely related to refuge than to food availability (Cocheret de la Morinière et al. 2004). Another study found that herbivore and zoobenthivore fish species were most likely attracted to complex mangrove systems for food, while piscivorous fish species were attracted to structure for rest, as well as to conduct ambush predation (Verweij et al. 2006). Hammond et al. (2007) examined the spatial distribution of dragonfly predators and tadpole prey in respect to the provision of food resources and predator avoidance behavior in the laboratory. They concluded that the avoidance of predators had a greater influence on the spatial distribution of tadpoles, than the influence of tadpoles had in affecting the spatial distribution of predatory dragonflies. In addition, the provision of food resources did not greatly affect prey distribution when predators were present.

The attraction of striped bass to structure within the mesocosms was low (< 30%). The striped bass used within this experiment may not have recognized the foraging potential of the medium and high complexity structures because of their relatively large body size (38 – 43 cm) compared to each structure on the 1 m^2 area tested, despite the high percentage of grass shrimp and utilization of white perch on each structure. While the overall utilization of structure by striped bass was low, it was lowest on the medium

complexity treatment, and differed significantly from the other treatments. The reasons for this seeming avoidance of the medium complexity structure are unclear. When only grass shrimp were present, they aggregated sufficiently on the medium complexity reef to seemingly be able to be a potential food source for striped bass. Grass shrimp densities were low on the medium complexity structure when both striped bass and white perch were present. However the presence of white perch on the structure should have also been sufficient to attract striped bass. It is unlikely that striped bass were more attracted to grass shrimp found outside than inside the zone of structural complexity. If this were the case, then I would expect that striped bass occurrence on the zone of structural complexity within the flat sand treatment to be similar to their occurrence on the medium complexity structure, because of enhanced grass shrimp utilization of the surface of the water within each of these complexity treatments, which it is not. It is possible that striped bass may have been attracted to the area around the medium complexity structure more than the structure itself. The occurrence of striped bass around this structure was 42% compared to 32% around the high complexity structure, although why striped bass would not venture onto the medium complexity structure itself is not clear, especially considering that there was little in the way of visual or physical barriers.

Striped bass spent less time on the zone of structural complexity during hours of darkness than when it was light. In the field, grass shrimp have been shown to move away from structure or into deeper waters at night because of the protection afforded by darkness (Clark et al. 2003). I did not observe any grass shrimp migration off the "reef" within this experiment despite the dark period within this experiment. Striped bass may have avoided the zone of structural complexity at night within this study because of an

innate behavior that takes advantage of prey migrating off structurally complex areas at night due to the relative safety from visual predators during hours of darkness.

Predation on structurally complex habitat

The value of habitat complexity is not only how well it attracts organisms, but also how successfully that refuge protects those organisms from predation. While more grass shrimp were found on the high complexity structure in the presence of white perch, there was no statistical difference in grass shrimp mortality among all structural complexity treatment levels. The similarity in predation rates between the flat sand and high complexity treatments is likely due to a shift in predatory behavior by white perch from an active search behavior to an ambush predatory behavior, which I interpreted from the behavioral observations made during video analysis

Grass shrimp were significantly attracted to increasing levels of structural complexity when only striped bass were present; this fact, however, did not always lead to increased refuge for grass shrimp. The highest rates of predation occurred on the medium complexity treatment, which attracted grass shrimp but provided no interstitial space for refuge. Within this treatment grass shrimp were observed moving between the structure and the surface of the water, which may indicate that neither area provided adequate refuge from predation. This movement may have also facilitated predation on grass shrimp by striped bass because they were more easily encountered and captured when they were traveling through the water column.

Crowder and Cooper (1982) found that bluegill inhabiting intermediate complexity habitats had better growth rates and higher predation rates than bluegill inhabiting low or high complexity structures. They postulated that predator feeding rates

are maximized in intermediate structures because of the adequate abundance and diversity of prey and easier access to them because of reduced structural complexity. It is important to recognize, however, that the term "Habitat Complexity" is relative in terms of faunal community density, individual body size, as well as the structural make-up of the habitat itself. A habitat considered "intermediate" by qualitative means within the context of a field study may actually function ecologically as a highly complex habitat. This makes the comparison of field studies and mesocosm studies difficult because they are using fundamentally different measures of habitat complexity.

When both white perch and striped bass were present there was a decrease in the predation on grass shrimp with increased levels of structural complexity. Both grass shrimp and white perch utilized the surface of the water as a refuge against predation in the flat sand treatment. Striped bass were observed chasing white perch within this treatment and successfully capturing one white perch in two of the five experimental runs. The preoccupation of striped bass with white perch at the surface of the water may also have inadvertently made grass shrimp at the surface of the water more susceptible to predation compared to when only striped bass were present. This is because striped bass were qualitatively observed entering the top layer of the water column more frequently to encounter white perch, which also put them in closer proximity to encounter and successfully capture grass shrimp. Grass shrimp predation on the medium complexity reef was lower than that of the flat sand treatment, but the reasons for this are unclear. As stated before, grass shrimp were not found in high abundance on the medium complexity structure because of the presence and behavior of the white perch, but instead used the water column and sides of the mesocosm as a refuge against predation. It is possible that

the increased usage of the mesocosm sides decreased the encounter rate between grass shrimp and striped bass, which lead to a reduction in shrimp mortality.

Shrimp mortality on the high complexity structure when both predators were present was almost identical to when only striped bass was present. This indicates that there was no additive effect of both fish predators on grass shrimp predation on the high complexity structure; although I can not differentiate predation rates individually for each fish predator species. Other studies have also reported interactions between prey, intermediate predator, and predator species under varying levels of habitat complexity.

Persson and Eklöv (1995) examined how levels of habitat complexity in experimental ponds influenced predator–prey relationships of piscivorous adult perch (*Perca fluviatilis*) and juvenile perch and roach (*Rutilus rutilus*). They found that the amount of juvenile perch and roach in the diet of adult perch decreased with partial refuge and was absent from their diets in the complete refuge treatment. The presence of predators resulted in a shift in the diet of juvenile perch that were feeding primarily on zooplankton in the absence of predators to feed primarily on macroinvertebrates in the presence of predators, which then altered the composition of the zooplankton community to larger sized organisms (Persson and Eklöv 1995).

Behavior of fish on structure

High levels of structural complexity decreased swimming activity of both white perch and striped bass. The swimming activity of white perch decreased as the level of structural complexity increased, regardless of the presence of prey or predators. This finding indicates that the presence of structurally complex habitat alone can alter the behavior of an intermediate fish predator, this fact, however, may be due to innate

behavioral preferences. From a foraging perspective, an active search pattern on structurally simple or intermediate complexity structure may increase the chance of encountering and capturing prey because these structures may have a lower abundance and diversity of prey items. While on high complexity structure it may be more energetically feasible to adapt an ambush predatory style due to a greater chance of prey accidentally crossing into the path of a waiting predator than trying to actively search through visual and physical barriers. From a refuge perspective, remaining motionless on an unstructured area may make a fish be more vulnerable to predation itself; if that is not part of its usual cryptic predatory avoidance behavior.

Only the highest complexity treatment decreased striped bass swimming activity. White perch and grass shrimp were easily observable within the water column and surface in both the flat sand and medium complexity treatments, so an active search pattern would likely result in maximum foraging efficiency. A high density of grass shrimp, and white perch were present on the high complexity structure with few individuals of either species observed off the structure. Striped bass likely employed some ambush behavior because their search was hampered visually by the structure and physically by the interstitial space within this complexity treatment (Savino & Stein 1982, Savino & Stein 1989). A decrease in swimming activity on high complexity structure may also be due to a decrease in a predator's visual field which corresponds to a decrease in their territorial area (Basquill & Grant 1998, Breau & Grant 2002).

I observed evidence of shoaling activity by white perch within this study. Because only two fish were used for each experimental run it is difficult to define this activity as shoaling per se because that term usually indicates a larger aggregation of

individuals. I attempted, however, to quantify and describe this behavior by recording how often the white perch occurred together within one-half body lengths of each other (Fig. 4.6). An increase in the level of structural complexity generally decreased the amount of time white perch spent together, except in the medium complexity treatment when striped bass were present. Within this treatment white perch occurred together 91% of the observed time, compared to 36% when neither prey nor predators were present and 26% when only prey were present. White perch clearly did not find medium complexity structure adequate as a refuge against potential predation by striped bass, and attempted to decrease potential predation by remaining close to each other. Within the flat sand treatments white perch were observed occurring together when no predators were present likely to increase foraging efficiency within this low complexity habitat. The relatively easy access of grass shrimp on the medium complexity structure may have been complex enough to limit shoaling activity in the absence of striped bass, but not complex enough to decrease shoaling behavior when striped bass were present. A decrease in shoaling activity of white perch on the high complexity structure, regardless of prey or predator presence, was likely due to enough refuge to safely pursue ambush behavior.

Other studies have also observed a decrease in shoaling activity with an increase in structural complexity of the habitat. Orpwood et al. (2008) found that minnows (*Phoxinus phoxinus*) increased shoaling activity in the presence of predatory pike (*Esox lucius*), however this behavior was diminished by the addition of complex habitat. Butler (1988) found that bluegill shoaling activity decreased in dense vegetation, while Eklöv (1997) reported that group size for perch decreased with increasing vegetation density. Shoaling activity may also increase individual foraging rates by individuals transferring

informational cues to each other when prey populations are patchy or scarce, as generally seen in low complexity habitats (Clark & Mangel 1986).

Conclusion

Increased structural complexity of a habitat generally increases the attraction and refuge potential of that habitat for associated fauna, while also influencing their behavior upon that habitat. Studies have suggested that intermediate complexities may be the most beneficial type of habitat for associated fauna because of refuge availability for prey and enhanced foraging opportunities for predators (Crowder & Cooper 1982, Grenouillet et al. 2002). This study indicates that this may not be the case when there is more than one trophic level present. The presence of three species of differing trophic levels within the medium level of structural complexity resulted in some very interesting interactions among them. White perch apparently were attracted to this structure because of the predation threat posed by striped bass. This medium level of structural complexity did not afford white perch adequate visual or physical protection from striped bass so they remained in motion, close to each other to enhance their individual safety. Increased movement of white perch and their possible shoaling behavior may also increase their foraging success on low complexity structures. Therefore, this behavior likely forced grass shrimp off the structure and into the water column. When striped bass were present within the water column and preoccupied by the presence of white perch, the grass shrimp were attracted to the sides of the mesocosm away from the structure. White perch were then left expending energy on a structure in which they had no opportunity to forage successfully, which may lead to a decrease in their condition over time.

These findings correspond somewhat to the risk reduction strategies put forth by Schmitz (2007), in which species at low trophic levels may escape predation by predators of higher trophic levels by intraguild predation among predators. Both of the predator species in this study have similar hunting modes and habitat preferences that overlap broadly with the grass shrimp prey species. Within the medium complexity treatment grass shrimp sought out a spatial refuge on the sides of the mesocosm walls because of the presence of white perch on the structure and striped bass in the water column, which may have led to a intraguild predation strategy that states in the absence of other prey, predators will hunt each other (Schmitz 2007). However, there was little evidence of this in the high complexity structure where a decrease in predation occurred more likely due to an increase in habitat complexity than any interaction between predators.

The hypothesis that intermediate complexities are more beneficial than high complexity structures for predators and prey are also being questioned by other studies. The density and diversity of prey on structurally complex habitats can be an order of magnitude higher than on structurally simple habitats (Bohnsack et al. 1994, Nagelkerken & van der Velde 2002, Rodney & Paynter 2006), and this enhanced prey density may result in greater foraging opportunities for predators even under high levels of structural complexity (Forrester & Steele 2004, Johnson 2006, Mattila et al. 2008, Canion & Heck 2009, Chapter 5). Studies in mesocosms generally investigate how individual aspects of habitat complexity influence species interactions with that habitat, while field studies typically investigate more complex interactions involving numerous trophic levels in an uncontrolled system. Greater attention is needed as to how these two types of studies may be better aligned. Field studies should start by quantifying the complexity of a particular

habitat, and the body size and density of its inhabitants, in relation to other habitats of a similar type to determine its complexity value. The value of mesocosm experiments is to consistently test which parameters are important in shaping predator– prey interactions on complex habitat or a variety of species, which can then be used as a metric to quantify habitat complexity in the field.

CHAPTER 4:

Tables

Table 4.1: Two-way ANOVA and two-way Repeated Measures ANOVA table for organism attraction to structure

Significance tables for 2-way ANOVA (a) and 2-way Repeated Measures ANOVA (b,c,d) analysis using the *MIXED Procedure* in SAS 9.1. Interactions between main effects of trophic complexity, structural complexity, and time which affect habitat utilization for shrimp (a,b), white perch (c), and striped bass (d) were examined. y = significant at $P < 0.05$; DF = degrees of freedom.

Effect	Treatment	Num DF	Den DF	F value	Pr > F	Significant?
a) % Shrimp on structure	Trophic complexity	3	46	3.81	0.02	y
	Structural complexity	3	46	60.82	< 0.0001	y
	Structural complexity × trophic complexity	6	46	4.24	0.002	y
b) % Shrimp at water surface	Trophic complexity	2	48	21.24	< 0.0001	y
	Structural complexity	2	48	21.72	< 0.0001	y
	Time	3	175	1.28	0.28	
	Structural complexity × trophic complexity × time	18	142	1.19	0.28	
	Trophic complexity × time	9	160	1.10	0.36	
	Structural complexity × time	6	169	1.64	0.14	
	Structural complexity × trophic complexity	6	48	3.02	0.01	y
c) % Occurrence of white perch on structure	Trophic complexity	2	40	5.31	0.01	y
	Structural complexity	2	40	34.15	< 0.0001	y
	Time	3	123	0.74	0.53	
	Structural complexity × trophic complexity × time	12	99	0.82	0.63	
	Trophic complexity × time	6	111	0.51	0.80	
	Structural complexity × time	6	117	0.85	0.53	
	Structural complexity × trophic complexity	4	36	0.83	0.52	
d) % Occurrence of striped bass on structure	Trophic complexity	1	26	1.63	0.21	
	Structural complexity	2	26	4.14	0.03	y
	Time	3	83	3.52	0.01	y
	Structural complexity × trophic complexity × time	6	68	1.16	0.34	
	Trophic complexity × time	3	74	1.62	0.19	
	Structural complexity × time	6	77	1.83	0.10	
	Structural complexity × trophic complexity	2	24	0.62	0.55	

Table 4.2: Shrimp usage of water surface, and fish attraction to structure by time period

The mean percentage (± SE) of grass shrimp at the water surface, and the mean percentage (± SE) of observations of white perch and striped bass on structure at each time period. Mean percentages were pooled across structural and trophic complexity factors. Different letters indicate a significant difference in back-transformed percentages within species (LSD pairwise comparisons; $P \leq 0.05$). Not significant = –.

Species	Time	Mean (%)	+ SE(%)	− SE(%)	P ≤ 0.05
a) Grass shrimp	Day 1	11	1.6	1.5	–
	Night	11	1.6	1.5	–
	Morning	11	2	1.9	–
	Day 2	13	1.9	1.8	–
b) White perch	Day 1	52	5.9	5.9	–
	Night	51.2	5.9	5.9	–
	Morning	54.5	5.9	5.9	–
	Day 2	57.2	5.8	5.9	–
c) Striped bass	Day 1	21.4	4.4	4	a
	Night	16.2	2.9	2.7	b
	Morning	19.8	3.4	3.2	ab
	Day 2	24.6	4.6	4.3	a

Table 4.3: Two-way Repeated Measures ANOVA for fish behavior on structure

Significance tables for 2-way Repeated Measures ANOVA analysis using the *Mixed Procedure* in SAS 9.1. Interactions between main effects for white perch (a,b) and striped bass (c,d) were examined. y = significant at $P < 0.05$; DF = degrees of freedom.

Effect	Treatment	Num DF	Den DF	F value	Pr > F	Significant?
a) % Occurrence of white perch moving on structure	Trophic complexity	2	40	0.80	0.46	
	Structural complexity	2	40	97.89	< 0.0001	y
	Time	3	123	0.69	0.56	
	Structural complexity × trophic complexity × time	12	99	1.06	0.40	
	Trophic complexity × time	6	111	0.33	0.92	
	Structural complexity × time	6	117	1.80	0.11	
	Structural complexity × trophic complexity	4	36	1.89	0.13	
b) % Occurrence of white perch together within the mesocom	Trophic complexity	2	36	2.35	0.11	
	Structural complexity	2	36	25.32	< 0.0001	y
	Time	3	118	0.92	0.43	
	Structural complexity × trophic complexity × time	12	94	1.38	0.19	
	Trophic complexity × time	6	106	0.57	0.75	
	Structural complexity × time	6	112	0.16	0.99	
	Structural complexity × trophic complexity	4	36	3.23	0.02	y
c) % Occurrence of striped bass moving on structure	Trophic complexity	1	26	0.07	0.79	
	Structural complexity	2	26	13.18	< 0.0001	y
	Time	3	81	0.74	0.53	
	Structural complexity × trophic complexity × time	6	66	1.22	0.31	
	Trophic complexity × time	3	72	0.32	0.81	
	Structural complexity × time	3	75	0.89	0.45	
	Structural complexity × trophic complexity	2	24	0.06	0.94	
d) % Occurrence of striped bass together within the mesocosm	Trophic complexity	1	26	0.00	0.97	
	Structural complexity	2	26	2.02	0.15	
	Time	3	83	1.72	0.17	
	Structural complexity × trophic complexity × time	6	68	0.67	0.67	
	Trophic complexity × time	3	74	1.18	0.32	
	Structural complexity × time	6	77	1.25	0.29	
	Structural complexity × trophic complexity	2	24	1.81	0.18	

CHAPTER 4:

Figures

Figure 4.1: Structurally complex PVC pipe habitats

Top view of constructed PVC pipe (A) medium complexity and (B) high complexity structures. Oval indicates an example of the PVC pipe baffles which extended horizontally perpendicular from the base (see Methods section)

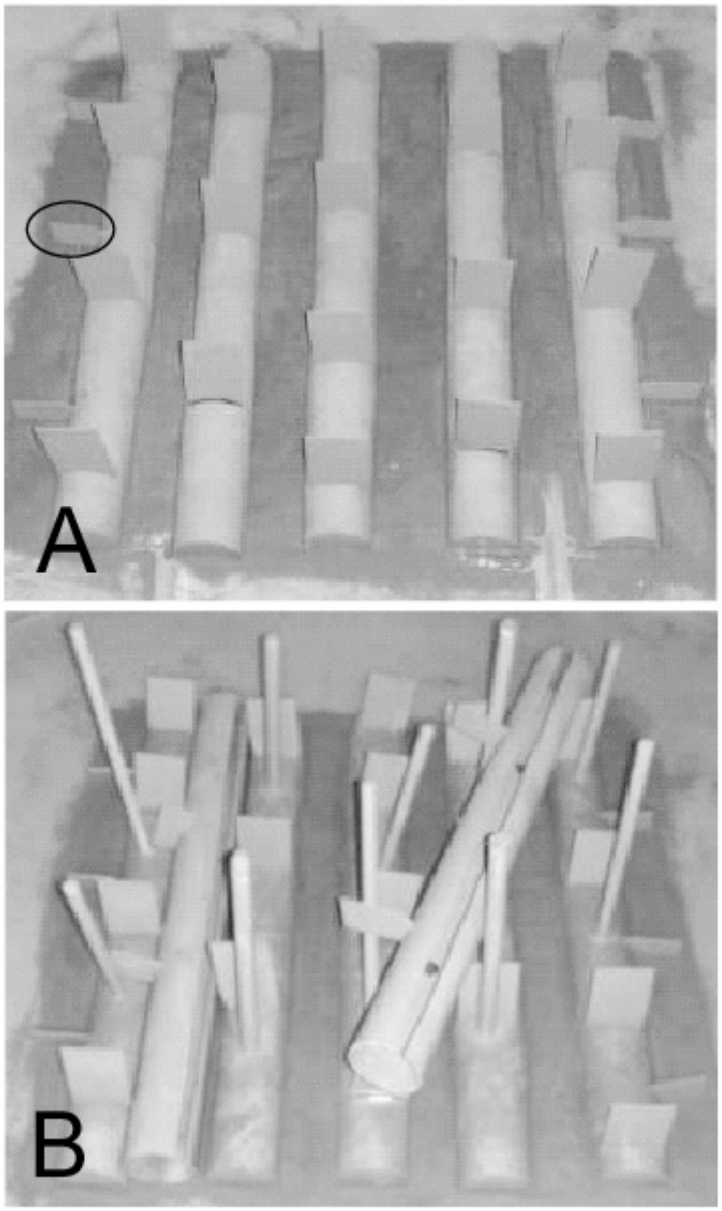


Figure 4.2: Grass shrimp attraction to structure and water surface

Back-transformed mean (n = 5) percentages (± SE) of shrimp attraction to the zone of structural complexity (A) and attraction to the top 5 cm of the water column in the experimental treatments examining habitat complexity. Black bars represent the flat sand treatment. Grey bars represent the medium complexity treatment. White bars represent the high complexity treatment. Different letters indicate a significant difference in back-transformed percentages among treatments (LSD pairwise comparisons; $P \leq 0.05$)

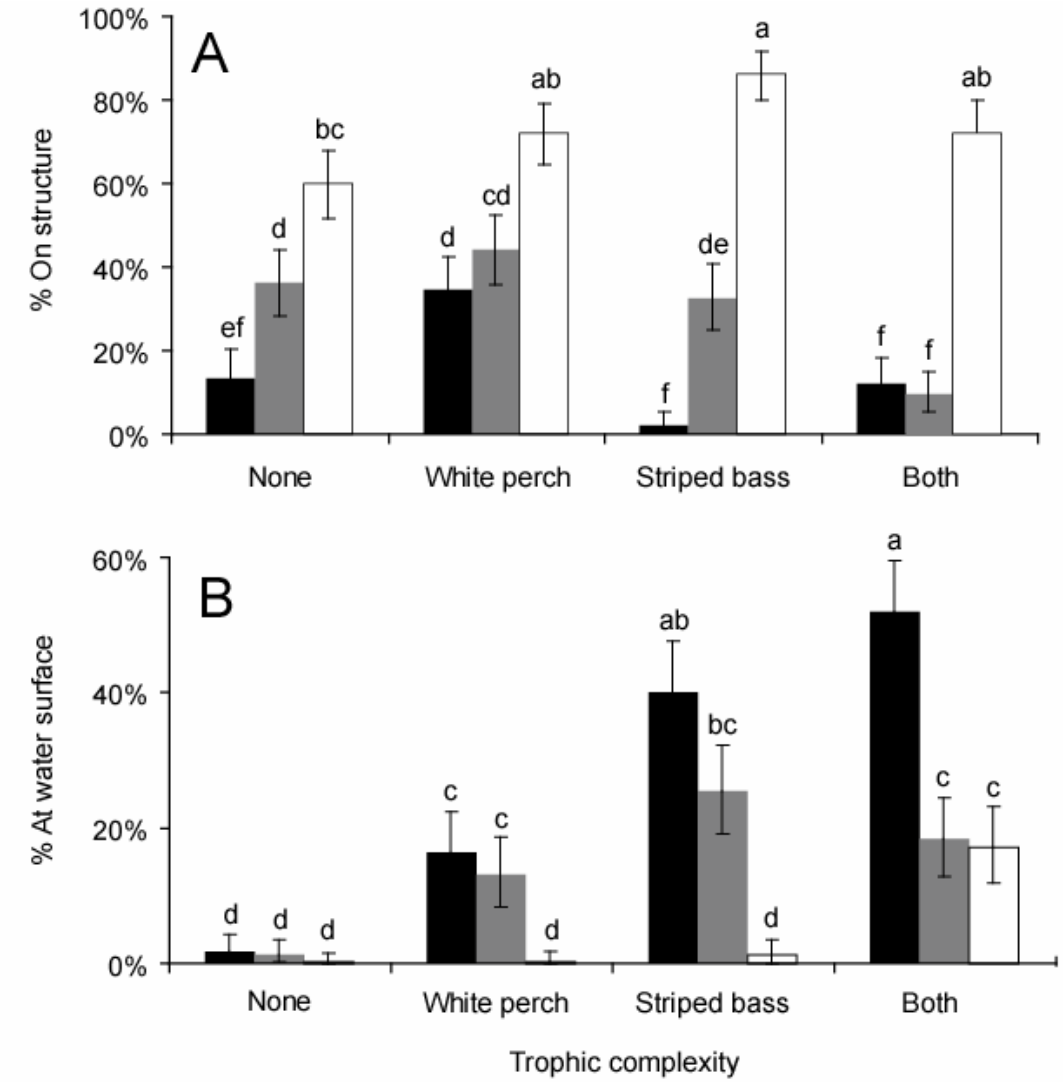


Figure 4.3: Grass shrimp mortality by fish predation

Back-transformed mean (n = 5) percentages (± SE) of predation of shrimp by white perch and striped bass predators. Black bars represent the sand control treatment. Grey bars represent the medium complexity treatment. White bars represent the high complexity treatment. Different letters indicate a significant difference in back-transformed percentages among treatments (LSD pairwise comparisons; $P \leq 0.05$).

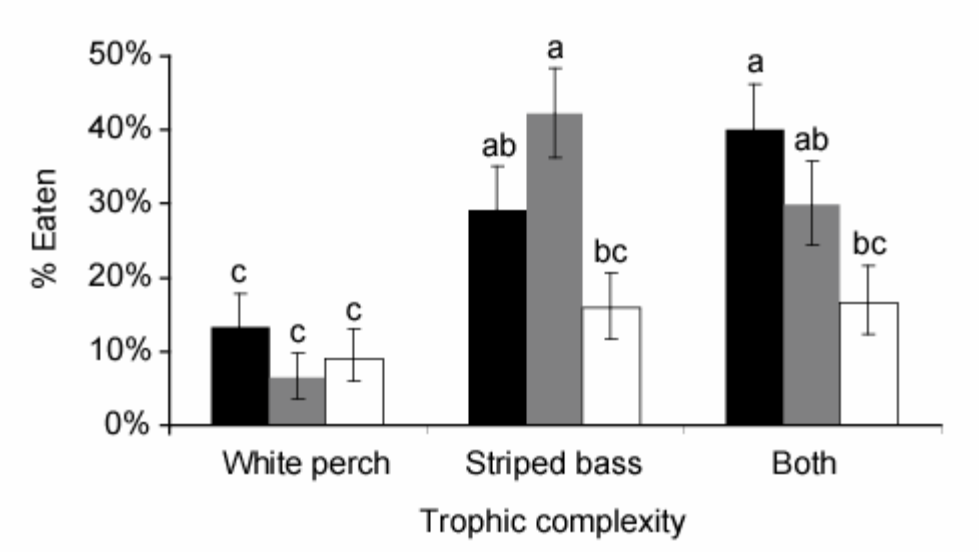


Figure 4.4: White perch attraction to structure

Back-transformed mean (n = 5) percentages (± SE) of white perch occurrence on the zone of structural complexity pooled across each trophic complexity level (A) pooled across each structural complexity level (B). Different letters indicate a significant difference in back-transformed percentages among treatments (LSD pairwise comparisons; $P \leq 0.05$).

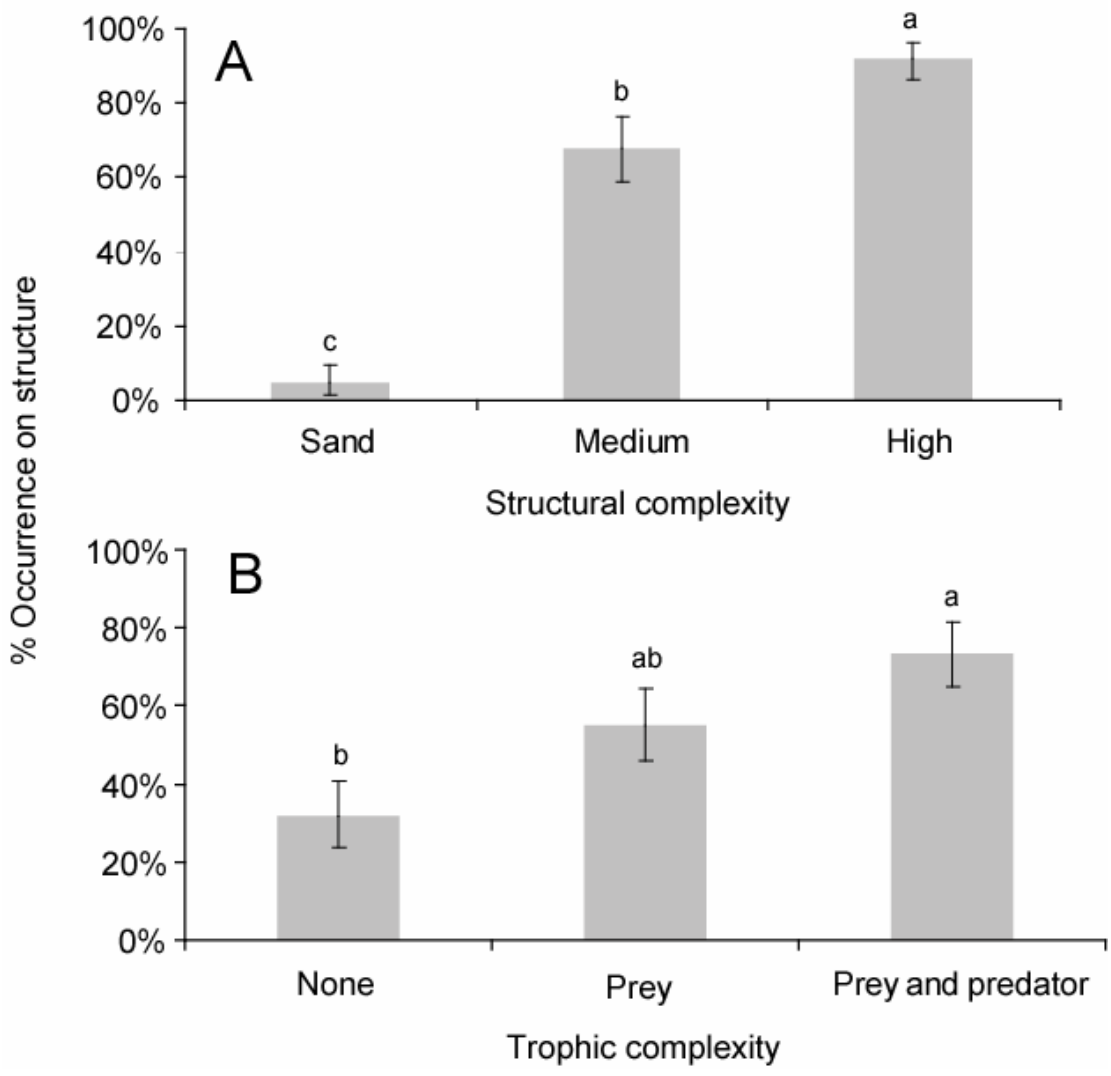


Figure 4.5: Movement of white perch on structure

Back-transformed mean (n = 5) percentages (± SE) of the movement of white perch while on the zone of structural complexity. Different letters indicate a significant difference in back-transformed percentages among treatments (LSD pairwise comparisons; $P \leq 0.05$).

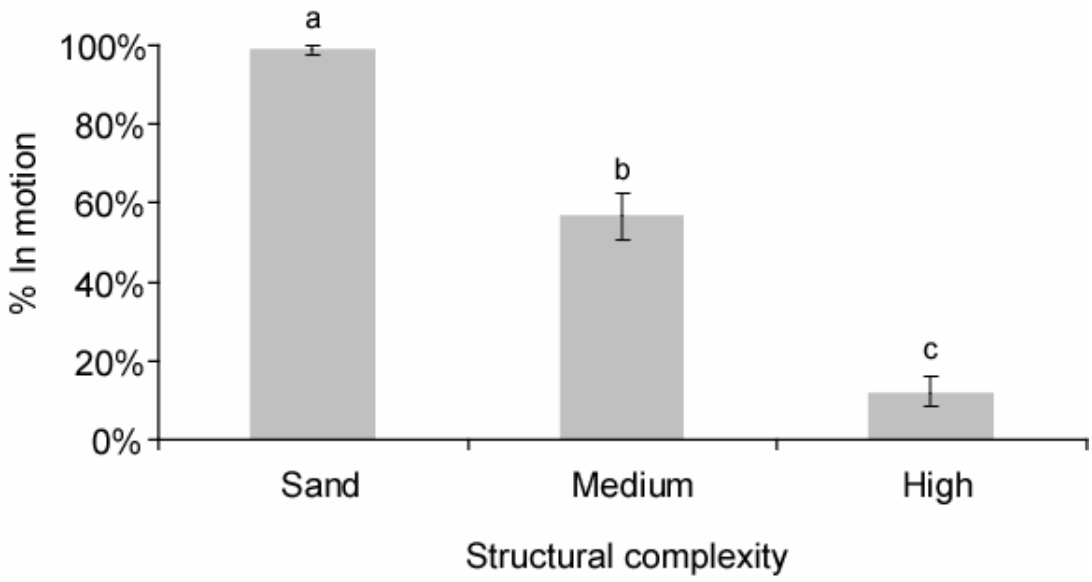


Figure 4.6: Behavior of white perch on structure

Back-transformed mean (n = 5) percentages (± SE) of white perch occurrence within ½ body length of each other within each structural complexity treatment across trophic complexity levels. Black stippled bar represents white perch only. White stippled bar represents white perch + grass shrimp. Grey bar represents white perch + grass shrimp + striped bass. Different letters indicate a significant difference in back-transformed percentages among treatments (LSD pairwise comparisons; $P \leq 0.05$).

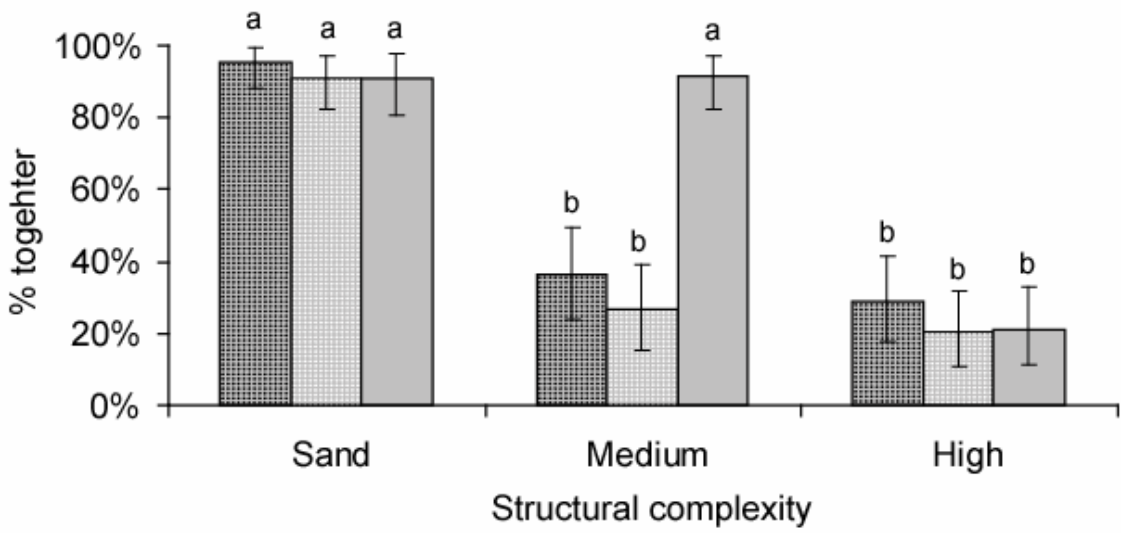


Figure 4.7: Striped bass attraction to and movement on structure

Back-transformed mean (n = 5) percentages (± SE) of striped bass occurrence on the zone of structural complexity (A) and the motion while within each zone (B) pooled across each trophic complexity level. Different letters indicate a significant difference in back-transformed percentages among treatments (LSD pairwise comparisons; $P \leq 0.05$).

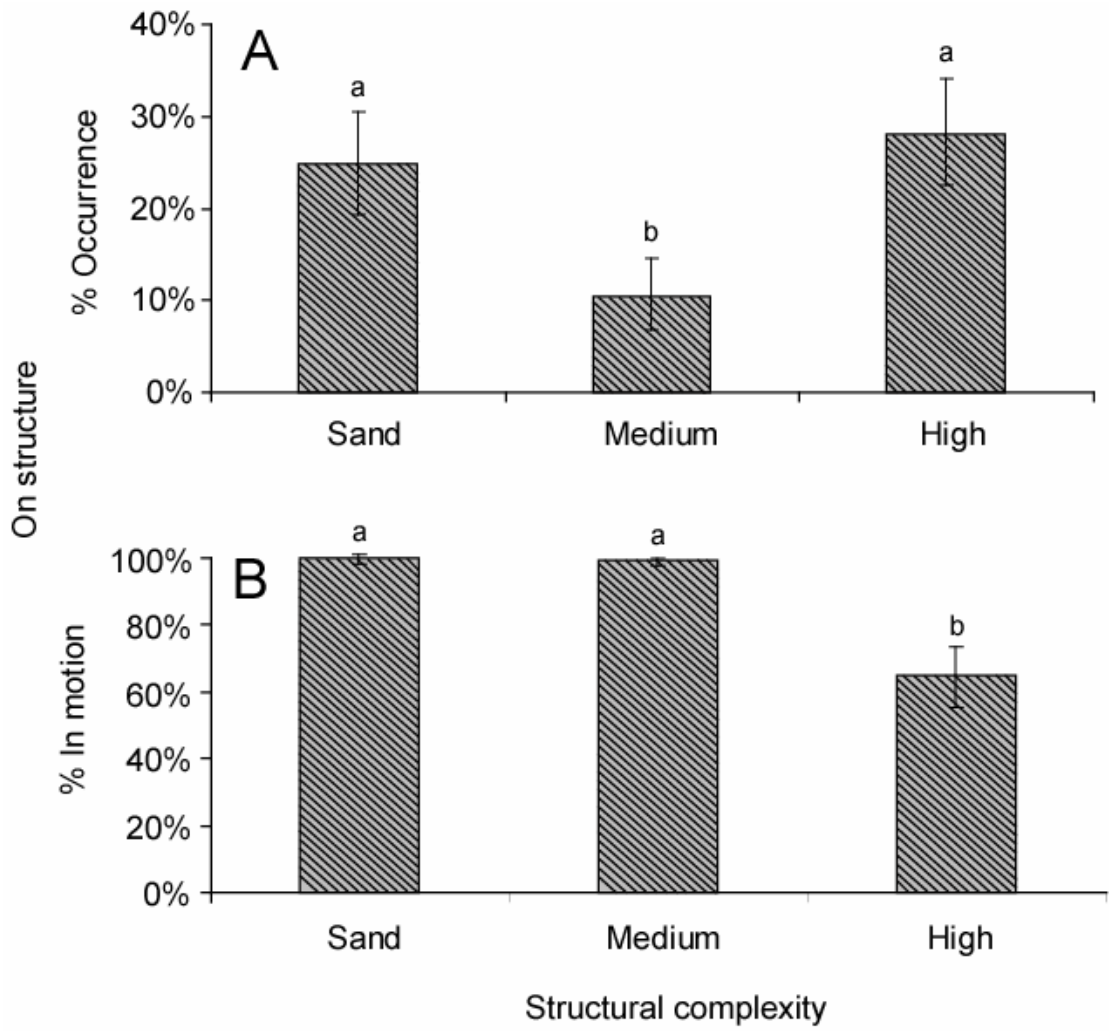
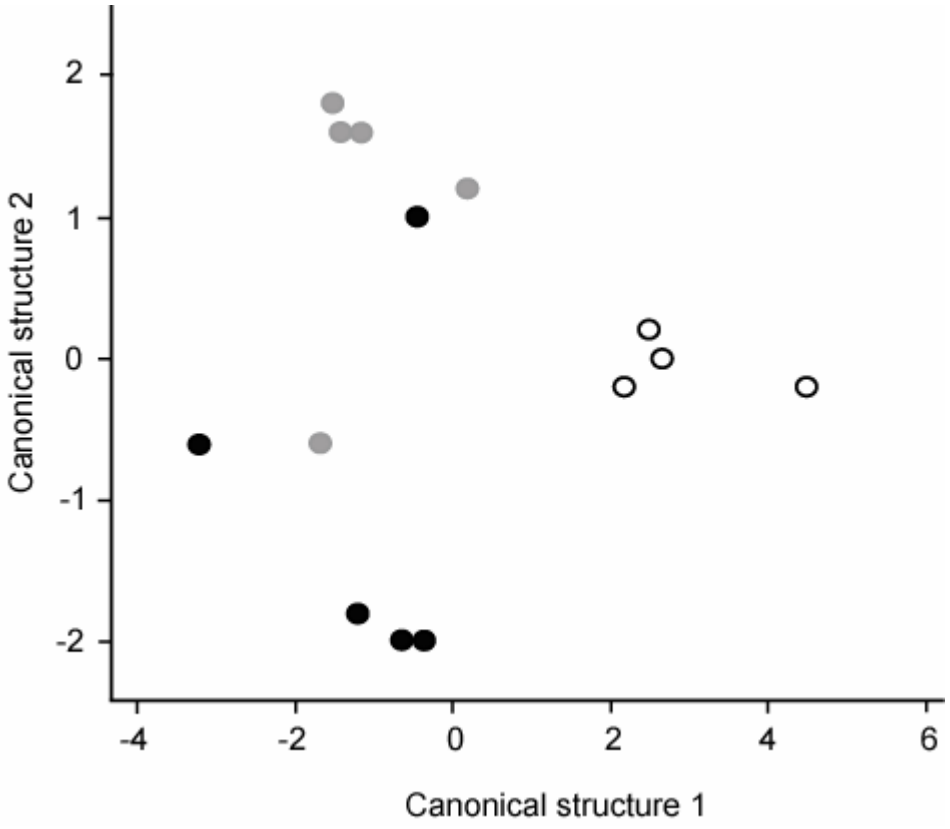


Figure 4.8: Interactions among predator and prey species attracted to structure

Plot of canonical structure 1 vs. canonical structure 2 representing the attraction of grass shrimp, white perch, and grass shrimp to the zone of structural complexity when examined together. Canonical structure 1 accounts for 79% of the total variance and is heavily correlated to grass shrimp attraction to the zone of structural complexity. Canonical structure 2 accounts for 21% of the variance and is correlated to white perch being the only species attracted to the zone of structural complexity. Black circles represent sand control. Grey circles represent medium complexity structure. White circles represent high complexity structure.



CHAPTER 5:

Grass Shrimp (*Palaemontes pugio*) Habitat Utilization is Influenced by Structural Complexity, Predation Threat, and Conspecific Density

ABSTRACT

Increased levels of structural complexity and organism density interact to influence predator–prey interactions between grass shrimp (*Palaemontes pugio*) and juvenile striped bass (*Morone saxatilis*) on complex aquatic habitat. Grass shrimp structure utilization was examined in laboratory mesocosms (2.5 m diameter) containing three levels of structural complexity (flat sand, medium, high) crossed with three grass shrimp prey densities (40, 100, or 250 individuals) and four striped bass predator densities (0, 2, 5, or 12 individuals). Habitat complexity was defined as an increase in surface area between the flat sand (1 m^2) and medium complexity (3.4 m^2) treatments, and as the absence or presence of interstitial space between the medium and high complexity treatments. The two highest complexity levels were constructed of PVC pipe. In the presence of striped bass, grass shrimp were attracted to the visual refuge provided by the surface area of the medium complexity structure, and were attracted to the physical refuge provided by interstitial space within the high complexity structure. This attraction, however, was reduced at the highest level of complexity when grass shrimp densities were high. In the absence of striped bass, grass shrimp attraction to the two structural complexity levels was similar because surface area was identical, and the utilization of interstitial space as a refuge was unnecessary. Striped bass density did not affect grass shrimp attraction to structure. Predation by striped bass on grass shrimp was similar among all complexity treatments, indicating that shrimp behavior did not result in enhanced survival.

INTRODUCTION

Structurally complex habitats are usually highly productive, with densities of fish and invertebrates often an order of magnitude higher than on structurally simple habitats (Russ 1991, Bohnsack et al. 1994, Jordan et al. 1996, Nagelkerken & van der Velde 2002, Adams et al. 2004, Rodney & Paynter 2006). Many invertebrate and fish species are attracted to complex habitat because it serves as a refuge against predation (Heck & Crowder 1991, Gotceitas & Colgan 1989, Hixon & Beets 1993, Steele 1999), provides food resources (Crowder & Cooper 1982, Adams et al. 2004, Verweij et al. 2006) that can enhance production (Polovina & Sakai 1989, Ebeling & Hixon 1991, Peterson et al. 2003), and provides a refuge from adverse environmental conditions (Kelly & Bothwell 2002, Cocheret de la Morinière et al. 2004).

Habitats created by ecosystem engineers (sensu Lawton & Jones 1995), such as oysters, coral, seagrass, and mangroves, are comprised of individuals of the constituent species attached to an existing substrate or grown in close proximity to each other; thereby creating a structurally complex matrix with numerous interstitial spaces of varying size and volume. Organisms that are vulnerable to predation often hide from predators within such matrices. Predators may have difficulty finding prey either due to visual obstacles or because of difficulty maneuvering effectively around physical barriers, limiting the chance of a successful attack and capture of prey items (Savino & Stein 1982, Person & Eklöv 1995, Macia et al. 2003). A predator may switch from an active to ambush predatory behavior in complex habitat in order to increase their chances of encountering a prey species (Savino & Stein 1982, Miner & Stein 1996), or to utilize

structure for foraging opportunities without the threat of being preyed upon themselves (Turner & Mittelbach 1990, Persson & Eklöv 1995, Johnson & Heck 2006).

Structurally complex habitats can act as an important pelagic-benthic shunt that deposits organic carbon and nitrogen into the sediment by way of waste products produced by ecosystem engineers and their associated fauna (Polunin 1988, Choat & Bellwood 1991, Dame 1996, Newell & Ott 1999). This residual organic matter is then consumed by a diversity of microorganisms and benthic invertebrates that form the base of the food web, which can increase the carrying capacity of the habitat for associated fauna (Ulanowicz & Tuttle 1992, Menge 2000, Norling & Kautsky 2007).

When species create extensive habitats, which provide enlarged surface areas and greater interstitial volumes, the carrying capacity of those habitats can also increase due to a greater availability of structure on which associated fauna are able to colonize, grow, and eventually reproduce (Abelson & Shlesinger 2002, Luckenbach et al. 2005). Species richness will also increase due to enhanced heterogeneity of that habitat (Kohn & Leviten 1976, Heck & Wetstone 1977, Diehl 1992, Rodney & Paynter 2006). As a result of increased attraction and enhanced secondary production, structurally complex habitat generally have high densities of both prey and predator species. Most studies, however, investigating interactions between predators, prey, and habitat complexity have maintained constant predator and prey densities while increasing the level of habitat complexity (Savino & Stein 1982, Gotceitas & Colgan 1989, Nelson & Bonsdorff 1990, Levin & Hay 2003, Davis et al. 2003, Adams et al. 2004, Shoji et al. 2007). The increase in the level of structural complexity provides enhanced prey refuge that may not actually occur when prey production is enhanced on highly complex structures. Such an

experimental design may lead to findings that overestimate the importance of complex habitat as a refuge for prey species, and underestimate the importance of complex habitat as a foraging area for predators.

Several studies have manipulated predator and prey densities and level of structural complexity to better understand trophic interactions on structurally complex habitats (Forrester & Steele 2004, Johnson 2006, Mattila et al. 2008, Canion & Heck 2009). These studies have shown that increased levels of structural complexity do not always decrease predation when predator and prey densities are increased proportionally with habitat complexity. Complex habitats contain niches, which have a finite carrying capacity, and when carrying capacity is exceeded a portion of the prey population becomes vulnerable to predation (Forrester & Steele 2004, Johnson 2006).

Grass shrimp (*Palaemontes pugio*) and striped bass (*Morone saxatilis*) are two species found in abundance around structurally complex habitats within temperate estuarine systems along the Atlantic Coast of North America (Posey et al. 1999, Coen et al. 1999, Clark et al. 2003, Davis et al. 2003, Rodney & Paynter 2006). Juvenile striped bass are voracious predators on grass shrimp (Clark et al. 2003), and grass shrimp have been shown to alter their habitat preferences in the presence of striped bass (Davis et al. 2003, Chapter 4). These attributes make these two species excellent organisms to investigate general predator – prey interactions on different complexity habitats.

The goal of my study was to examine how habitat complexity, predator and prey densities, and the combination of these factors influence predation risk. I hypothesized that when predator and prey densities were held constant, increased levels of structural complexity would enhance attraction of the prey species to structure and decrease

predation. However, as the density of prey and predator were increased concomitant to structural complexity the attraction of the prey species would be limited by the amount of space available as a refuge. Predation rates would be high when there was no available refuge, and remain similar as the level of structural complexity was increased.

MATERIALS AND METHODS

I explored the effect of three factors: habitat complexity, prey abundance and predator avoidance on the attraction of grass shrimp to structure. Increased level of structural complexity was defined as an increase in surface area and interstitial space over three complexity levels: a flat sand treatment, and medium, and high complexity 3-dimensional structures constructed from PVC pipe. This experiment was purposely not designed as a 3 way factorial study because time and budget constraints did not allow for the large number of trials needed for such a design. Rather this study was conducted as a center point screening design involving a series of one-way factorial experiments (Table 5.1). Utilization of habitat of differing levels of structural complexity was determined by the percentage of grass shrimp on each structure, and at the surface of the water at the end of each experimental trial. The effectiveness of each level of structural complexity as a refuge was determined by the percentage of grass shrimp surviving at the end of each experimental trial.

Study System

Experimental trials were conducted at Horn Point Laboratory between July and October 2010 within three 4164 L circular fiberglass tanks (diameter 2.5 m) filled with 2 μm filtered ambient Choptank River water to a depth of 0.55 m. Water temperature ranged from 20.4 –27.2ºC and salinity ranged from 10.4 – 13.5. Each tank was randomly

assigned a treatment with one of three structural complexities paired with a predetermined density of grass shrimp and/or juvenile striped bass (Table 5.1). Each treatment was replicated 5 times within the period from July – October 2010.

Grass shrimp (body length, 1.5 – 2.5 cm) were collected from the Choptank River and placed into a holding tank supplied with flow-through raw ambient Choptank River water and fed fish flake food (Wardley: Goldfish flake food) *ad libidum*. Grass shrimp surviving predation were reused in other treatments because of the high number of individuals needed (n = 7,650). This supply was supplemented with new individuals on a weekly basis. Individual grass shrimp were never reused in consecutive trials.

Fifty juvenile striped bass (21 – 26 cm; fork length) reared at Horn Point Laboratory's fish hatchery were kept in a holding tank between experimental trials and fed a maintenance ration of pelleted food. Pre-trials showed that hatchery juvenile striped bass reared on an artificial diet still fed voraciously on live grass shrimp. Test fish were chosen randomly from the holding tank and starved in a separate tank for 20 h before being placed into experimental mesocosms. Striped bass were reused over the course of the experiment because of the high number of individuals (n = 300) needed and limited availability of new stock. Individual striped bass were never reused in consecutive trials to limit learned behavior and reduce fish handling stress.

Habitat Complexity

The flat sand treatment consisted of a 0.95 m^2 area of fine aquarium sand that covered 21%, and rose 1 cm above, the bottom of the mesocosm. This complexity level was designed to provide no structural refuge to shrimp for protection against predation.

The medium complexity structure (Fig. 5.1A) had a surface area of 3.4 m^2 and protruded 45 cm from the bottom of the tank. This complexity was comprised of split 10 cm diameter PVC pipes capped at each end so that the internal space of the pipe could not be used as a refuge. Affixed vertically to each split pipe were two capped 1.3 cm diameter PVC pipes, as well as three or four flat PVC baffles. This assembly comprised one pipe unit. Each pipe unit was placed parallel to each other with a 5 cm gap and pressed gently into a 1 m^2 sand-bed. The end pipe units had three flat PVC pipe baffles that extended horizontally perpendicular from their base. Two open ended 10 cm diameter PVC pipes 1 m in length were placed on top of the structure to achieve the desired surface area. This complexity was designed to provide a visual refuge against predation, but not provide interstitial space for grass shrimp to use as a physical refuge. Striped bass were physically capable of accessing the entirety of this structure's surface, including inside the two 10 cm diameter PVC pipes.

The high complexity structure (Fig. 5.1B) also had a surface area of 3.4 m^2 and protruded 45 cm from the bottom of the tank. This complexity was composed of the same elements as the medium complexity structure except the two 10 cm diameter PVC pipes 1 m in length were replaced with seven 3.8 cm diameter PVC pipes of various lengths. These pipes kept the surface area of the medium and high complexity structure equal, but created interstitial space within the high complexity structure designed to exclude striped bass and provided physical refuge for grass shrimp against predation.

Predator and Prey Density

A density of 100 grass shrimp per tank was used to assess shrimp preference for structural complexity in the absence of striped bass predators (Table 5.1; Category I). A

moderate density of five striped bass and 100 grass shrimp per mesocosm was used to determine how differences in the level of structural complexity alone influenced grass shrimp habitat utilization and predation mortality (Table 5.1; Category II).

To determine the relationship between predator and prey density and level of structural complexity, the density of grass shrimp and striped bass added to the treatments was increased as the level of structural complexity was enhanced (Table 5.1; Category III). The density in the flat sand treatment consisted of two striped bass and 40 grass shrimp (low). The medium complexity treatment consisted of five striped bass and 100 grass shrimp (moderate). The high complexity treatment consisted of 12 striped bass and 250 grass shrimp (high). These densities were chosen so that each treatment had a consistent predator–prey ratio of approximately 1:20, which facilitated comparison among treatments.

The 12 striped bass in the high complexity treatment approximates the density of juvenile striped bass aggregated around high complexity structure on an oyster reef (Breitburg 1999). Grass shrimp densities used for this experiment were higher than the reported mean density of grass shrimp in the mesohaline region of Chesapeake Bay, USA. According to calculations based on Rodney and Paynter (2006), the mean density of grass shrimp on a high complexity oyster reef is 30 individuals m^{-2} and the mean density of grass shrimp found on a low complexity, moribund oyster reef is two individuals m^{-2}. Fish predators in the field will forage on a range of organisms in addition to grass shrimp. Therefore the increase in shrimp density within this experiment over published field conditions attempted to reflect such greater access to food availability in all complexities examined.

To further examine interactions among predator and prey densities, and structural complexity levels, additional trials involving further density combinations were conducted. Densities of 250 grass shrimp and 12 juvenile striped bass were tested on the medium complexity structure to examine the relationship of enhanced predator and prey densities on a single complexity structure (Table 5.1; Category IV). In order to determine if prey density or predator density had a greater influence on the attraction of grass shrimp to structure, two additional density treatments were added to the high complexity structure. These treatments consisted of five juvenile striped bass and 250 grass shrimp, and 12 juvenile striped bass and 100 grass shrimp (Table 5.1; Category V).

Methods and Analysis

Grass shrimp were added into each tank 15–18 h before the striped bass to allow for acclimatization before the threat of predation. Experimental trials were run for 34 h after striped bass were with a 12 h light, 12 h dark, 10 h light photoperiod. Grass shrimp that were utilizing the surface water in a tank as a refuge were enumerated twice during each light period. A previous study showed there was no significant difference in the number of grass shrimp at the surface of the water between the light and dark periods in the presence of striped bass predators (Chapter 4); therefore grass shrimp in the surface of the water were not enumerated at night during this experiment.

At the end of the experimental period a 1 m^3 wire cage covered in nylon mesh was placed over the structure to prevent grass shrimp from moving into and out of the zone of structural complexity. Striped bass were removed from the mesocosm, the water drained, and the number of shrimp on and off the structure was enumerated. Video was taken intermittently in order to qualitatively assess structure utilization by striped bass.

The percentage of shrimp associated with each habitat was calculated by dividing the number of shrimp found inside the mesh cage by the total number of remaining shrimp within the mesocosm at the end of each experimental trial. The percentage of shrimp eaten by striped bass was calculated by subtracting the average number of shrimp missing from no predator treatments from the number of shrimp missing from each predator treatment. That value was then divided by the total number of shrimp released into the mesocosm at the beginning of each experimental trial. Missing shrimp within the no predator treatments averaged 1.7 individuals, which indicated nearly complete recapture of grass shrimp within the mesocosms.

The effect of habitat complexity on the attraction of grass shrimp to structure was tested by transforming the data through arcsine transformation and performing an Analysis of Variance (ANOVA) on the eight treatments comprising categories I – III. An ANOVA was also performed on the arcsine transformed data in category IV to determine the effects of predator:prey ratio at the medium complexity treatment. To determine the effect of predator:prey density at the high complexity treatment a two-way ANOVA was performed on the arcsine transformed data of category V. Post-hoc Least Significant Difference (LSD) multiple means comparisons tests were used to determine differences among treatments. Arcsine values (mean, ± SE) were back-transformed to percentages (mean, ± SE). All means are back-transformed, and standard errors are asymmetrical around the mean. These same statistical analyses were performed to determine differences in predation rates among the treatments within each of the category combination described above.

To test the differences in shrimp utilization of the surface water among treatments the number of shrimp near the surface of the water during the first light period was divided by the total number of shrimp introduced into the mesocosm at the beginning of the experimental trial. A separate percentage was calculated by dividing the number of shrimp near the surface of the water during the second light period by the total number of remaining shrimp at the end of the experimental trial. Percentages were arcsine transformed and Repeated Measures ANOVAs were performed to evaluate differences due to treatments and photoperiods for categories I – III and category IV. A two-way Repeated Measures ANOVA was performed on the arcsine transformed data of category V. Arcsine values (mean, ± SE) were back-transformed to mean percentages.

RESULTS

The effect of habitat complexity on the attraction of grass shrimp to structure (Table 5.1; Categories I – III) was significantly different among the eight treatments (ANOVA, $F_{7, 32} = 15.70$; $P < 0.0001$). The proportion of grass shrimp attracted to structure in the absence of striped bass was similar between the medium and high complexity structures (LSD, $t_{32} = 0.20$; $P = 0.85$), but significantly greater in both of those treatments than the proportion attracted to the flat sand treatment ($t_{32} = 2.60$; $P = 0.02$) (Fig. 5.2A; Category I).

When striped bass were present, and predator and prey densities were moderate and uniform across complexity treatments (Table 5.1; Category II), the attraction of grass shrimp to structure increased as the level of structural complexity increased (Fig. 5.2A). Significantly more grass shrimp were attracted to the high complexity structure than were attracted to the medium complexity structure ($t_{32} = 3\ .54$; $P = 0.001$) or flat sand

treatment (t_{32} = 7.82; P < 0.0001). The medium complexity structure attracted significantly more grass shrimp than the flat sand treatment (t_{32} = 4.29; P = 0.0002). When striped bass and grass shrimp densities were enhanced concurrently with level of structural complexity (Table 5.1; Category III), there was a significantly greater proportion of shrimp attracted to the medium complexity structure than were attracted to the flat sand treatment (t_{32} = 4.42; P = 0.0001). There was also a trend towards a greater proportion of grass shrimp being attracted to the high complexity structure (68%) than to the medium complexity structure (54%); however this difference was not significant (t_{32} = 1.23; P = 0.23) (Fig 2A).

In general, the presence of predators tended to influence the use of structure by grass shrimp. Within the flat sand treatment, grass shrimp were not attracted to the sand bed regardless of striped bass presence or absence (Fig 2A). A greater proportion of grass shrimp tended to be attracted to the medium complexity structure in the presence of striped bass (54%) than were attracted to this structure in the absence of striped bass (40%), this trend, however, was not significant (t_{32} = 0.85; P = 0.40). When grass shrimp density was moderate, a significant proportion of them were attracted to the high complexity structure in the presence of striped bass compared to when striped bass were absent (t_{32} = 4.23; P = 0.0002). When grass shrimp and striped bass densities were high, a significantly lower proportion of grass shrimp were attracted to the high complexity structure compared to when predator and prey densities were moderate (t_{32} = 2.31; P = 0.028).

The effect of habitat complexity on the proportion of grass shrimp eaten (Table 5.1; Categories 1 – III) within these eight treatments was not significant (ANOVA, $F_{4, 20}$

= 2.2; P = 0.11). However, the proportion of grass shrimp eaten within the medium and high complexity treatments was lower than the proportion of grass shrimp eaten within the flat sand treatment. These results were deemed important enough to warrant testing pre-planned comparisons of individual treatments using post-hoc multiple comparison analysis with a significance level set at P = 0.05.

This additional analysis showed that a significantly greater proportion of grass shrimp were eaten by striped bass on the flat sand sand than on the medium complexity structure (Fig. 5.2B), regardless of predator (LSD, $t_{20} = 2.35$; P = 0.03) or prey ($t_{20} = 2.29$; P = 0.03) density. There was no significant difference in the proportion of shrimp eaten by striped bass between the medium and high complexity structures either when prey and predator densities were moderate ($t_{20} = 0.90$; P = 0.38) or when prey and predator densities were high ($t_{20} = 0.60$ P = 0.59). Although this difference was not significant, there was a trend towards a lower proportion of grass shrimp preyed upon in the high complexity treatment relative to the flat sand treatment.

Effect of Predator:Prey Ratio at Medium Complexity

As grass shrimp and striped bass densities were increased the proportion of grass shrimp attracted to the medium complexity structure tended to decrease (Fig. 5.3A; Category IV), this result however, was not statistically significant (ANOVA, $F_{2, 12} = 2.59$; P = 0.12). There was no significant difference in the proportion of grass shrimp eaten among the predator and prey densities (Fig. 5.3B) ($F_{2, 12} = 1.24$; P = 0.32).

Effect of Predator:Prey Density at High Complexity

There was no interaction between predator and prey density on the attraction of grass shrimp to structure within the high complexity treatment (Two-way ANOVA, $F_{1,16}$

= 0.24; P = 0.63), nor was there an affect of predator density ($F_{1,16}$ = 0.32; P = 0.58). There was an effect of prey density on the attraction of grass shrimp to structure within the high complexity treatment (Fig. 5.4A; Category V) in which proportionally fewer grass shrimp were attracted to the high complexity treatment when their population density was high ($F_{1,16}$ = 5.71; P = 0.03). There was no significant interaction ($F_{1,16}$ = 0.43; P = 0.52) or difference in the proportion of grass shrimp eaten on the high complexity treatment regardless of prey ($F_{1,16}$ = 2.67; P = 0.12) or predator ($F_{1,16}$ = 1.11; P = 0.31) density (Fig. 5.4B).

Surface Water

The effect of habitat complexity on the attraction of grass shrimp to the surface of the water (Table 5.1; Categories I – III) was significantly different among the eight treatments examined (Repeated Measures ANOVA, $F_{7, 32}$ = 2.56; P = 0.033). There was no difference between sampling periods ($F_{1, 39}$ = 1.04; P = 0.31). In the absence of a predator (Table 5.1; Category I) no grass shrimp were observed to utilize the surface of the water. When prey and predator density was moderate (Table 5.1; Category 2) grass shrimp were significantly attracted to the surface of the water within the flat sand treatment (23%) compared to the high (0%) complexity treatment (LSD, t_{32} = 2.75; P = 0.01). There was no significant difference between the flat sand treatment and medium complexity treatment (t_{32} = 1.21; P = 0.24) or the medium and high complexity treatments (t_{32} = 1.54; P = 0.13). Grass shrimp only utilized the surface of the water when the structure within in the mesocosm did not provide adequate refuge from predation due to a lack of complexity and/or when grass shrimp or striped bass densities were high.

As grass shrimp and striped bass densities were increased (Table 5.1; Category IV) the proportion of grass shrimp forced to the surface of the water within the medium complexity treatment increased (Repeated Measures ANOVA, $F_{2, 12} = 5.04$; $P = 0.03$). There was time effect ($F_{1, 14} = 1.26$; $P = 0.28$). There was a significant difference between the proportion of grass shrimp present at the surface of the water in the low density treatment (0%) and the high density treatment (34%) (LSD, $t_{12} = 3.17$; $P = 0.01$). There was, however, no difference between the low and medium (7.9%) density treatments ($t_{12} = 1.38$; $P = 0.19$), or the medium and high density treatments ($t_{12} = 1.78$; $P = 0.10$).

There was no interaction between predator and prey density on the presence of grass shrimp near the surface of the water (Two-way Repeated Measures ANOVA, $F_{1,16} = 0.02$; $P = 0.90$) within the high complexity treatment (Table 5.1; Category V). There was, however, an interaction between striped bass density and the proportion of grass shrimp in the surface of the water between the first and second light periods ($F_{1,18} = 4.72$; $P = 0.04$). Significantly more grass shrimp were found at the surface of the water during the first light period (10%) than the second light period (4%) when a high density of striped bass were present (LSD, $t_{18} = 3.03$; $P = 0.01$). There was no overall effect of the density of predators on the presence of grass shrimp in the surface of the water ($t_{17} = 2.18$; $P = 0.16$), while there was significantly more grass shrimp found at the surface of the water when the density of prey was high ($t_{17} = 14.28$; $P = 0.0015$).

DISCUSSION

Results from these experiments indicate that the use of structured habitat by prey was significantly affected by the level of structural complexity, the presence of predators, and the density of conspecifics. There were also interactions among these variables that

suggest that responses to predation risk can alter habitat selection. There was no effect of predator density on prey distribution. I interpret these results to indicate that shrimp were primarily attracted to habitat for its structural complexity, while the threat from predation enhanced that attraction and the density of conspecifics reduced that attraction.

In the absence of the predation threat a greater proportion of grass shrimp were attracted to a structured habitat instead of an unstructured one. This finding is consistent with observations from the field where grass shrimp are often found in high densities on structurally complex habitats, such as oyster reefs and seagrass beds, but are generally found in low abundance or absent on structurally degraded habitats or sand flats (Posey et al. 1995, Clark et al. 2003, Rodney & Paynter 2006). There was no difference in the attraction of grass shrimp to the two structurally complex treatments that were identical in surface area; though only the high complexity treatment contained interstitial space. Proportionally fewer grass shrimp were attracted to the high complexity structure when striped bass were absent (43%) compared to when striped bass were present (90%). This result indicates that in the absence of an immediate threat from predation the presence of interstitial space was not an important factor in attracting grass shrimp to structure. Earlier studies (Orth et al. 1984, Moore & Hovel 2010, Chapter 4) indicate that the magnitude of surface area influenced the attraction of structural habitats to grass shrimp and other benthic and epifaunal invertebrates, even when that attraction increases their vulnerability to predation over time (Stoner 1980).

Interstitial space increased grass shrimp attraction to structure when the threat of predation existed. When striped bass were present a significantly higher proportion of grass shrimp (90%) were attracted to the high complexity structure than the medium

complexity structure (54%) presumably because of the perceived refuge provided by interstitial space. The importance of interstitial space in increasing the perceived predation refuge value of habitat is further illustrated by the difference in grass shrimp attraction to high complexity structure in the absence (43%) and presence (90%) of striped bass predators. A positive correlation between interstitial space and organism density has also been demonstrated in a wide range of species and habitats (Hacker & Steneck 1990, Hixon & Beets 1993, Charbonnel et al. 2002, Adams et al. 2004, Forrester & Steele 2004), and macroinvertebrate densities are generally more correlated to the average amount of interstitial space available as a refuge than an increase in structural surface area (Warfe et al. 2008).

Many studies inadvertently increase the surface area of a structure concurrently with an increase in interstitial space. An increase in surface area may lead to an enhanced carrying capacity of a habitat because there is more physical space to inhabit, as well as because the increased rugosity of the structure could decrease the visual field of an organism and therefore its territorial area (Basquill & Grant 1998). What makes my study different is that an increase in interstitial space was not accompanied by an increase in surface area, allowing the influence of these two physical characteristics to be directly compared against one another. Increased surface area of a habitat attracts large numbers of organisms regardless of predatory threat. However, an increase in interstitial space alone is not sufficient to attract increased faunal densities to structure in the absence of predators. The effectiveness of a habitat in attracting and protecting fauna is best served by increasing both the surface area and interstitial space within a structure.

Predator and Prey Density

The value of high complexity structure as a refuge for grass shrimp was diminished as shrimp density increased concurrently with the level of structural complexity. This was evidenced by a reduction in the proportion of grass shrimp in the high density treatment utilizing the structure relative to when shrimp density was moderate. There are several possible explanations for this finding.

The density of striped bass swimming through and utilizing the high complexity structure may have decreased the perceived refuge value of this structure, resulting in grass shrimp seeking refuge near the surface water or elsewhere within the mesocosm. Predatory fish species are attracted to high complexity structure in part because of enhanced foraging opportunities afforded by aggregated prey communities (Coen et al. 1999, Cocheret de la Morinière et al. 2004), and increased habitat complexity has also been shown to alter predatory behavior (Savino & Stein 1982, Person & Eklöv 1995, Chapter 4). These two factors may minimize the value of structure as a refuge for prey species under an intense threat of predation. Qualitative video analysis of striped bass within this experiment indicated that there was no comparative change in fish behavior or attraction to the high and medium complexity treatments regardless of their density relative to the density of grass shrimp.

Another possible explanation is that the enhanced density of grass shrimp in the high complexity treatment may have exceeded the structure's threshold to provide adequate refuge for a portion of the grass shrimp population. Grass shrimp grass shrimp utilization of the high complexity structure within the high density treatment averaged 120 out of 186 of the remaining individuals. Numerically fewer shrimp utilized the high

complexity structure within this treatment than within a comparative treatment in which only the striped bass density was reduced. Within that treatment grass shrimp utilization of the high complexity structure averaged 151 out of 214 the remaining individuals. These absolute grass shrimp densities show that while the carrying capacity of high complexity structure in the high organism density treatment may have been close to saturated it was likely not surpassed.

As the density of grass shrimp and striped bass increased concurrently on the medium complexity structure, the percentage of shrimp attracted to that structure tended to decrease. In the low density treatment, 70% of grass shrimp utilized the medium complexity structure as refuge against predation, while only 30% of the grass shrimp population utilized this complexity as a refuge when shrimp densities were high. Interestingly, the number of individual grass shrimp on the structure in the medium (n = 43) density treatment was not dramatically different than the number of grass shrimp in the high (n = 53) density treatment. This may indicate that the capacity of the medium complexity structure to attract individual grass shrimp may have been saturated at densities around the medium density treatment.

The lack of attraction of grass shrimp to the structure could also have been due to the increased density of striped bass within the treatment. Qualitative analysis of striped bass behavior in my study did not indicate any change in the attraction or behavior between the two treatments. The large number of striped bass within the high density treatment resulted in striped bass passing through the structure more frequently than in the medium density treatment.

Structurally simple habitats may serve to attract low densities of prey organisms. The possibility of population growth on these habitats may be limited by the lack of the structural complexity to attract new individuals through immigration or retain new recruits from *in situ* production. Foraging opportunities by fish predators on these habitats may be unsuccessful because the low density of organisms decreases the chance of a successful encounter and capture, while the lack of foraging may actually serve to enhance the refuge for extant prey populations (Holt 1987). These results may also indicate that when a threshold refuge capacity is reached, complex habitats may provide a spillover of prey to other structures through the emigration of new recruits as well as enhanced foraging opportunities for fish predators.

In the high level of structural complexity treatment the density of conspecifics had a greater influence on the attraction of grass shrimp to structure than did the predator density. While the presence of predators is increases grass shrimp attraction to high complexity structure, the density of that predator does not exert a significant influence on that attraction even under elevated prey densities (Kneib and Stiven 1982, Heck et al. 2000). Any reduction in the density of prey on a structure in the presence of a predator is likely due to increased predation and not by decreased movement by the prey species onto that habitat.

The threat of predation, coupled with an increase in the prey population on a structurally complex habitat, may foster interspecific competition for available refuge space within the structure (Holt 1987). When prey densities were moderate within the high complexity treatment a greater proportion of the grass shrimp population utilized the structure regardless of predator density. When prey densities were high, however, there

was more competition for space within the structure forcing a greater proportion of the shrimp population to utilize the surface of the water or the sides of the tank as a potential refuge. Predation rates were similar and relatively low within these treatments regardless of the density of prey or predators. These low predation rates may have lessened the per capita risk of a grass shrimp choosing an area that provided substandard refuge when their densities were high. It is possible that if predation pressure was more intense, grass shrimp may have chosen the risks associated with overcrowding of a refuge rather than the risks associated with increased predation. Another possible explanation is that striped bass may have been satiated with prey in the high density treatments minimizing their influence over grass shrimp behavior because of reduced foraging activity.

Perceived vs. Real Refuge

Grass shrimp were attracted to intermediate and high complexity structure in the presence of striped bass. They also had a higher per capita attraction to the high versus the intermediate complexity structure within the mesocosms under some predator and prey density treatments. I interpret the use of structure within the mesocosms to reflect a perception by grass shrimp that such habitat was a refuge from predation. Predation rates on grass shrimp have been found to decrease as habitat complexity increases in both mesocosm (Davis et al. 2003) and manipulative field experiments (Clark et al. 2003). In this study, however, the increased occupation of structure did not always provide actual refuge for grass shrimp. The highest predation rate occurred in the flat sand treatment (48%), presumably due to the lack of structural complexity to provide real refuge. In contrast, the difference in the predation rate of striped bass on grass shrimp was not

statistically different between the medium (15%) and high (27%) complexity treatments, regardless of predator and prey density.

Structural complexity can increase the attraction of both prey and predatory fish to habitat, potentially increasing encounter rates with prey and, thus, increasing predation rates where the protection afforded by the refuge habitat is not absolute (Crowder and Cooper 1982). The mere presence of a structure within my experiment provided protection from striped bass predation. While increasing levels of structural complexity were apparently perceived by grass shrimp differently in terms of refuge potential, the protection against striped bass predation afforded by the two levels of structural complexity were broadly similar.

Predation rates have been shown to decrease as the level of structural complexity increases due to enhanced refuge when prey and predator populations are held constant (Savino & Stein 1982, Persson and Eköv 1995). When prey and predator populations are increased concurrently with the level of structural complexity, predation rate is influenced more by density-dependent (Forrester and Steele 2004, Mattilia et al. 2008, Canion and Heck 2009) and behavioral (Johnson 2006) factors than by the level of structural complexity. Prey species are often more abundant and active within high complexity structure due to an apparent increased attraction and production. This attraction makes them more susceptible to predation because of increased encounter and capture rates from predators who are also more abundant on habitats with high levels of structural complexity.

Conclusion

My findings suggest that the value of habitat as a refuge for prey is dependent on the level of habitat complexity, the presence of predatory species, and the density of conspecific organisms. Prey species may initially benefit in newly established complex habitats such as artificial reefs, restored oyster reefs, or re-established seagrass beds because the level of structural complexity of the habitat exceeds the amount of refuge needed by the prey population to protect against predation. Over time, prey populations on structurally complex habitats may become denser because of immigration and recruitment to, and production on, the structure (Forrester & Steele 2004, Norling & Kautsky 2007). Predators may benefit when prey populations exceed the refuge capacity of a habitat, which then provides enhanced foraging opportunities through an increase in encounter and capture rates (Johnson 2006). Restoration and conservation efforts on structurally complex habitats should seek to weigh the necessity of increased structural surface area with the importance of interstitial space. This necessity will provide prey with an enhanced refuge that allows for increased productivity while still providing ample foraging opportunities for predators.

CHAPTER 5:

Tables

Table 5.1: Experimental categories and grass shrimp utilization of surface water as a refuge.

Complexity and density treatment combinations within experimental categories. The ratio of striped bass to grass shrimp is presented for each treatment combination. Surface % is back-transformed mean (n = 5) percentage (± SE) of shrimp utilizing the surface water as a refuge against predation. Different letters indicate a significant difference in back-transformed percentages within experimental categories (LSD pairwise comparisons; $P \leq 0.05$). Not significant = —. The (*) indicates that replicates were not duplicated and have already been reported in another experimental category.

	Category	Complexity	# of fish	# of shrimp	Ratio	Surface %	+ SE (%)	- SE (%)	P ≤ 0.05	Replicates
		Sand	0	100		0.0	0.0	0.0	—	5
I	– Grass shrimp, no striped bass predators	Medium	0	100		0.1	1.6	0.0	—	5
	– Structural complexity increased	High	0	100		0.0	0.0	0.0	—	5
	– Grass shrimp and striped bass density	Sand	5	100	1:20	23.2	10.4	9.0	a	5
II	held constant	Medium	5	100	1:20	7.9	7.3	5.1	ab	5
	– Structural complexity increased	High	5	100	1:20	0.0	0.0	0.0	b	5
	– Grass shrimp and striped bass density	Sand	2	40	1:20	14.2	9.0	7.0	—	5
III	increased together	Medium	5	100	1:20	7.9	7.3	5.1	—	*
	– Structural complexity increased	High	12	250	1:21	8.0	7.3	5.1	—	5
	– Grass shrimp and striped bass density	Medium	2	40	1:20	0.0	0.0	0.0	b	5
IV	increased together	Medium	5	100	1:20	7.9	7.3	5.1	b	*
	– Structural complexity held constant	Medium	12	250	1:21	33.6	11.3	10.4	a	5
	– Grass shrimp and striped bass density	High	5	100	1:20	0.0	0.0	0.0	—	*
V	increased separately	High	12	100	1:8	0.6	3.1	0.5	—	5
	– Structural complexity held constant	High	5	250	1:50	4.0	5.7	3.3	—	5
		High	12	250	1:21	8.0	7.3	5.1	—	*

CHAPTER 5:

Figures

Figure 5.1: PVC pipe habitats within mesocosms

Top and side views of constructed PVC pipe (A) medium complexity and (B) high complexity structure.

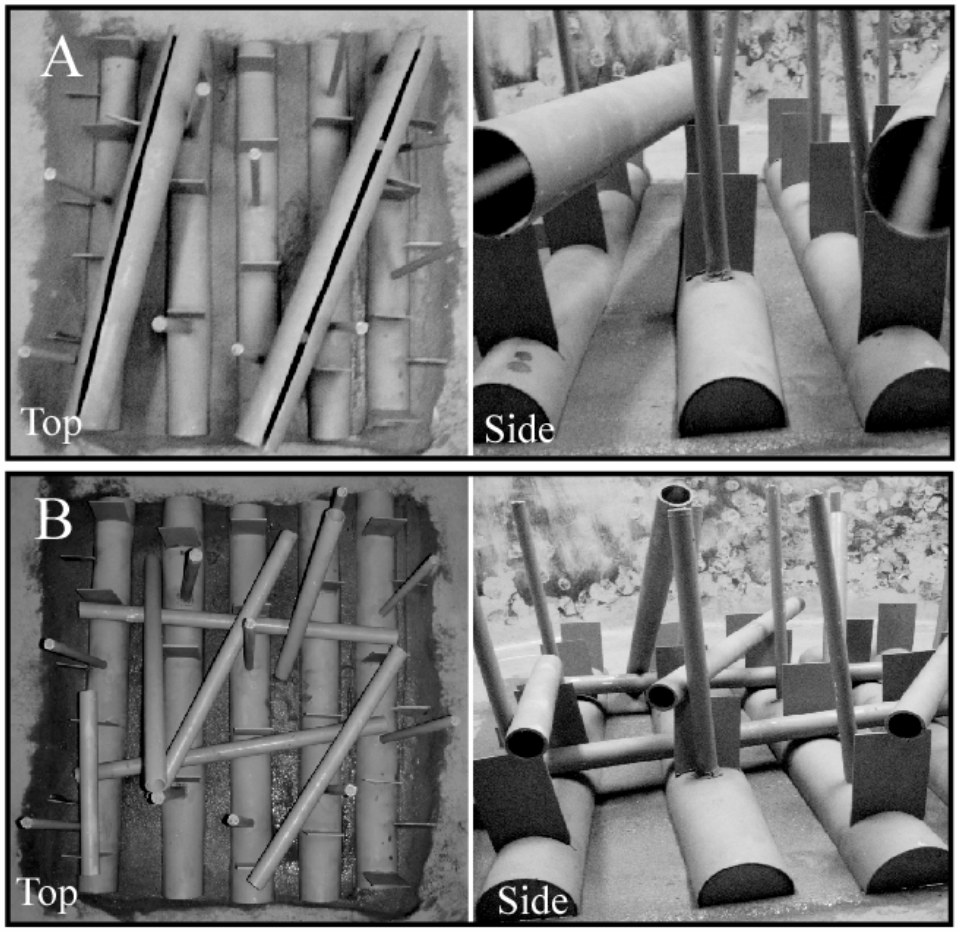


Figure 5.2: Grass shrimp utilization of habitat and predation rates

Back-transformed mean (n = 5) percentages (± SE) of shrimp attraction to differing levels of structural complexity (A) and predation by striped bass (B) in experimental treatments examining habitat complexity. White bars are results for abundance on structure in the absence of predation (I). Grey bars indicate results under moderate density of shrimp and striped bass (II). Black bars indicate results for increase in shrimp and striped bass density with enhanced structural complexity (III). The (*) indicates treatments duplicated graphically for ease of comparison. Different letters indicate a significant difference in back-transformed percentages among treatments (LSD pairwise comparisons; $P \leq 0.05$)

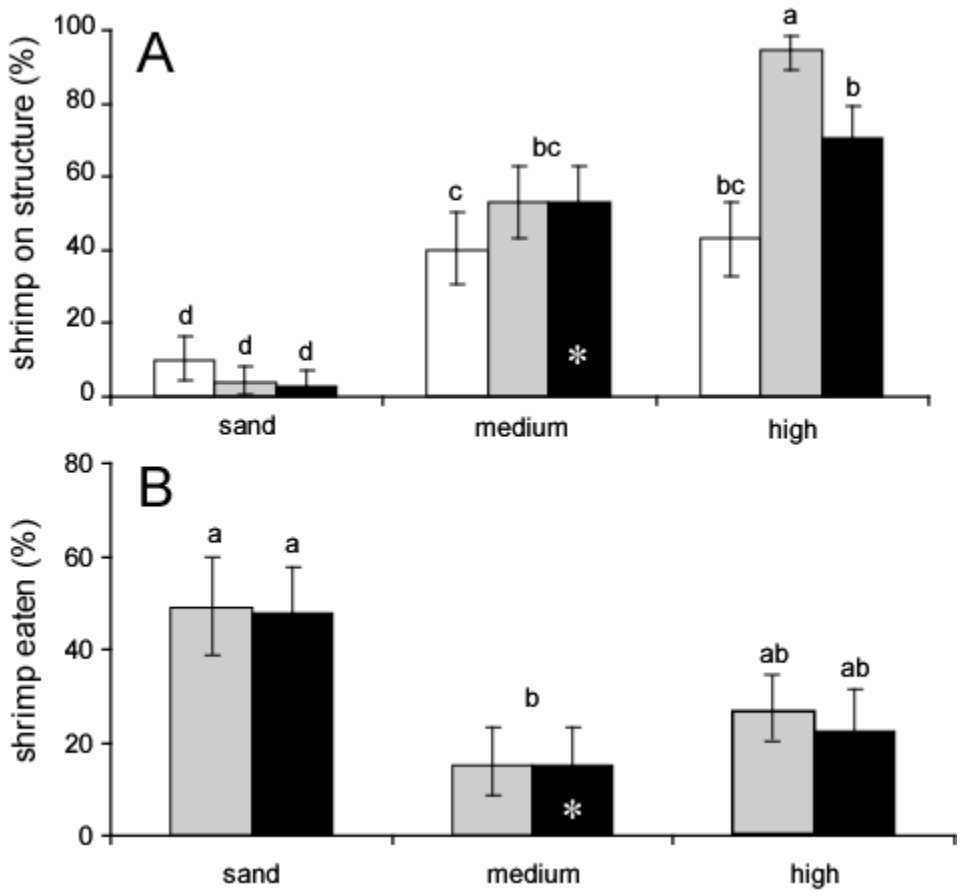


Figure 5.3: Effect of predator : prey ratio on medium complexity habitat

Back-transformed mean (n = 5) percentages (± SE) of shrimp attracted to structure (A) and predation by striped bass (B) on medium complexity structure with increasing densities of both shrimp and striped bass (experimental category IV). Differences between treatments were not significant (ANOVA; $P > 0.05$)

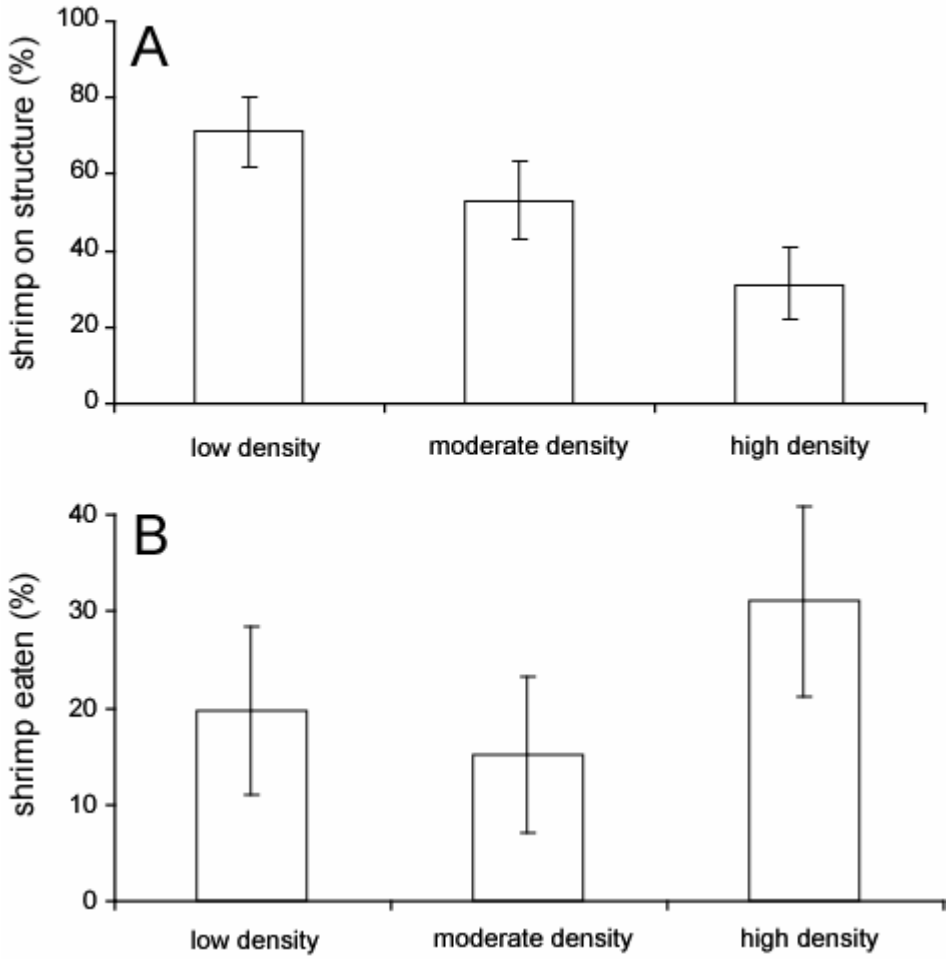
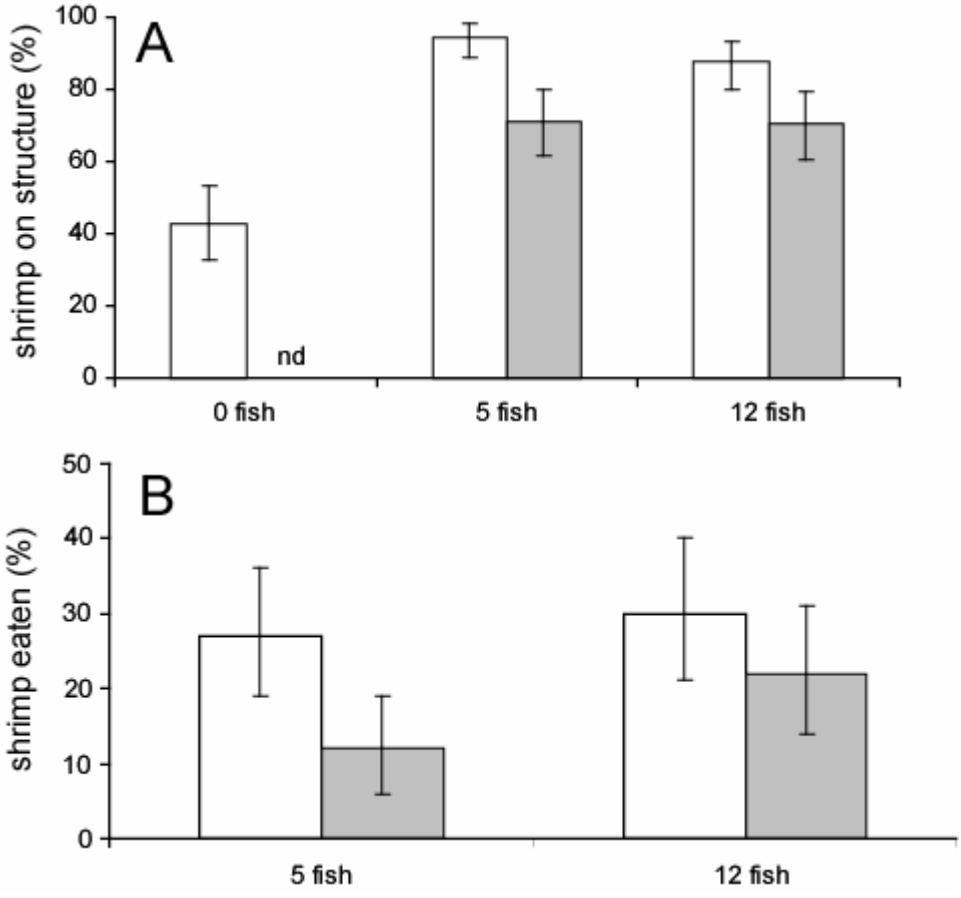


Figure 5.4: Effect of predator : prey density on high complexity habitat

Back-transformed mean (n = 5) percentages (± SE) of shrimp utilizing structure (A) and predation by different densities of striped bass (B) on high complexity structure (experimental category V). White bars indicate a density of 100 shrimp. Grey bars indicate a density of 250 shrimp. The overall effect of striped bass density on grass shrimp distribution was not significant (Two-way ANOVA; $P > 0.05$). The overall effect of grass shrimp density on its distribution was significant ($F_{1,6} = 5.71$; $P = 0.03$). There was no difference in predation rates between treatments. No data = nd.



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