7-28-2014

Effect of Temperature Changes on Competitive and Predator-Prey Interactions in Coastal Epi-Benthic Communities

Joshua P. Lord
University of Connecticut - Avery Point, joshua.p.lord@gmail.com

Effect of Temperature Changes on Competitive and Predator-Prey Interactions in Coastal Epi-Benthic Communities

Joshua Pratt Lord, PhD

University of Connecticut, 2014

Changes in temperature with global warming can facilitate biological invasions, cause species range shifts, alter community composition, and affect the growth rates and competitive abilities of many marine and terrestrial species. However, few studies take into account latitudinal variability in thermal responses or interactions between species. This dissertation focuses on the impact of temperature on predator-prey and competitive relationships in coastal epibenthic marine communities. Eastern oysters (*Crassostrea virginica*) are economically and ecologically important, but their relationship with a major predator, the oyster drill *Urosalpinx cinerea*, is not well understood. Three chapters of this dissertation quantify the effect that increased temperature has on oyster shell growth and metabolism, drill feeding, and oyster inducible defenses. I found that oysters produced thicker shells at experimentally and latitudinally warmer temperatures, and that oyster drills consumed 60% more oysters with just a 4°C increase in seawater temperature above ambient conditions. Interspecific competition was assessed using marine epibenthic fouling communities, which are dominated by invasive species. Species' growth responses to warmer temperature were positive in the northern portions of their latitudinal ranges and negative near the southern limits of their distributions, and I linked this thermal response to competitive outcomes. I also quantified the spread of invasive fouling species and found both summer temperature and commercial shipping to play a large role in the number of invasive fouling species in studied regions along both coasts of North America.

Effect of Temperature Changes on Competitive and Predator-Prey Interactions in Coastal Epi-Benthic Communities

Joshua Pratt Lord

B.A., Colby College, 2008

M.S., University of Oregon, 2010

A Dissertation

Submitted in Partial Fulfillment of the

Requirements for the Degree of Doctor of Philosophy

at the

University of Connecticut

2014

APPROVAL PAGE

Doctor of Philosophy Dissertation

Effect of Temperature Changes on Competitive and Predator-Prey Interactions in Coastal Epi-Benthic Communities

Presented by

Joshua Pratt Lord, B.A., M.S.

Major Advisor _____
Robert B. Whitlatch

Associate Advisor _____
J. Evan Ward

Associate Advisor _____
Hans G. Dam

Associate Advisor _____
Richard W. Osman

University of Connecticut

2014

PREFACE

Each chapter of this dissertation stands alone, but the general introduction and conclusion tie together the overall theme of the work. More detailed introductions are present at the beginning of each chapter; this creates some repetition but facilitates publication while still showing the connection between sections[1]. All chapters describe effects of temperature on individual species or interspecific interactions, but each chapter focuses on a specific element of this: oyster inducible defenses (Chapter 1), oyster drill feeding (Chapter 2), changes in oyster shell thickness and metabolism with latitude (Chapter 3), fouling community thermal growth responses and competition (Chapter 4), and impact of temperature on invasive fouling species (Chapter 5).

[1] All chapters are formatted for the journals in which they are published, in press, or in review: Marine Biology (Chapters 1 and 3), Journal of Experimental Marine Biology and Ecology (Chapter 2), Ecology (Chapter 4), and Diversity and Distributions (Chapter 5). The introduction and general conclusions are formatted in the same way as Chapter 5.

ACKNOWLEDGMENTS

I completed this dissertation with the support and cooperation of many people at the University of Connecticut and around the country. My advisor, Dr. Robert Whitlatch, provided me with academic and financial support, and always encouraged me to pursue the large projects that are a major part of this dissertation but seemed far-fetched at the time. His assistance with planning and designing these projects and with incorporating them into my dissertation was invaluable; it would have been much easier for him to discourage these efforts but he did the opposite and it made all the difference.

My committee was vital in the planning of different parts of my dissertation; Dr. Evan Ward provided valuable expertise on oyster biology that greatly improved the first 3 chapters, Dr. Richard Osman used his expansive knowledge of tunicates and large-scale research projects to assist in planning and analysis of the final 2 chapters, and Dr. Hans Dam kept the research focused on specific hypotheses, something that is difficult with large-scale projects.

All of the latitudinal-scale projects that I conducted with oysters and with fouling communities would not have been possible without the support of many institutions and individuals in these locations that often provided their facilities and expertise free of charge. There are too many people to describe here, but they are mentioned in the acknowledgments of individual chapters. The faculty and staff in the UConn Marine Science Department were extremely helpful, particularly those involved with supporting all of my travel, Janet Laflamme and Pat Evans, and those involved with my experiment planning, Charlie Woods and Jeff Godfrey. Financial support for my projects was provided by my advisor, by several pre-doctoral

fellowships through the Marine Science Department, and by grants from CT SeaGrant (PD-12-17, PD-14-04, PD-14-12).

Fellow graduate students, technicians, and undergraduate interns with Team Benthos assisted me greatly with this project. In particular, Jeremy Calini provided invaluable help with many of my projects over my entire tenure at the University of Connecticut, and he is a great worker and friend.

Most of all, I want to thank Zair Burris, who helped me design many of my experiments, always patiently let me bounce crazy ideas off of her, tolerated my absence of all of my research trips, and always inspires me personally and professionally.

TABLE OF CONTENTS

LIST OF TABLES		**viii**
LIST OF FIGURES		**ix**
INTRODUCTION		**1**
CHAPTER 1	Inducible defenses in the eastern oyster *Crassostrea virginica* Gmelin in response to the presence of the predatory oyster drill *Urosalpinx cinerea* Say in Long Island Sound	**13**
CHAPTER 2	Latitudinal patterns of shell thickness and metabolism in the eastern oyster *Crassostrea virginica* along the east coast of North America	**33**
CHAPTER 3	Impact of temperature and prey shell thickness on feeding of the oyster drill *Urosalpinx cinerea* Say	**59**
CHAPTER 4	Predicting competitive shifts and responses to climate change based on latitudinal distributions of species assemblages	**95**

CHAPTER 5 Influence of seawater temperature and shipping on the spread and establishment of invasive marine fouling species **127**

GENERAL CONCLUSIONS **156**

LIST OF TABLES

Chapter 2

Table 2.1. Feeding rates of *U. cinerea* at different temperatures **54**

Chapter 3

Table 3.1 Site characteristics for all winter and summer oyster sampling sites **85**

Table 3.2 Correlations of oyster shell thickness or metabolism with temperature **86**

Chapter 4

Table 4.1 Regions and physical characteristics of fouling thermal experiment sites **119**

Table 4.2 Equations and fit statistics for tunicate growth responses to temperature **120**

Table 4.3 Competitive scores for pairs of fouling species **121**

Chapter 5

Table 5.1 Site characteristics for all 80 fouling community survey sites **145**

Table 5.2 Mean summer temperature and fouling species richness in 16 sub-regions **146**

Table 5.3 Diversity, shipping and species richness in 8 regions **147**

LIST OF FIGURES

Chapter 1

Figure 1.1 Growth (shell weight) of oysters in predator and control treatments **30**

Figure 1.2 Growth (shell area) of oysters in predator and control treatments **31**

Figure 1.3 Inferred shell thickness of oysters in predator and control treatments **32**

Chapter 2

Figure 2.1 Feeding rates of *U. cinerea* on large and small oysters **55**

Figure 2.2 Seasonal changes in feeding rate of *U. cinerea* **56**

Figure 2.3 Elevated feeding rates of *U. cinerea* in heated treatments **57**

Figure 2.4 Positive correlation between *U. cinerea* feeding and water temperature **58**

Chapter 3

Figure 3.1 Correlation between measured oyster shell weight and immersed weight **87**

Figure 3.2 Map of all oyster survey and experiment sites **88**

Figure 3.3 Correlation between inferred and measured oyster shell thickness **89**

Figure 3.4 Correlation between oyster respiration in different types of respirometers **90**

Figure 3.5 Shell weight and thickness of *C. virginica* at different temperatures **91**

Figure 3.6 Correlation between *C. virginica* shell thickness and seawater temperature **92**

Figure 3.7 Exponential relationship between *C. virginica* respiration and temperature **93**

Figure 3.8 Comparison of heated and ambient metabolic responses of *C. virginica* **94**

Chapter 4

Figure 4.1 Map of fouling community experiment and survey sites **122**

Figure 4.2 Growth rates of fouling species at heated and control temperatures **123**

Figure 4.3 Correlation between latitudinal position and response to higher temperature **124**

Figure 4.4 Thermal performance curves for several fouling species **125**

Figure 4.5 Correlation between competitive ability and relative growth rate **126**

Chapter 5

Figure 5.1 Map of fouling community survey sites **148**

Figure 5.2 Relationship between temperature and number of species **149**

Figure 5.3 Correlation between shipping and number of invasive species **150**

Figure 5.4 Surface plot of invasive species richness with temperature and shipping **151**

Figure 5.5 Map of global shipping traffic **152**

Figure 5.6 Occurrence within latitudinal range for native and invasive species **153**

Figure 5.7 Dendrogram of similarity of fouling assemblages between regions **154**

Figure 5.8 Correlation between distance and pairwise similarity between sites **155**

Introduction

Climate change can cause localized extinctions, shifts in marine and terrestrial communities and is expected to strongly influence ecosystem dynamics, facilitate bioinvasions and cause species range shifts over the next 100 years (Loehle 1998, Sagarin 1999, Helmuth et al. 2002, Hansen et al. 2006, IPCC 2007, Herborg et al. 2009, Sorte and Stachowicz 2011). Coastal seawater temperatures are expected to rise 2-4°C in the northwest Atlantic and 1.5-3°C in the northeast Pacific ocean by 2100, which has particular significance for benthic communities because temperature is the primary factor driving latitudinal shifts in composition of these communities (Engle and Summers 1999, IPCC 2007). Many studies attempting to predict the impact of global warming on marine organisms have been conducted in a single location (e.g. Sagarin 1999, Dijkstra et al. 2011, Sorte and Stachowicz 2011) or have focused on a single species (e.g. Manzi 1970, Dame 1972, Amui-Vedal et al. 2007, Epelbaum et al. 2009, Saunders and Metaxas 2009). Harley et al. (2006) described two of the major goals of future climate research as incorporating community-levels effects and determining the influence of scale on responses to climate. Gilman et al. (2010) explained that studies on individual species do not incorporate interactions between species, and the authors propose a "community module approach" focusing on 2-6 strongly interacting species. In addition, many climate studies fail to establish causation with temperature changes because long-term or latitudinal results are largely correlative and do not incorporate experiments that could elucidate the mechanisms behind observed patterns (Somero 2012). To fill in these gaps, this work focused on the impacts of temperature on complex predator-prey and competitive interactions between species over a

latitudinal gradient. I examined the influence of temperature on the predator-prey relationship between the eastern oyster *Crassostrea virginica* and one of its major predators, the oyster drill *Urosalpinx cinerea.* I did this by measuring oyster growth rates and drill feeding rates at experimentally elevated temperatures. I also assessed community-level effects of increased temperature on the epibenthic fouling community, which is an ideal system for this type of work because it is space limited and driven by differential growth rates and competition between species (Sorte and Stachowicz 2011).

1. Predator-Prey Interactions

The eastern oyster *Crassostrea virginica* is an economically and ecologically important species in estuaries along the east coast of the United States. Oysters are ecosystem engineers that provide refuge from predation for juveniles of many species, remove particulates from the water, and couple the benthic and pelagic environments (Coen et al. 2007). Eastern oysters are present over a wide geographic and temperature range, from New Brunswick to Brazil, although there can be some genetic differentiation between locations within this range (Dittman et al. 1998). Oyster metabolism is strongly linked to temperature, as multiple studies have found higher metabolic rates at experimentally elevated temperatures within individual locations (Dame 1972, Shumway and Koehn 1982, Cranford et al. 2011). However, it has not been established how *C. virginica* or other oyster species respond metabolically to increased temperature in different parts of their geographic range.

Higher temperature also increases oyster shell growth, especially in juveniles that allocate all of their excess energy to growth (Dame 1972, Waldbusser et al. 2011). Shells of *C. virginica* are made of calcite, a softer, less dense, and less soluble form of calcium carbonate than

aragonite, and environmental factors exert control over the carbonate chemistry that limits shell production (Trussell and Etter 2001). Shell deposition can only occur when waters are supersaturated with calcium carbonate (CO_3^{2-}); in addition, ion activity coefficients of calcium and carbonate ions decrease with higher salinity, leading to increased saturation state (Mook and Koene 1975, Mucci 1983). Precipitation is expected to increase and pH is expected to decrease with climate change, which could have detrimental effects on shell formation because lower pH and salinity both decrease saturation state (Waldbusser et al. 2011). However, warmer temperatures have a positive impact on shell deposition because the solubility of both forms of calcium carbonate decrease at higher temperatures, facilitating shell deposition and counteracting some of the negative influences of pH and low salinity (Mucci 1983, Morse and Mackenzie 1990, Waldbusser et al. 2011). Decreased solubility of $CaCO_3$ at warmer temperatures is a likely mechanism behind increased oyster growth rates at experimentally higher temperatures and could also lead to higher growth rates at lower latitudes (Dame 1972, Waldbusser et al. 2011).

Many shell-producing organisms also produce thicker shells in response to the presence of predators, a response referred to as an inducible defense (e.g. Trussell and Etter 2001, Trussell et al. 2003, Newell et al. 2007). In the presence of the blue crab *Callinectes sapidus* and the mud crab *Rhithropanopeus harrisii*, oysters *C. virginica* and *C. ariakensis* produced significantly thicker shells, highlighting the capacity of these oysters to invest in an inducible defense when exposed to predators (Newell et al. 2007).

One of the major predators of the eastern oyster *C. virginica* is the Atlantic oyster drill *Urosalpinx cinerea*, which uses a combination of physical (radula rasping) and chemical (viscic acid) techniques to drill holes in shells of prey (Carriker 1969). Unlike natissid gastropods which only drill in one specific location on prey shells (stereotypy), *U. cinerea* is a muricid gastropod,

which will drill on any available portion of the shell (Federighi 1931, Carriker 1969). In the 1890s, this predator destroyed 90% of the oyster population in the major oyster producing location of Hampton Roads, VA, and caused over $1 million of damage to the oyster industry in Long Island Sound (Federighi 1931). It is native to the east coast of the United States and demonstrates direct development, meaning that it has internal fertilization and then produces eggs capsules that are cemented to rock or shell and from which juveniles eventually drill out and crawl away. The lack of a pelagic larval phase is not a typical characteristic for invasive species, but oysters are often transported great distances through the aquaculture industry, and *U. cinerea* or their egg capsules are often attached to oyster shells that are being transported (Carlton 1992, Eno et al. 1997). Therefore, oyster drills have been introduced in this manner to the west coast of the United States, Europe, and the Netherlands Oosterschelde estuary, a major source of European oysters (Carlton 1992, Eno et al. 1997, Faasse and Ligthart 2007, Buhle and Rueshink 2009). It is considered a threat to oyster aquaculture and to natural populations in these locations and has inhibited native oyster restoration efforts in Willapa Bay, Washington.

Oyster drills consume several shelled species of invertebrates, including barnacles, mussels, clams, snails, and oysters, with highest feeding rates during the summer months (Hanks 1957, Carriker 1969, Franz 1971). They are dormant during the winter, with minimal food intake at temperatures lower than 10°C, indicating a strong temperature dependence in relation to feeding (Hanks 1957, Manzi 1970, Franz 1971). This species of drill prefers spat or juvenile oysters over adults, presumably because of their thin shells, and they can consume up to 200 spat per season, a number that could increase with global warming (Federighi 1931, Hanks 1957). Because of the strong effect of temperature on oyster drill feeding and on oyster growth and

metabolism, this predator-prey relationship was an ideal system to examine trophic shifts that could occur in response to increased seawater temperatures.

2. Competitive Interactions

The impact of temperature on competitive and community-level interactions was assessed in the epibenthic fouling community, which is largely composed of tunicates, bryozoans, and sponges that grow on artificial substrates such as boat hulls, marina pilings, docks, and aquaculture equipment. These organisms have an extraordinary economic impact, as they can account for up to 30% of the costs in some shellfish aquaculture operations and over $21 million annually for the mollusk aquaculture industry (Adams et al. 2011). Ship hull fouling costs the US Navy alone over $15 million annually, or an estimated $1 billion over the next 15 years due to mitigation efforts and increased fuel costs associated with these species (Schultz et al. 2011). Fouling communities in most locations are dominated by invasive species that spread between marinas largely due to human-mediated transport via boat traffic and transport of aquaculture organisms and equipment (Molnar et al. 2008, Sorte and Stachowicz 2011). Invasive species in these communities have proliferated over the last 50 years, as several studies have reported large shifts in fouling communities resulting from increased abundance of these species (Sagarin 1999, Dijkstra et al. 2011, Sorte and Stachowicz 2011). Fouling communities are ideal systems to study competition and interspecific interactions as suggested by Gilman (2010) and Harley (2006) because they are space-limited, which enhances the importance of differential growth rate and competition in driving community composition (Sebens 1986, Sorte and Stachowicz 2011).

Dominant invasive species can numerically or physically outcompete native species, causing local extinctions, reducing overall species diversity, and homogenizing species pools

(Vitousek et al. 1996, Vitousek et al. 1997, Hejda et al. 2009). Global warming is likely to facilitate invasions as well, because introduced species are often more tolerant of a wide range of environmental variables than native species. Many of the same factors that make species successful invaders make them well-suited to deal with climate change (Helmuth et al. 2002, Sorte et al. 2011). Many invasive fouling species are competitively dominant, such as the colonial tunicate *Diplosoma listerianum*, which has overgrown and outcompeted native species for space after being recently introduced in the UK (Vance et al. 2009). Another colonial tunicate that has had a large ecological and economic impact is the encrusting tunicate *Didemnum vexillum*, which is native to Japan (Stefaniak et al. 2009). This species has carpeted benthic environments like cobble-bottom habitats that are typically uninhabited by colonial fouling species (Mercer et al. 2009). There are only a few species of snail that appear to prey on *D. vexillum* in its invaded range, further exacerbating its ecological impact (Carman et al. 2009).

The invasion of *D. vexillum* has recently included cold-water habitats on the west coast of North America such as Sitka, Alaska, where this species has become established (and quarantined) in a single harbor (Cohen et al. 2011). Beyond biodiversity concerns, *D. vexillum* can overgrow mussel, crab, and oyster aquaculture cages, and early eradication is much cheaper than mitigation when dealing with the spread of invasive species (Denny 2008, Dijkstra and Nolan 2011). Even solitary tunicates such as *Ciona intestinalis* can grow at such high densities that they cause breaking of rope lines or direct mussel mortality (Daigle and Herbinger 1999). The highest estimate of the cost of biofouling to the aquaculture industry is $3 billion per year (Fitridge et al. 2012).

While limited control of fouling tunicates and bryozoans can be exhibited by predators such as snails and sea urchins, temperature is likely to exert the strongest control on abundance

of these species (Lodeiros and Garcia 2004, Carman et al. 2009, Whitlatch and Osman 2009). Temperature can influence the reproductive timing of tunicates (Reinhardt et al. 2012) and can have positive or negative impacts on growth rates of different fouling species (Amui-Vedal et al. 2007, McCarthy et al. 2007, Saunders and Metaxas 2009). Individual experiments with bryozoans *Cryptosula pallasiana* and *Membranipora membranacea* indicated higher growth rates at warmer temperatures (Amui-Vedal et al. 2007, McCarthy et al. 2007, Saunders and Metaxas 2009). However, all thermal growth experiments with fouling organisms such as tunicates, bryozoans and sponges have only been conducted in one location each. Therefore, 'species' growth rates and responses to higher temperatures only represent results of experiments conducted in one location. They do not consider differential responses to temperature within species ranges, which would be useful to establish the mechanism behind latitudinal patterns (Somero 2012).

The overall goal of this dissertation was to determine the local and latitudinal-scale effects of increased temperatures on the relationships between strongly interacting species. The predator-prey relationship between the oyster *C. virginica* and oyster drill *U. cinerea* impacts population dynamics of both species, so understanding how temperature affects the link between these species is fundamental to predicting how both with respond to global warming. Chapter 1 focuses on a laboratory experiment that shows an inducible shell thickening response by the oyster *C. virginica* in the presence of the predator *U. cinerea*. Chapter 2 describes changes in *U. cinerea* feeding rates and preference at experimentally elevated temperatures over the course of a feeding season. Chapter 3 looks at the larger scale connection between temperature and oysters, examining oyster shell thickness and metabolic response to increased temperature along a large latitudinal gradient. Chapter 4 presents results from fouling community growth experiments

conducted over a wide geographic range and links these results to shifting competitive outcomes. Chapter 5 describes the factors influencing species diversity and invasions in the fouling community, assessing the role of temperature in introductions and range expansions of invasive species.

References:

Adams C.M., Shumway S.E., Whitlatch R.B., & Getchis T. (2011) Biofouling in marine molluscan shellfish aquaculture: a survey assessing the business and economic implications of mitigation. *Journal of the World Aquaculture Society*, **42**, 242–252.

Amui-Vedel A.-M., Hayward P.J., & Porter J.S. (2007) Zooid size and growth rate of the bryozoan *Cryptosula pallasiana* Moll in relation to temperature, in culture and in its natural environment. *Journal of Experimental Marine Biology and Ecology*, **353**, 1–12.

Buhle E. & Ruesink J. (2009) Impacts of invasive oyster drills on Olympia oyster (*Ostrea lurida* Carpenter 1864) recovery in Willapa Bay, Washington, United States. *Journal of Shellfish Research*, **28**, 87–96.

Carlton J. (1992) Introduced marine and estuarine mollusks of North America: an end-of-the-20th-century perspective. *Journal of Shellfish Research*, **11**, 489–505.

Carman M. (2009) Limited value of the common periwinkle snail *Littorina littorea* as a biological control for the invasive tunicate *Didemnum vexillum*. *Aquatic Invasions*, **4**, 291–294.

Carriker M. (1969) Excavation of boreholes by the gastropod, *Urosalpinx*: an analysis by light and scanning electron microscopy. *American Zoologist*, **9**, 917–933.

Coen L., Brumbaugh R., Bushek D., Grizzle R., Luckenbach M., Posey M., Powers S., & Tolley S. (2007) Ecosystem services related to oyster restoration. *Marine Ecology Progress Series*, **341**, 303–307.

Cohen C.S., McCann L., Davis T., Shaw L., & Ruiz G. (2011) Discovery and significance of the colonial tunicate *Didemnum vexillum* in Alaska. *Aquatic Invasions*, **6**, 263–271.

Cranford P., Ward J., & Shumway S. (2011) Bivalve filter feeding: variability and limits of the aquaculture biofilter. *Shellfish aquaculture and the environment* (ed. by S. Shumway), pp. 81–124.

Daigle R. & Herbinger, C. (2009) Ecological interactions between the vase tunicate (*Ciona intestinalis*) and the farmed blue mussel (*Mytilus edulis*) in Nova Scotia, Canada. *Aquatic Invasions*, **4**, 177–187.

Dame R. (1972) The ecological energies of growth, respiration and assimilation in the intertidal American oyster *Crassostrea virginica*. *Marine Biology*, **17**, 243–250.

Denny C. (2008) Development of a method to reduce the spread of the ascidian *Didemnum vexillum* with aquaculture transfers. *ICES Journal of Marine Science*, **65**, 805–810.

Dijkstra J. & Nolan R. (2011) Potential of the invasive colonial ascidian, *Didemnum vexillum*, to limit escape response of the sea scallop, *Placopecten magellanicus*. *Aquatic Invasions*, **6**, 451–456.

Dijkstra J.A., Westerman E.L., & Harris L.G. (2011) The effects of climate change on species composition, succession and phenology: a case study. *Global Change Biology*, **17**, 2360–2369.

Dittman D., Ford S., & Haskin H. (1998) Growth patterns in oysters, *Crassostrea virginica* , from different estuaries. *Marine Biology*, **132**, 461–469.

Engle V. & Summers J. (1999) Latitudinal gradients in benthic community composition in Western Atlantic estuaries. *Journal of Biogeography*, **26**, 1007–1023.

Eno N., Clark R., & Sanderson W. (1997) *Non-native marine species in British waters: a review and directory.* Joint Nature Conservation Committee, Peterborough.

Epelbaum A., Herborg L.M., Therriault T.W., & Pearce C.M. (2009) Temperature and salinity effects on growth, survival, reproduction, and potential distribution of two non-indigenous botryllid ascidians in British Columbia. *Journal of Experimental Marine Biology and Ecology*, **369**, 43–52.

Faasse M. & Ligthart M. (2007) The American oyster drill, *Urosalpinx cinerea* (Say, 1822), introduced to The Netherlands – increased risks after ban on TBT? *Aquatic Invasions*, **2**, 402–406.

Federighi H. (1931) Salinity death-points of the oyster drill snail, *Urosalpinx cinerea* Say. *Ecology*, **12**, 346–353.

Fitridge I., Dempster T., Guenther J., & de Nys R. (2012) The impact and control of biofouling in marine aquaculture: a review. *Biofouling*, **28**, 649–69.

Franz D. (1971) Population age structure, growth and longevity of the marine gastropod *Urosalpinx cinerea* Say. *The Biological Bulletin*, **140**, 63–72.

Gilman S.E., Urban M.C., Tewksbury J., Gilchrist G.W., & Holt R.D. (2010) A framework for community interactions under climate change. *Trends in ecology & evolution*, **25**, 325–31.

Hanks J. (1957) The rate of feeding of the common oyster drill, *Urosalpinx cinerea* (Say), at controlled water temperatures. *Biological Bulletin*, **112**, 330–335.

Hansen J., Sato M., Ruedy R., Lo K., Lea D.W., & Medina-Elizade M. (2006) Global temperature change. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 14288–14293.

Harley C.D.G., Hughes R.A., Hultgren K.M., Miner B.G., Sorte C.J.B., Thornber C.S., Rodriguez L.F., Tomanek L., & Williams S.L. (2006) The impacts of climate change in coastal marine systems. *Ecology letters*, **9**, 228–41.

Hejda M., Pyšek P., & Jarošík V. (2009) Impact of invasive plants on the species richness, diversity and composition of invaded communities. *Journal of Ecology*, **97**, 393–403.

Helmuth B., Harley C.D.G., Halpin P.M., O'Donnell M., Hofmann G.E., & Blanchette C. a (2002) Climate change and latitudinal patterns of intertidal thermal stress. *Science*, **298**, 1015–7.

Herborg L.-M., O'Hara P., & Therriault T.W. (2009) Forecasting the potential distribution of the invasive tunicate *Didemnum vexillum*. *Journal of Applied Ecology*, **46**, 64–72.

IPCC (2007) Climate Change 2007: Synthesis Report. 52.

Lodeiros C. & García N. (2004) The use of sea urchins to control fouling during suspended culture of bivalves. *Aquaculture*, **231**, 293–298.

Loehle C. (1998) Height growth rate tradeoffs determine northern and southern range limits for trees. *Journal of Biogeography*, **25**, 735–742.

Manzi J. (1970) Combined effects of salinity and temperature on the feeding, reproductive, and survival rates of *Eupleura caudata* (Say) and *Urosalpinx cinerea* (Say)(Prosobranchia: *Biological Bulletin*, **138**, 35–46.

McCarthy A., Osman R.W., & Whitlatch R.B. (2007) Effects of temperature on growth rates of colonial ascidians: A comparison of *Didemnum* sp. to *Botryllus schlosseri* and *Botrylloides violaceus. Journal of Experimental Marine Biology and Ecology*, **342**, 172–174.

Mercer J., Whitlatch R.B., & Osman R.W. (2009) Potential effects of the invasive colonial ascidian (*Didemnum vexillum*) on pebble-cobble bottom habitats in Long Island Sound, USA. *Aquatic Invasions*, **4**, 133–142.

Molnar J.L., Gamboa R.L., Revenga C., & Spalding M.D. (2008) Assessing the global threat of invasive species to marine biodiversity. *Frontiers in Ecology and the Environment*, **6**, 485–492.

Mook W. & Koene B. (1975) Chemistry of dissolved inorganic carbon in estuarine and coastal brackish waters. *Estuarine and Coastal Marine Science*, **3**, 325–336.

Morse J.W. & Mackenzie F.T. (1990) *Geochemistry of Sedimentary Carbonates.* Elsevier B.V., New York.

Mucci A. (1983) The solubility of calcite and aragonite in seawater at various salinities, temperatures, and one atmosphere total pressure. *American Journal of Science*, **283**, 780–799.

Newell R.I.E., Kennedy V.S., & Shaw K.S. (2007) Comparative vulnerability to predators, and induced defense responses, of eastern oysters *Crassostrea virginica* and non-native *Crassostrea ariakensis* oysters in Chesapeake Bay. *Marine Biology*, **152**, 449–460.

Reinhardt J.F., Osman R., & Whitlatch R. (2012) A phenological mid-domain analysis of non-native and native species recruitment richness. *Journal of Ecosystem & Ecography*, **02**, 4–9.

Sagarin R., Barry J., Gilman S., & Baxter C. (1999) Climate-related change in an intertidal community over short and long time scales. *Ecological Monographs*, **69**, 465–490.

Saunders M.I. & Metaxas A. (2009) Effects of temperature, size, and food on the growth of *Membranipora membranacea* in laboratory and field studies. *Marine Biology*, **156**, 2267–2276.

Schultz M., Bendick J., Holm E., & Hertel W. (2011) Economic impact of biofouling on a naval surface ship. *Biofouling*, **27**, 87–98.

Sebens K. (1986) Spatial relationships among encrusting marine organisms in the New England subtidal zone. *Ecological Monographs*, **56**, 73–96.

Shumway S. & Koehn R. (1982) Oxygen consumption in the American oyster *Crassostrea virginica*. *Marine Ecology Progress Series*, **9**, 59–68.

Somero G.N. (2012) The physiology of global change: linking patterns to mechanisms. *Annual Review of Marine Science*, **4**, 39–61.

Sorte C.J. & Stachowicz J. (2011) Patterns and processes of compositional change in a California epibenthic community. *Marine Ecology Progress Series*, **435**, 63–74.

Sorte C.J., Jones S.J., & Miller L.P. (2011) Geographic variation in temperature tolerance as an indicator of potential population responses to climate change. *Journal of Experimental Marine Biology and Ecology*, **400**, 209–217.

Stefaniak L., Lambert G., & Gittenberger A. (2009) Genetic conspecificity of the worldwide populations of *Didemnum vexillum* Kott, 2002. *Aquatic Invasions*, **4**, 29–44.

Trussell G., Ewanchuk P., & Bertness M. (2003) Trait-mediated effects in rocky intertidal food chains: predator risk cues alter prey feeding rates. *Ecology*, **84**, 629–640.

Trussell G.C. & Etter R.J. (2001) Integrating genetic and environmental forces that shape the evolution of geographic variation in a marine snail. *Genetica*, **112-113**, 321–37.

Vance T., Lauterbach L., & Lenz M. (2009) Rapid invasion and ecological interactions of *Diplosoma listerianum* in the North Sea, UK. *Marine Biodiversity Records*, **2**, 1–5.

Vitousek P., D'Antonio C., Loope L.L., & Westbrooks R. (1996) Biological invasions as global environmental change. *American Scientist*, **84**, 468–478.

Vitousek P., Mooney H., Lubchenco J., & Melillo J. (1997) Human domination of Earth's ecosystems. *Science*, **277**, 494–499.

Waldbusser G.G., Voigt E.P., Bergschneider H., Green M.A., & Newell R.I.E. (2011) Biocalcification in the eastern oyster (*Crassostrea virginica*) in relation to long-term trends in Chesapeake Bay pH. *Estuaries and Coasts*, **34**, 221–231.

Whitlatch R.B. & Osman R.W. (2009) Post-settlement predation on ascidian recruits: predator responses to changing prey density. *Aquatic Invasions*, **4**, 121–131.

Chapter 1

Inducible defenses in the eastern oyster *Crassostrea virginica* Gmelin in response to the presence of the predatory oyster drill *Urosalpinx cinerea* Say in Long Island Sound[1]

Abstract

The response of the eastern oyster *Crassostrea virginica* to the presence of the oyster drill *Urosalpinx cinerea* was examined from July to September 2011. Several aspects of oyster growth were measured, including wet weight, shell weight and left (cupped) shell area for oysters collected near Groton, Connecticut (41.32036 N, -72.06330 W). Wet weight and shell weight growth were significantly higher in the presence of the predator *U. cinerea*, while tissue weight showed no difference from the control. The control group showed more shell area growth and a much lower ratio of shell weight growth to shell area growth. Differences in shell weight to area ratio indicated that *C. virginica* dramatically shifted from lateral shell growth to shell thickening in the presence of *U. cinerea*. This inducible defense has not been previously shown for *C. virginica* and could play an important role in the predator-prey interaction between these two species.

Keywords: phenotypic plasticity; size; growth; shell; prey response

[1]Lord J.P. & Whitlatch R.B. (2012) Inducible defenses in the eastern oyster Crassostrea virginica Gmelin in response to the presence of the predatory oyster drill Urosalpinx cinerea Say in Long Island Sound. *Marine Biology*, **159**, 1177–1182.

Introduction

Inducible defenses in response to predation occur in a variety of taxa, including terrestrial plants (Karban and Myers 1989), marine seaweeds (Van Alstyne 1988; Cronin and Hay 1996) and benthic invertebrates (e.g., Harvell 1986; Lively et al. 1986). In most gastropods and bivalves, the shell is the primary defense mechanism and is often modified to defend against predators (Vermeij 1982; Palmer 1985; Appleton and Palmer 1988; Leonard et al. 1999; Trussell and Etter 2001). Gnaus (1974) found that shell thickness decreased with increasing latitude due to elevated $CaCO_3$ deposition rate and higher number of predators in tropical ecosystems. In the intertidal zone of the eastern United States the invasive green crab *Carcinus maenas* has induced thicker shells in multiple prey species including the whelk *Nucella lapillus* (Vermeij 1982; Trussell et al. 2003), the blue mussel *Mytilus edulis* (Leonard et al. 1999), and the snail *Littorina obtusata* (Trussell and Etter 2001). In sand flats of the southeast US, *Busycon* whelks induce changes in the shell growth of the clam *Mercenaria mercenaria* (Nakaoka 2000). These prey species display phenotypic plasticity in that individuals are able to alter their shell growth patterns, in some cases within a couple months (Leonard et al. 1999; Trussell and Etter 2001).

While several studies have focused on inducible shell defenses, few have examined the response to drilling predators and none have tested the shell plasticity of oysters. The eastern oyster *Crassostrea virginica* is common in intertidal and shallow subtidal zones along the northwest Atlantic coast and plays a large part in estuarine ecosystems (Dame 1972). It has great commercial importance in the eastern United States and Canada, where it is farmed as far north as the Gulf of Saint Lawrence in Canada (Singh and Zouros 1978). Eastern U.S. populations have become genetically differentiated between estuaries including the Delaware River, James River, and Long Island Sound, where they grow to different sizes (Dittman 1998). Oyster

growth and shell morphology is more variable than most bivalves, since their axes of growth are not well defined (Palmer and Carriker 1979). The shells of oyster have high phenotypic plasticity, as they can alter shell growth patterns based on substrate, temperature, current, turbidity, and pollution, among other factors (Palmer and Carriker 1979, Polson et al. 2009). While growth varies with location and environmental conditions, *C. virginica* displays continuous growth throughout its life and size is closely tied to fitness (Singh and Zouros 1978).

A major predator of *C. virginica* is the oyster drill *Urosalpinx cinerea*, which can have a dramatic effect on oyster populations. In 1894, *U. cinerea* destroyed 90% of *C. virginica* at oyster farms in Hampton Roads, Virginia, and resulted in one million dollars of damage to oysters in Long Island Sound (Federighi 1931). The oyster drill has recently invaded both Europe and the west coast of North America and shown the ability to consume other oyster species found in these locations (Buhle and Ruesink 2009; Faasse and Ligthart 2009). While *U. cinerea* prefers to prey on oysters, it also consumes blue mussels (*Mytilus edulis*,) slipper limpets (*Crepidula fornicata),* barnacles (*Balanus balanoides*) and bivalves (*Argopecten irradians*, *Mya arenaria*) (Hanks 1957; Carriker 1969; Franz 1971; Pratt 1974; Ordzie and Garofalo 1980). The oyster drill feeds preferentially on smaller oysters and those with thinner shells, although it can drill adult oysters (Federighi 1931; Buhle and Ruesink 2009). The boring process is largely chemical and is facilitated by the application of viscic acid from the accessory boring organ (Carriker 1969). It can take *U. cinerea* up to six days to drill an adult oyster, but by focusing primarily on smaller individuals, one drill can consume up to 200 oysters per year (Federighi 1931).

Despite the frequent co-occurrence of *Crassostrea virginica* and *Urosalpinx cinerea*, little is known about the non-lethal interactions between these species. The goal of this study

was to assess the impact of predation stress on the growth patterns of *C. virginica* by measuring several aspects of *C. virginica* growth with and without the presence of *U. cinerea*. Changes in shell thickness or the energy allocation between shell growth and tissue growth could affect the susceptibility of *C. virginica* to predation. The eastern oyster has not been shown to have inducible defenses, but given the preference of *U. cinerea* for thin-shelled oysters, a shell thickening response could serve as an effective defense. Therefore, we hypothesized that *C. virginica* exposed to *U. cinerea* for an extended period would produce thicker shells. Since *Nucella lapillus* and *Littorina littorea* have both been shown to produce thicker shells at the expense of linear shell growth (Trussell et al. 2003), we also hypothesized that shell thickening in *C. virginica* would result in reduced lateral shell growth.

Materials and methods

Crassostrea virginica were collected (n = 80) intertidally and subtidally between Jupiter Point and Bluff Point in Groton, Connecticut, USA (41.32036 N, -72.06330 W). *Urosalpinx cinerea* were collected (n = 56) in the intertidal zone less than one kilometer from Avery Point, Groton, Connecticut. Both species were collected on June 25, 2011 and were held in flowing seawater tables for 10 days in the University of Connecticut Avery Point Rankin Laboratory prior to the start of the experiment. Epibionts were removed and oysters were individually labeled with Hallprint© shellfish tags super-glued to the side of the shells. Oysters were 3-8 cm in length, with a mean length of 5.5 cm, wet weight of 24.4 grams, and dry shell weight of 15.7 grams.

Several aspects of shell size were measured for *C. virginica* and *U. cinerea* on July 2nd and 3rd 2011 and again at the end of the experiment on September 4th and 5th 2011. Wet weight

was measured on an electronic balance to the nearest 0.01g after specimens were patted dry with paper towels. Immersed weight was also measured in order to estimate shell weight, using methods described by Palmer (1982). A tray was suspended in a bucket of water from the electronic balance and then specimens were placed in this tray to measure immersed weight to 0.01 g. In order to estimate shell weight, 20 specimens of each species were sacrificed, tissue was dissected out, then shells were patted dry with paper towels and weighed. Afterward, shells were placed in a muffle furnace at 500 °C for two hours to remove any remaining organic material and moisture. Dry weight of the shells was then measured and two regressions were plotted, describing the relationships of both dry shell weight (DSW = 1.443 * immersed weight, $r^2 = 0.994$) and wet shell weight (WSW = 2.071 * immersed weight, $r^2 = 0.992$) to immersed shell weight. Coefficients of determination were similar to those described by Palmer (1982) who detailed this immersed weight method. The wet shell weight regression equation was used to estimate wet shell weight from immersed weight for all specimens before and after the experiment. Wet shell weight was used because oyster total wet weight (shell + tissue) was measured, so wet shell weight allowed for the calculation of tissue growth. In addition to these measurements, photos were also taken of the left side of the oysters before and after the experiment, allowing for the digital measurement of shell area in ImageJ© image analysis software.

The experimental units were 12 large round (2.25m diameter, 1m tall) mesocosms with flow-through seawater outside of the Rankin Laboratory. Since the tanks were constantly supplied with raw seawater pumped out of Long Island Sound, oysters were not given additional food. Because chemical predator cues were being tested, six mesocosms were randomly assigned to have predators (*U. cinerea*) and six were used as controls. Oysters were kept in

small (12 cm diameter, 20 cm tall) cylindrical plastic containers with both ends cut out and replaced with 4 millimeter plastic mesh. The predation treatment included four *U. cinerea* inside a smaller plastic cylinder with mesh ends within the prey container. Since this smaller cylinder took up approximately 40% of the space in the container, predator treatments included three oysters per container and controls included five oysters per container. This kept *C. virginica* density constant between treatments, accounting for the smaller volume and ensuring that oysters were not space-limited in the containers. Predators were supplied with two live *C. virginica* within the predator cage to feed on each week, so that neither control nor predator treatments exceeded five oysters per container. Two containers were placed in each of the mesocosms for a total of 12 containers for both the predator and control treatments.

Since the mesocosms were outdoors, the experimental organisms were exposed to natural light cycles and temperature fluctuations over the course of the experiment. The experiment ran for a period of two months from July 4th to September 4th 2011. Temperatures in 3 tanks of each treatment were elevated by immersion heaters (for an unrelated experiment) and were recorded with temperature probes connected to a computer but did not vary more than an average of one degree between tanks over the course of the experiment. Containers rested on the bottom of each of the mesocosms and were kept clear of fouling algae by *Littorina littorea* snails that grazed freely within the mesocosms.

Once measurements were made after the end of the experiment, a one-way ANOVA was employed to compare differences in shell growth between containers and tanks within each treatment. Two-sample T-tests (two-tailed) were run in Minitab© to compare differences in total growth, shell growth, shell area growth, and tissue growth between treatments.

Results

Comparisons of oyster wet shell weight growth showed no significant difference (Two sample t test, $t_{78} = 0.19$, $p = 0.84$) between heated and non-heated tanks. An ANOVA comparing growth between all mesocosms of the same treatment revealed no significant differences in shell growth within control treatments (ANOVA, $F_{(5,46)} = 0.30$, $p = 0.83$) and within predator treatments (ANOVA, $F_{(5,28)} = 0.80$, $p = 0.50$). Because of the lack of differences within treatments, data from all tanks were pooled to allow for a more powerful comparison of the effect of predation on *C. virginica* growth. Underwood (1997) suggested that data from nested designs such as this can safely and effectively be pooled when the null hypothesis cannot be rejected even with an alpha critical value of 0.25. Since p-values within treatments for the current experiment were found to exceed 0.50, we pooled the data from the different tanks within each treatment. Eleven oysters died during the two month period of the experiment, two from the predator treatment (5.6%) and nine from the control treatment (15%). An additional 7 oysters from the control group were not included in the analysis because of data outliers (greater than three standard deviations from mean) due to very small changes in area over the course of the experiment, which resulted in extraordinarily high weight/area ratios that skewed the means. Therefore, for all analyses, the sample size for the predator treatment was 34 and for the control treatment was 46.

Significant differences were found between oysters in predator and control treatments in terms of shell weight growth and shell thickness, as well as total wet weight growth over the course of the experiment. Oysters exposed to *U. cinerea* predation stress increased their wet weight significantly more than the controls, with a predator-treatment mean increase of 1.137g (4.67% of initial weight) and control mean of 0.787 g (3.22%) (Two sample t test, $t_{78} = 2.03$, $p =$

0.046) (Fig. 1.1). This increase was almost exclusively due to a significantly greater increase in wet shell weight for *C. virginica* exposed to predators (X ± SE = 0.818 ± 0.140 g, N = 34, 3.90% increase) compared to the control (X ± SE = 0.399 ± 0.086 g, N = 46, 1.90%) (Two sample t test, $t_{78} = 2.67$, $p = 0.009$) (Fig. 1.1). Wet shell weight was used because it was measured in the same manner as total wet weight and thus could be directly used to calculate wet tissue weight. The difference in dry shell weight between predator (mean = 0.570, 3.64% increase) and control (mean = 0.278, 1.78%) treatments was also highly significant ($t_{78} = 2.67$, $p = 0.009$). There was no significant difference in tissue (non-shell) growth between the two treatments over the experimental period (control mean = 0.388g, predation mean = 0.319g, $p = 0.50$).

Despite the greater shell weight gain in the predator treatment, oysters in the control group displayed more lateral areal shell growth of the left side of the shell (predator mean = 1.10 cm^2, control mean = 1.44 cm^2), but this difference was not significant with a critical value of 0.05 (Two sample t test, $t_{78} = 1.61$, $p = 0.11$) (Fig. 1.2). To estimate the amount of shell deposition allocated to thickening the shell, shell thickness was approximated as wet shell weight (g) per square centimeter of cupped (left) shell area. Figure 3 shows significant differences in shell thickness (ratio of shell weight growth to shell area growth) between control ($\bar{x}$ ± SE = 0.19 ± 0.24 g/cm^2, N = 46) and predator ($\bar{x}$ ± SE = 1.91 ± 0.71 g/cm^2, N = 34) treatments (Two sample t test, $t_{78} = 2.57$, $p = 0.012$), with oyster exposed to predators demonstrating a 10-fold increase. Similar results were found with thickness estimated from the ratio of dry shell weight growth to shell area growth, with a control mean of 0.14 g/cm^2 and predator mean of 1.33 g/cm^2 (Two sample t test, $t_{78} = 2.57$, $p = 0.012$). Variability shown in error bars of figure 3 was relatively high due to the inclusion of two variables: left shell area and shell weight, but did not adversely affect the significance of the results.

Discussion

The eastern oyster *Crassostrea virginica* showed a distinct response to predation stress induced by the oyster drill *Urosalpinx cinerea*. The cue involved is likely chemical and not tactile because predator and prey were kept spatially segregated within experimental containers. It is unknown whether the chemosensory response of *C. virginica* was due solely to the presence of *U. cinerea* or was related to attacks on oysters within the predator cages. The clam *Mercenaria mercenaria* changed shell growth patterns solely due to chemical cues of a *Busycon* whelk (not damaged conspecifics), so we predict that the response of *C. virginica* is also a result of chemical cues from the predator *U. cinerea* (Nakaoka 2000). The response to a chemical cue is similar to those described for crab predator stress induction on mussels (Leonard et al. 1999) and snails (Vermeij 1982; Palmer 1985; Trussell and Etter 2001).

The presence of the predatory whelk *U. cinerea* caused an increase in the wet weight growth of *C. virginica* (Fig. 1.1), but the difference between the predator treatment and the control was entirely explained by shell weight growth patterns (Fig. 1.1). Since there was no significant difference in tissue (non-shell) growth between predator and control treatments, it appears that the energy required to produce increased shell mass was not due to a change in allocation between shell and tissue growth. If this had been the case, increased shell weight would have been accompanied by a decrease in tissue growth during the two month span of this study. However, it is possible that tissue growth could show different long-term patterns with regard to shell deposition, since body growth is limited by the size of the shell (Trussell and Etter 2001).

The higher rate of *C. virginica* shell weight growth (Fig. 1.1) in the predator treatment indicates that calcification rate was likely not a limiting factor. The fact that shell weight growth

increased without a corresponding decrease in tissue weight growth rate could be due to several factors beyond the scope of this study, including increased feeding rate, increased metabolism, changes in allocation to reproductive tissue, or other physiological changes that could enhance shell deposition rate (McIntosh and Townsend 1996; Schmitz et al. 1997; Turner et al. 2000; Peacor and Werner 2001).

Greater lateral shell growth in the control treatment (Fig. 1.2) is most likely due to the switch to shell-thickening processes in the predator treatment due to the perceived threat of *U. cinerea* predation. Lateral shell growth in the absence of predators indicates that this is the typical summer growth pattern for *C. virginica*, which makes sense because lateral growth increases the volume of the oyster, unlike shell thickening. This increased internal size allows for greater tissue growth, higher feeding rates and higher fecundity (Peters 1983; Sebens 1987; Arendt 1997).

Shell thickness was approximated from the ratio of shell weight to left shell area. This is not a direct measure of thickness but does indicate the amount of shell material per unit area; since bivalve shells are deposited in accretionary layers shell density cannot be altered after layers are deposited. Therefore, higher shell weight growth (Fig. 1.1) and lower shell area growth (Fig. 1.2) in predator-exposed *C. virginica* indicated a clear increase in interior shell deposition or shell thickness. Even if there were a disparity in new shell deposit density between the two treatments, the ten-fold difference in shell weight growth to shell area growth ratio (Fig. 1.3) is so large that small density alterations would not impact the overarching shift from lateral shell growth to shell thickening. Given the two-month period of this study, it is unknown if *C. virginica* shell thickness would continue to increase with many months or years of exposure to

U. cinerea, or if there is an optimization of shell thickness after which there is little benefit to continued thickening with regard to *U. cinerea* predation.

While the full extent and timing of *C. virginica* response to *U. cinerea* is unclear, it is clear that oyster inducible defenses allow it to produce thicker shells in response to the threat of predation by *U. cinerea*. As with other types of phenotypic plasticity and inducible defenses, the ability of *C. virginica* to only produce more shell and thicker shell in the presence of predators provides several potential energetic advantages (Vermeij 1982; Appleton and Palmer 1988; Leonard et al. 1999; Trussell and Etter 2001). By not always producing thick shells, the eastern oyster may be able to put more energy into lateral shell growth (Fig. 1.2) that rapidly increases the size of the organism and thus has many ecological benefits such as increased feeding rate and reproduction (Peters 1983; Sebens 1987). Allocation of energy to processes other than shell-thickening could allow for greater lateral shell growth directly or provide energy necessary for the growth of the mantle, which facilitates lateral shell extension (Ren and Ross 2001).

Since oysters did not grow as much in terms of shell weight in the absence of predators, they may also be able to allocate more energy towards tissue growth, reproduction, feeding, or other non-defensive energetic costs. Feeding rate could have other implications as well, as Bourdeau (2010) found that an inducible shell thickening response in a marine snail (*Nucella lamellosa*) was actually a behavioral response that resulted in decreased feeding in the presence of predators. While oysters may alter feeding rates in response to the threat of predation, they do not need to move around to feed as snails do and feeding may not increase the threat of predation by a slow-moving drill (unlike snails with crab predation). Since *C. virginica* in this experiment displayed a response to a drilling predator, the most probable reason behind increased shell thickness is that it may decrease mortality rate in an environment with exposure to *U. cinerea.*

Since the presence of *U. cinerea* induced shell thickening and inhibited lateral shell growth, we predict that *C. virginica* populations in areas with high densities of *U. cinerea* would be smaller in terms of shell area than similar populations with few *U. cinerea*. Calcification rate changes with temperature as well and several mollusks have been shown to grow shell faster in warmer temperatures, so it is possible that the inducible defenses of *C. virginica* change with latitude (Gnaus 1974; Trussell and Etter 2001; Kawai 2009; Miyaji 2010). If this is the case, then environmental changes such as increased ocean temperatures or dissolved CO_2 levels could impact the shell production of *C. virginica* and facilitate (warming) or inhibit (CO_2) the ability of this oyster to produce thicker shells in response to the presence of predators such as *U. cinerea* (Dame 1972; Newell and Kofoed 1977; Gazeau et al. 2010; Thomsen and Melzner 2010). Recent invasion of Europe and western North America by *U. cinerea* highlights the importance of understanding prey response to this whelk, as multiple species of oysters will be exposed to this novel predation threat over the next several years as the distribution of *U. cinerea* spreads (Buhle and Ruesink 2009).

Acknowledgements

Thanks to Jeremy Calini and Wendy Turek, who were instrumental in the design, setup and execution of this experiment. Additional thanks to Zair Burris for assistance with pre-experimental measurements. The project was funded by grants from Connecticut Sea Grant and U.S. EPA – STAR programs.

References

Appleton RD, Palmer AR (1988) Water-borne stimuli released by predatory crabs and damaged prey induce more predator-resistant shells in a marine gastropod. Proc Nat Acad Sci USA 85:4387-4391.

Arendt JD (1997) Adaptive intrinsic growth rates: an integration across taxa. Q Rev Biol 72:149-177.

Bourdeau PE (2010) An inducible morphological defence is a passive by-product of behaviour in a marine snail. Proc R Soc B 277:455-462.

Buhle ER, Ruesink JL (2009) Impacts of Invasive Oyster Drills on Olympia Oyster (*Ostrea lurida* Carpenter 1864) Recovery in Willapa Bay, Washington, United States. J Shell Res 28:87-96.

Carriker MR (1969) Excavation of boreholes by the gastropod, *Urosalpinx*: an analysis by light and scanning electron microscopy. Am Zool 9(3): 917-933.

Cronin G, Hay ME (1996) Induction of seaweed chemical defenses by amphipod grazing. Ecology 77:2287-2301.

Dame, RF (1972) The ecological energies of growth, respiration and assimilation in the intertidal American oyster *Crassostrea virginica*. Mar Biol 17:243-250.

Dittman DE, Ford SE, Haskin HH (1998) Growth patterns in oysters, *Crassostrea virginica*, from different estuaries. Mar Biol 132:461-469.

Faasse M, Ligthart M (2009) American (*Urosalpinx cinerea*) and Japanese oyster drill (*Ocinebrellus inornatus*) (Gastropoda:Muricidae) flourish near shellfish culture plots in the Netherlands. Aquat Inv 4:321-326.

Federighi H (1931) Studies on the oyster drill. US Department of Commerce Bureau of Fisheries. Bulletin # 4.

Franz DR (1971) Population age structure, growth and longevity of the marine gastropod *Urosalpinx cinerea* Say. Bio Bull 140:63-72.

Gazeau F, Gattuso JP, Dawber C, Pronker AE, Peene F, Peene J, Heip CHR, Middelburg JJ (2010) Effect of ocean acidification on the early life stages of the blue mussel *Mytilus edulis*. Biogeosciences 7:2051-2060.

Hanks JE (1957) The rate of feeding of the common oyster drill, *Urosalpinx cinerea* (Say), at controlled water temperatures. Bio Bull 112(3):330-335.

Harvell CD (1986) The ecology and evolution of inducible defenses in a marine bryozoan: cues, costs, and consequences. Am Nat 128(6):810-823.

Karban R, Myers JH (1989) Induced plant responses to herbivory. Ann Rev Ecol Syst 20:331-348.

Kawai K (2009) Shell growth, reproduction and mortality of *Ovula ovum* in southern Kyushu, Japan. J Moll Stud 75:35-40.

Leonard GH, Bertness MD, Yund PO (1999) Crab predation, waterborne cues, and inducible defenses in the blue mussel, *Mytilus edulis*. Ecology 80(1):1-14.

Lively CM (1986) Predator-induced shell dimorphism in the acorn barnacle *Chthamalus anisopoma*. Evolution 40:232-242.

McIntosh AR, Townsend CR (1996) Interactions between fish, grazing invertebrates and algae in a New Zealand stream: a trophic cascade mediated by fish-induced changes to grazer behaviour? Oecologia 108(1):174-181.

Miyaji T, Tanabe K, Matsushima Y, Sato S, Yokoyama Y, Matsuzaki H (2010) Response of daily and annual shell growth patterns of the intertidal bivalve *Phacosoma japonicum* to Holocene coastal climate change in Japan. Palaeogeogr Palaeocl 286:107-120.

Nakaoka M (2000) Nonlethal effects of predators on prey populations: predator-mediated change in bivalve growth. Ecology 81(4):1031-1045.

Newell RC, Kofoed LH (1977) Adjustment of the components of energy balance in the gastropod *Crepidula fornicata* in response to thermal acclimation. Mar Biol 44:275-286.

Ordzie CJ, Garofalo GC (1980) Predation, attack success, and attraction to the bay scallop, *Argopecten irradians* (Lamarck) by the oyster drill, *Urosalpinx cinerea* (Say). J Exp Mar Biol Ecol 47(1):95-100.

Palmer AR (1981) Do carbonate skeletons limit the rate of body growth? Nature 292:150-152.

Palmer AR (1982) Growth in marine gastropods: a non-destructive technique for independently measuring shell and body weight. Malacologia 23:63-73.

Palmer AR (1985) The adaptive value of shell variation in *Thais lamellosa*: effect of thick shells on vulnerability to prey and to preferences by crabs. Veliger 27:349-356.

Palmer AR (1992) Calcification in marine molluscs: how costly is it? Proc Nat Acad Sci USA 89:1379-1382.

Palmer AR, Carriker MR (1979) Effects of cultural conditions on morphology of the shell of the oyster. Proc Natl Shellfish Assoc 69:58-72.

Peacor SD, Werner EE (2001) The contribution of trait-mediated indirect effects to the net effects of a predator. Proc Nat Acad Sci 98(7):3904-3908.

Peters RH (1983) The ecological implications of body size. Cambridge University Press, New York.

Polson MP, Hewson WE, Eernisse DJ, Baker PK, Zacherl DC (2009) You say *conchaphila,* I say l*urida*: molecular evidence for restricting the Olympia oyster (*Ostrea lurida* Carpenter 1864) to temperature western North America. J Shell Res 28:11-21.

Pratt DM (1974) Behavioral defenses of *Crepidula fornicata* against attack by *Urosalpinx cinerea*. Mar Biol 27:47-49.

Ren JS, Ross AH (2001) A dynamic energy budget model of the Pacific oyster *Crassostrea gigas*. Ecol Model 142:105-120.

Sebens KP (1987) The ecology of indeterminate growth in animals. Ann Rev Ecol Syst 18:371-401.

Schmitz OJ, Beckerman AP, O'Brien KM (1997) Behaviorally mediated trophic cascades: effects of predation risk on food web interactions. Ecology 78(5):1388-1399.

Singh SM, Zouros E (1978) Genetic variation associated with growth rate in the American oyster (*Crassostrea virginica*). Evolution 32(2):342-353.

Thomsen J, Melzner F (2010) Moderate seawater acidification does not elicit long-term metabolic depression in the blue mussel *Mytilus edulis*. Mar Biol 157:2667-2676.

Trussell GC, Etter RJ (2001) Integrating genetic and environmental forces that shape the evolution of geographic variation in a marine snail. Genetica 112-113:321-337.

Trussell GC, Ewanchuk PJ, Bertness MD (2003) Trait-mediated effects in rocky intertidal food chains: predator risk cues alter prey feeding rates. Ecology 84(3):629-640.

Turner AM, Bernot RJ, Boes CM (2000) Chemical cue modify species interactions: the ecological consequences of predator avoidance by freshwater snails. Oikos 88(1):148-158.

Underwood AJ (1997) Experiments in ecology: their logical design and interpretation using analysis of variance. University Press, Cambridge.

Van Alstyne KL (1988) Herbivore grazing increases poly-phenolic defenses in an intertidal brown alga, *Fucus distichus*. Ecology 69:655-663.

Vermeij GJ (1982) Phenotypic evolution in a poorly dispersing snail after arrival of a predator. Nature 299:349-350.

Figure Captions

Figure 1.1. Comparison of wet weight and wet shell weight gain over a period of two months between *C. virginica* in the control treatment and those exposed to the predator *U. cinerea*. Both wet weight and wet shell weight growth were significantly higher when oysters were exposed to predation (±SE bars)

Figure 1.2. Comparison of left shell area growth over a period of two months between *C. virginica* in the control treatment and those exposed to the predator *U. cinerea*. Oysters in the absence of *U. cinerea* grew more in terms of left shell area but not significantly more ($p = 0.11$) (±SE bars)

Figure 1.3. Comparison of wet shell weight growth to shell area growth ratio between *C. virginica* in the control treatment and those exposed to the predator *U. cinerea*. Oysters in the presence of *U. cinerea* for two months showed a ten-fold increase in this approximation of shell thickness and had a significantly higher wet shell weight growth to shell area growth ratio than oysters in the control group (±SE bars)

Figure 1.1 (see caption on p. 29)

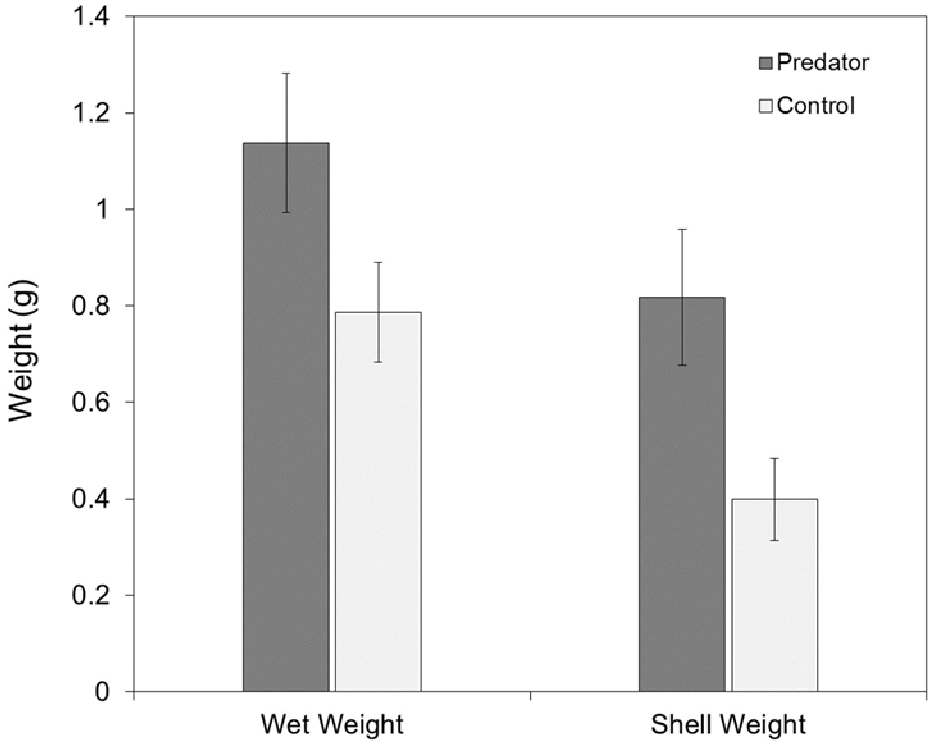


Figure 1.2 (see caption on p. 29)

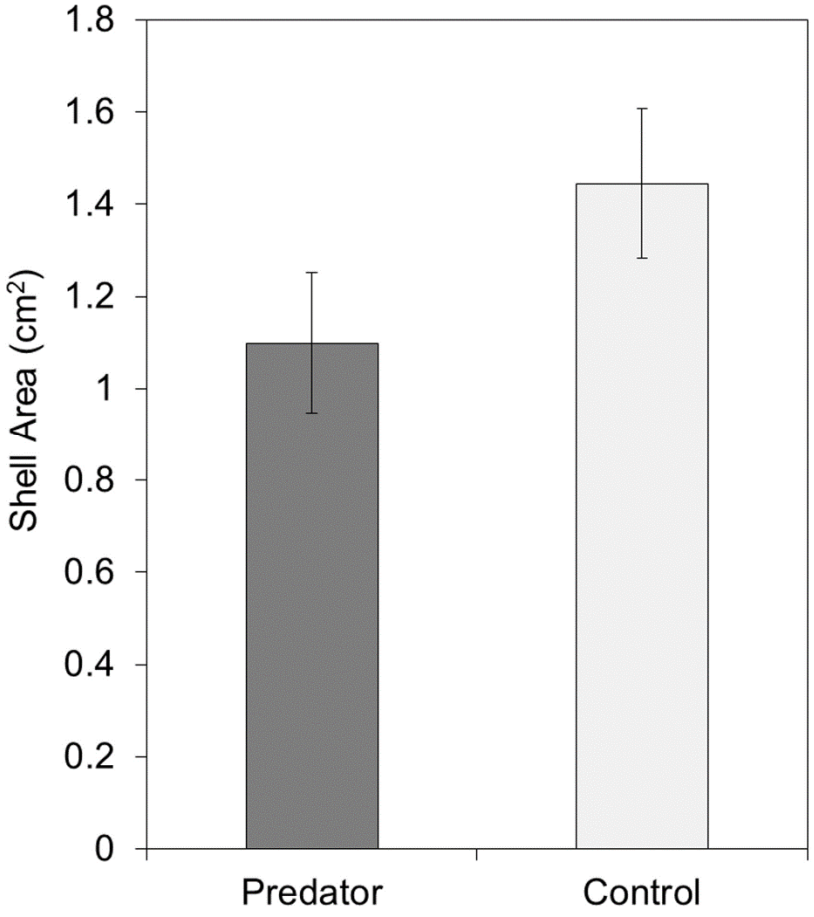
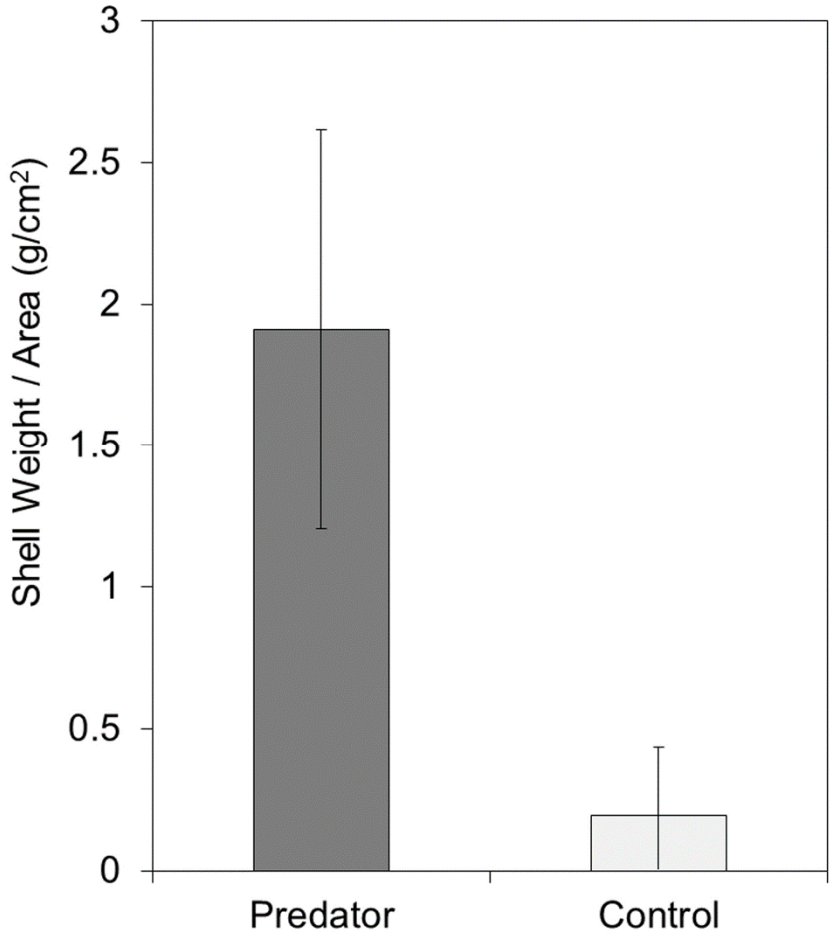


Figure 1.3 (see caption on p. 29)



Chapter 2

Impact of temperature and prey shell thickness on feeding of the oyster drill *Urosalpinx cinerea* Say[2]

Abstract

Feeding rates and selection of the oyster drill *Urosalpinx cinerea* were investigated with several long term temperature alteration experiments. Oyster drill prey selection was assessed in terms of prey (oyster) size and shell thickness. Drills fed on oysters with thinner shells 86% of the time and selectively preyed on smaller (1g wet weight) oysters during colder months and larger (10g wet weight) oysters during warmer months. Analysis of *U. cinerea* seasonal feeding patterns showed virtually no feeding during winter months (January to March) and a significant increase to over 1 gram of prey tissue per week during summer (July to September). A nine month experiment compared feeding rates of *U. cinerea* at two different temperature levels: ambient seawater (control) and temperatures elevated 4°C above ambient. Oyster drills showed a 60% increase in feeding rate in the warmer treatment, especially during late summer, fall and early winter. Both temperature manipulation and annual feeding experiments revealed a significant temperature dependence of *U. cinerea* feeding rates. Seasonal or long-term seawater temperature

---

[2]Lord J. & Whitlatch R. (2013) Impact of temperature and prey shell thickness on feeding of the oyster drill Urosalpinx cinerea Say. *Journal of Experimental Marine Biology and Ecology*, **448**, 321–326.

changes related to climate change could strongly affect the predator-prey relationship between *U. cinerea* and its prey.

Keywords: feeding; oyster; oyster drill; season; shell thickness; temperature

Introduction

Climate change is expected to have a variety of impacts on marine coastal and estuarine communities as seawater temperatures increase, with many changes due to modified interactions between species (Gilman et al., 2010; Kordas et al., 2011; Stachowicz et al., 2002). Changes in temperature can affect community composition in multiple ways, both directly and indirectly. Mortality due to temperatures above the thermal limit of a species is a direct result and is especially common in intertidal habitats that are regularly exposed to thermal extremes (Helmuth et al., 2002). Reduced performance can also be a direct result of elevated temperatures, as metabolic stress may be induced. Indirect effects of temperature on communities can be tied to changes in growth rate, notably in calcifying organisms that increase growth rates due to decreased solubility of calcium carbonate in warmer waters (Morse and Mackenzie, 1990). This has been observed in groups of organisms including clams, oysters, barnacles and others (Dame, 1972; Irie, 2005; Kanazawa and Sato, 2008; Kawai, 2009; Schöne et al., 2006). Feeding is also strongly related to temperature in a variety of organisms including stinkbugs (Mahdian et al., 2006), zooplankton (Loiterton et al., 2004), fish (Elliott and Leggett, 1996), crabs (Sanchez-Salazar et al., 1987), and sea stars (Pincebourde et al., 2008; Sanford, 1999). Changes in predation rate with temperature are well-documented in marine gastropods as well (Edwards and Huebner, 1977; Garton and Stickle, 1980; Hanks, 1957; Manzi, 1970).

The predator-prey relationship between the eastern oyster *Crassostrea virginica* Gmelin and oyster drill *Urosalpinx cinerea* Say is relevant not only to oyster populations but to the oyster aquaculture industry and oyster restoration projects. Oysters are ecologically important ecosystem engineers that provide feeding habitat, larval hatcheries, sediment stabilization and benthic-pelagic coupling, among other valuable ecosystem services (Coen et al., 2007). Several species of crabs and oyster drills prey on *C. virginica*, which has evolved inducible defenses to ameliorate this predation threat. Eastern oysters produce thicker shells in the presence of predators including the blue crab *Callinectes sapidus*, mud crab *Rhithropanopeus harrisii*, and oyster drill *U. cinerea* (Lord and Whitlatch, 2012; Newell et al., 2007) and show a size-dependent morphological response to the mud crab *Panopeus herbstii* (Johnson and Smee, 2012). Presumably, thicker shells lead to lower predation rates similar to those observed for crabs in response to littorine snail inducible defenses (Trussell et al., 2003). Oysters can also produce thicker shells in response to elevated temperatures due to higher calcification rates in warmer water (Waldbusser et al., 2007).

The oyster drill *U. cinerea* is a major oyster predator throughout its range, which is rapidly expanding due to introductions facilitated by oyster transport. These drills can consume a wide array of prey including clams, scallops, mussels, slipper limpets and barnacles, but feed most notably on oysters (Carriker, 1969; Franz, 1971; Ordzie and Garofalo, 1980). Oyster drills have had a large historical impact on oyster populations, causing over $1,000,000 of damage to oysters in Long Island Sound in 1894 and destroying 90% of the oysters in Hampton Roads, Virginia, around the same time (Federighi, 1931). Their impact continues today on natural and artificial reefs, as they have been a factor inhibiting restoration of the Olympia oyster in Willapa Bay, Washington, USA (Buhle and Ruesink, 2009). The range of *U. cinerea* has expanded with

accidental introductions from its native range on the east coast of North America to include the west coast of North America, the United Kingdom, and the Netherlands (Carlton, 1992; Eno et al., 1997; Faasse and Ligthart, 2007). Oyster drills can consume multiple species of oyster including the Olympia (*Ostrea lurida*), eastern (*C. virginica*), Pacific (*C. gigas*), and flat oyster (*Ostrea edulis*) (Buhle and Ruesink, 2009).

A single *Urosalpinx cinerea* can consume up to 200 juvenile oysters (spat) in one season, with an average of one adult oyster per week during the summer (Federighi, 1931; Hanks, 1957; Manzi, 1970). Short-term experiments at constant experimental temperatures have resulted in higher feeding rates at warmer temperatures (Manzi, 1970). This suggests that drill feeding patterns may be altered by seasonal and long-term temperature changes as well. During winter along the northeastern US coast, *U. cinerea* move and feed very little (Carriker, 1969). The temperature below which feeding stops varies between studies but generally ranges from 6.5 to 10°C (Carriker, 1969; Hanks, 1957). Similar temperature-related dormancy is displayed by other gastropods including the oyster drill *Stramonita haemastoma* (Garton and Stickle, 1980) and moon snail *Polinices duplicatus* (Edwards and Huebner, 1977). Since the coastal waters off New England are predicted to experience a 2-4°C increase in seawater temperature by 2100 (IPCC, 2007), climate changes could result in longer feeding seasons for *U. cinerea* and these other gastropods.

In order to test the impact of prey shell thickness and water temperature on *U. cinerea* feeding patterns, four hypotheses were tested: (1) *U. cinerea* will feed primarily on thin-shelled oysters; (2) drills will consume smaller oysters over larger oysters as described by Federighi (1931) regardless of seasonal changes in water temperature, (3) at temperatures elevated 4°C above seasonal ambient water temperatures, *U. cinerea* will consume more oysters than the

control; (4) at elevated temperature, *U. cinerea* will have a longer feeding season compared to control conditions. Addressing these hypotheses will provide insight into alterations in the predator-prey relationship between oysters and oyster drills and how they may be altered with changing climate.

Materials and Methods

Prey Shell Thickness

In order to determine the effect of prey shell thickness on feeding of the oyster drill *Urosalpinx cinerea*, 200 oysters were collected near Jupiter Point, Connecticut, USA (41.31958 N, 72.055828 W), in January 2012. All oysters were tagged with Hallprint® plastic shellfish tags, photographed, weighed on an electronic balance and weighed immersed in water. The immersed weight measurements were correlated with shell weights from 20 dissected oysters to establish a linear relationship between immersed weight and shell weight (shell weight = 1.544 x immersed weight) in order to estimate shell weights for live oysters (after Brookes and Rochette, 2007; Palmer, 1982). Photographs of the left (cupped) shell of the oysters were measured in terms of area using the image analysis program ImageJ (NIH). From these measurements, shell thickness was estimated by dividing shell weight by shell area; this estimation method has been found to accurately represent shell thickness in eastern oysters (Lord and Whitlatch, 2012). From 200 measured oysters, individuals with the 50 thickest (mean 1.16 g/cm^2) and 50 thinnest (mean 0.50 g/cm^2) shells were selected for this experiment. There was no significant difference in left shell area between the two groups (thick mean = 17.5 cm^2, SE = 0.59, thin mean = 17.2 cm^2, SE = 0.38).

Oyster drills were collected intertidally at Avery Point, Groton, Connecticut, USA, in January 2012, with a mean weight of 1.61g and 2.13cm in length. Experiments were conducted in flowing seawater tanks at the University of Connecticut Avery Point Rankin Lab. One oyster drill was placed in each of 14 plastic containers (15cm diameter, 20 cm height) with ends made of 0.6 cm aperture plastic mesh, which allowed water to flow through the container. In each container, three thick-shelled and three thin-shelled oysters were haphazardly placed on the bottom with a single *U. cinerea* that had been starved for two weeks prior to the start of the experiment. Experiments ran from February 1 through June 6, 2012, with seawater temperatures ranging from 6°C in February to 15°C in June. During this time every container was checked weekly for oyster mortality. All dead oysters over the course of the experiment showed drill holes in their shell, definitively indicating the cause of mortality. Each week, oysters were added to the containers to maintain the ratio of 3 thick and 3 thin-shelled oysters for each drill to choose from. At the end of the experiment, the percent of total *U. cinerea* predation that occurred on thick and thin-shelled oysters was calculated individually for each container. Percentage data are not normally distributed, so percentages of thick and thin-shelled oyster predation were arc-sine transformed and then compared statistically with a paired t-test.

Prey Size

Oyster drill feeding was quantified at ambient seawater temperatures in a flowing seawater table in order to determine feeding rates over an entire year and to test prey size selection. One oyster drill was placed in each of 12 cylindrical mesh-ended containers (6cm diameter, 10 cm length) with 6 oysters. Drills were provided with a choice of three small (~1g wet wgt., 2 cm length) and three large (~10g wet wgt., 5 cm length) oysters from the start of the

experiment in February 2012 through the end of June, a five month experimental period for the size choice experiment. Feeding experiments were continued through January 2013 for a complete one year record of feeding rates, but only large oysters were used after June 2012. Experiments were checked weekly, with any dead oysters replaced with live oysters of similar size. This experiment was conducted solely in flowing seawater at ambient temperatures and the numbers of small and large oysters consumed was compared with a paired t-test.

Temperature and Predation

This experiment assessed the impact of elevated temperature on *U. cinerea* feeding rates. Oyster drills were collected intertidally at Avery Point in Groton, Connecticut, and oysters were obtained from Fisher's Island Oyster Farm. Experimental units were 16 cylindrical tanks (15 cm diameter, 30 cm height), each aerated and with an independent seawater supply from the flowing seawater system in the Rankin Lab at the University of Connecticut Avery Point. Each tank contained two drills and 5 oysters (~10g, 5 cm length), with an additional drill and three oysters in a small (6 cm diameter, 10 cm height) container with mesh ends within each tank. This smaller container was used in order to accurately quantify individual *U. cinerea* feeding rates. The isolated drills in small containers were the only ones used in the statistical comparison of feeding rates between temperature treatments, since drill-specific feeding rates could be quantified. There was no prey size component to this experiment in order to standardize feeding rates and drilling effort across treatments.

Temperature was controlled with water baths surrounding the experimental tanks; control tanks were held in a large (1m x 2m) flowing seawater table at ambient seawater temperature. Elevated temperature tanks were kept in 30 cm x 50 cm insulated containers with 1000-watt aquarium heaters that used digital Aqualogic® temperature controllers to maintain water

temperatures at 4 °C above ambient seawater temperatures. The 4 °C interval was selected because it is the maximum ocean temperature increase expected in along the east coast of North America in the next 100 years and because it would push summer water temperatures above those occasionally experienced in the field. There was no water exchange with the heated or control water baths, so tanks remained independent, and only differentiated by the seawater temperature. Tanks were constantly aerated and water was changed daily in order to keep oysters alive until they were consumed by drills.

Temperature feeding experiments ran for seven months between June 15, 2012, and January 8, 2013. Tanks were checked weekly for predation from June-September, after which they were checked bi-weekly because *U. cinerea* feeding rates had slowed. Every time feeding was checked, shells of consumed oysters were removed and new oysters were placed in the experimental unit to maintain the same number of prey over the duration of the experiment. Shell wet weight was measured for consumed oysters and was converted to wet tissue weight through a linear regression between shell weight and tissue weight from 20 dissected oysters (tissue wt. = 0.283 x shell wt). Therefore, feeding was measured in both number of oysters consumed per week and tissue weight of oysters consumed per week. Temperature was also recorded at every measurement interval in order to plot feeding rate as a function of temperature for both control and elevated temperature treatments. Since individual drills often did not feed for 1-2 weeks following consumption of a large oyster, feeding data were combined into one month bins for analysis of feeding patterns. This method more accurately captured feeding patterns and minimized excessive influence of zero values in each feeding and measurement interval. Feeding rates in heated and control treatments for each month were statistically compared with t-tests to test for increased feeding at warmer temperatures.

Results

Prey Shell Thickness

During the four month experiment, oyster drills (*U. cinerea*) consumed a significantly higher percent of thin-shelled (86%) than thick-shelled (14%) oysters (Paired t-test, $t = 6.50$, $p < 0.001$). A total of 45 oysters were drilled and eaten by *U. cinerea* and 38 of these individuals were in the thin shelled group, with only 7 thick-shelled oysters eaten over the course of the experiment.

Prey Size

Oyster drills showed a strong and significant selection of smaller oysters (~1g wet wgt.) from February 15 through May 16, 2012 (small mean consumption = 2.25 oysters (SE=0.37), large mean = 0.42 (SE=0.19)) (two-tailed paired T-test, $T_{11} = -4.52$, $p < 0.001$) (Fig. 2.1). No larger (~10g wet wgt.) oysters were consumed until water temperatures rose above 10°C in mid-April, then *U. cinerea* fed on both prey sizes during late April and May (Fig. 2.1). As water temperatures rose above 15 °C in June, drills switched to preying primarily on larger oysters from June 6-27 (small mean consumption = 0.083 oysters (SE=0.08), large mean = 1.17 (SE=0.24)) (two-tailed paired T-test, $T_{11} = 4.17$, $p < 0.01$) (Fig. 2.1).

Clear seasonal patterns were found over the course of the year-long *U. cinerea* feeding experiment, suggesting that feeding is linked to temperature (Fig. 2.2). Very little predation occurred over the winter (<0.05 g/week) when water temperatures were < 10 °C, but feeding increased slowly with increasing temperatures in the spring to a peak in the summer months (~1 g/week) (Fig. 2.2). Feeding rates in spring and fall were similar and were between 0.15 and 0.5 g

of prey tissue per week, less than half of summer feeding levels (Fig. 2.2). Greater prey consumption occurred during July, August and September than the rest of the year combined.

Temperature and Predation

Oyster drills in the elevated temperature treatment did not show a significant change in feeding over the first 3-4 months (May to August) of the experiment (T-tests for each month, $p > 0.2$) (Fig. 2.3). However, *U. cinerea* showed a higher feeding rate in the elevated temperature treatment during the fall in September (T-test, $T_{14} = -2.05$, $p = 0.030$), October (T-test, $T_{14} = -1.55$, $p = 0.071$), and December (T-test, $T_{14} = -2.24$, $p = 0.021$) (Fig. 2.3). The overall summer feeding rate was not significantly different ($p > 0.4$) between treatments, but fall showed significantly higher feeding in the heated treatment (control mean = 0.20 g tissue / week, heated mean = 0.36 g tissue / week) (T-test, $T_{14} = -2.78$, $p = 0.007$). The largest difference between heated and control treatments occurred in the early fall, when control temperatures dropped rapidly but the heated treatment remained in the summer temperature range (Fig. 2.3). While the difference between treatments was highest in early fall, the percent increase in feeding from control to elevated temperatures was highest in early winter when *U. cinerea* displayed a five-fold increase in feeding rate in the warmer treatment.

The year-long feeding experiment at ambient seawater temperatures also showed little feeding at temperatures <10°C and strong temperature dependence of feeding (Fig. 2.4). When all bi-weekly data points were combined for the entire year, they highlighted a roughly linear relationship between temperature and feeding rate (feeding rate $F = 0.509 + 0.062T$, $r^2 = 0.80$). Based on the strong relationship between temperature and feeding rate during all seasons for *U. cinerea* (Fig. 2.4), annual estimates of oyster consumption were made for oysters of different

sizes. These estimates were made from the regression between temperature and feeding rate (grams of prey tissue per week) using the established relationship between tissue weight and total wet weight. Annual oyster predation was predicted at current temperatures and at temperatures elevated 2 and 4°C (Table 2.1). Under present temperature conditions, a single *U. cinerea* can be expected to consume approximately 106 oyster spat per year, a number that would increase to 137 and 169 per year with temperature increases of 2° and 4°C, respectively. An overall increase in feeding rate of 30% at +2°C and 60% at +4°C is predicted for the oyster drill (Table 2.1).

Discussion

All of the experimental results provide insight into the feeding behavior of *Urosalpinx cinerea* and the mechanisms behind the predation patterns of this species. The first hypothesis, suggested by Federighi (1931) and by the evolution of inducible defenses (Lord and Whitlatch, 2012; Newell et al., 2007), was that oysters with thinner shells are more susceptible to predation by *U. cinerea*. The hypothesis was supported by data from the shell thickness experiment which indicated a much higher predation rate on thin- than thick-shelled oysters. This vulnerability could manifest itself as either a feeding preference by the predator or reduced drilling success as a result of thicker shells. The latter reason is more likely since *U. cinerea* prey selection is tied to prey respiratory metabolites, the production of which is size-dependent (Blake, 1960). Since thin and thick-shelled oysters were of similar sizes, they likely produced similar levels of metabolic byproducts that could attract drill predation. In addition, failed (partial) drill holes were common on thick-shelled oysters, indicating that drill attempts were made but that *U. cinerea* eventually aborted these attempts. However, this was not conclusive since oysters were field collected and thus had drill marks prior to the start of the experiment. In addition, thick-shelled oysters that

were rarely consumed and thus rarely replaced remained exposed to *U. cinerea* for much longer than thin-shelled oysters, confounding any counts of failed drill attempts. Regardless of the prey selection mechanism, the inducible shell-thickening defense shown by the eastern oyster *Crassostrea virginica* appears to be an effective defense against predation by drilling predators such as *U. cinerea* (Lord and Whitlatch, 2012; Newell et al., 2007).

The second hypothesis that oyster drills should select smaller over larger oysters appears to be season-dependent. Drills fed solely on smaller oysters during the winter and early spring months, before switching to mainly larger oyster prey during late spring and summer (Fig. 2.1). This foraging pattern is likely a result of physiological changes with temperature, as *U. cinerea* is largely dormant during the winter months and feeds little during early spring (Fig. 2.2) (Carriker, 1969; Franz, 1971; Hanks, 1957). Slower winter-time metabolic rates likely reduce the ability of the drills to be active predators on larger oysters, as low drilling speeds could lead drills to give up before penetrating large prey. As temperatures increase, energetic demand and metabolic rate increase, allowing oyster drills to attack and consume larger prey.

Increased metabolism probably increases drilling speed, decreases handling time and increases the energetic efficiency of feeding on larger prey, causing the spring-time switch from smaller to larger oysters (Fig. 2.1). While the summer predation on larger oysters (Fig. 2.1) may be due to high energetic demands, it is most likely a result of the higher prey shell surface area and higher levels of metabolites produced by larger oysters (Blake, 1960). The metabolites would attract *U. cinerea* and the higher surface area would skew predation towards larger oysters simply by increasing encounter rates. This metabolite and shell area difference would not be relevant in the winter months when drills are actively selecting smaller oysters either by avoiding larger oysters or by failed drill attempts. Regardless of the mechanism, there was a clear shift

from consuming solely small oysters during the winter to larger prey sizes in spring and summer. The larger oysters in this experiment were still fairly small relative to market-size or several-year old oysters, but they were 10-20 times larger than the smaller experimental oysters, so the size difference was substantial. This type of prey size switching is known to occur in some species of ants as well, which consume smaller prey under stressful environmental conditions that limit movement (Traniello et al., 1984). Lizards and fish can also change prey size preferences with season, either due to temperature or changes in prey densities (Avery and Mynott, 1990; Diaz and Carrascal, 1993; Werner and Hall, 1974).

Feeding rates of *U. cinerea* varied strongly with season and were most likely tied to changes in temperature, although reproductive cycles could play a minor role (Fig. 2.2) (Hanks, 1957; Manzi, 1970). Little feeding occurred <10°C, a result that is similar to minimum feeding temperatures observed in other studies on this species (Carriker, 1969; Franz, 1971; Hanks, 1957). None of these studies showed complete dormancy of the drill as was observed in the snail *Polinices duplicatus* which does not feed at all for four winter months (November-February), (Fig. 2.2) (Edwards and Huebner, 1977). It should be noted, however, that Long Island Sound winter water temperatures (Jan-Mar) in 2012 were unusually warm and did not drop below 7°C. Mean winter water temperature over the past 15 years in Long Island Sound has varied between 2.2° to 7.5°C. Since Carriker (1955) found that oyster drills became dormant when water temperatures were <6.5°C, it is possible that colder winter temperatures would have resulted in dormancy and a complete lack of feeding.

The hypothesis that *U. cinerea* would have higher feeding rates at experimentally elevated temperatures during all seasons was partially supported from the temperature feeding experiments. There was a significantly higher feeding rate in heated (+4°C) than in the unheated

control treatments in late summer, fall, and winter, but not at the start of the experiment during late spring and early summer (Fig. 2.3). The lack of a difference between treatments from May to July could be related to the time it takes for the drills to fully acclimate to the elevated temperatures, although little is known about snail acclimation rates. Elevated *U. cinerea* feeding rates in late summer and fall in heated treatments relative to controls were pronounced, but the mechanism behind this pattern is unclear (Fig. 2.3). The difference in feeding rate was likely due increased metabolism, activity and metabolic demands at warmer temperatures. The strong temperature dependence shown by *U. cinerea* in year-long and temperature experiments (Figs. 2, 3) is similar to patterns displayed by several other temperate marine predators including green crabs (Sanchez-Salazar et al., 2007), sea stars (Sanford, 1999), and gastropods (Edwards and Huebner, 1977; Garton and Stickle, 1980).

The fourth hypothesis that *U. cinerea* would have a longer feeding season at higher temperature treatments was generally supported; the largest relative difference in feeding rate between heated and control treatments was in the winter. This occurred as drills in the control tanks slowed down feeding rates earlier than those in the heated treatment. However, feeding in the control group only stopped a few weeks before the heated group because of a fairly rapid ambient water temperature decline in winter. Therefore, the impact of feeding season length is small (1-2% of total change in feeding) relative to the large increase in feeding rates at elevated temperatures during the summer and fall.

Both annual feeding and temperature experiments showed a strong dependence of feeding rate on temperature, indicating that temperature is the primary control on *U. cinerea* feeding (Fig. 2.4). Most previous studies of feeding rates and temperature either quantified seasonal patterns (e.g. Edwards and Huebner, 1977) or manipulated temperature at constant

levels (e.g. Garton and Stickle, 1980; Sanford, 1999). This study provided both seasonal feeding patterns (Fig. 2.2) and long-term alteration of seawater temperatures relative to ambient temperatures (Fig. 2.3). Thermal response experiments that incorporated seasonal temperature fluctuations suggest that seawater temperature controls seasonal feeding patterns and could drive inter-annual variability in feeding rates as well due to the strong linear relationship between feeding and temperature (Fig. 2.4). Even small increases of 2°C or 4°C on a monthly or potentially even decadal scale can result in large increases in oyster drill feeding rates (Table 2.1). Ocean temperatures along the Atlantic coast of northern North America are predicted to increase 2-4°C by 2100, so the feeding changes described and predicted by this study could be realized within 50-100 years (IPCC, 2007). An increase of 2°C or 4°C could result in a *U. cinerea* feeding rate rise of 30% or 60%, respectively (Table 2.1). This sharp increase in feeding rate with incremental temperature increase also occurs in the sea star *Pisaster ochraceus*, which showed a 25% increase in feeding with temperature change from 9°C to 12°C (Sanford, 1999).

Higher *U. cinerea* feeding rates predictably increase the estimated number of oysters consumed per year per individual oyster drill, including a rise from an average of 106 to 169 juvenile oysters (spat) per year with 4°C higher temperatures (Table 2.1). It is unclear what precise effect this would have on oyster populations, but the impact could be felt in many locations, since *U. cinerea* is invasive in the UK, Netherlands, and on the west coast of the US (Carlton, 1992; Eno et al., 1997; Faasse and Ligthart, 2007). The range of this predator is still expanding and it has shown the ability to consume all species of oysters throughout its native and introduced range (Buhle and Ruesink, 2009; Eno et al., 1997; Faasse and Ligthart, 2007). Since *U. cinerea* impacts commercial oyster cultures and oyster restoration projects, changes in water temperature could have a strong impact on the ecological and economic impact of this species

(Buhle and Ruesink, 2009; Eno et al., 1997; Federighi, 1931). While future changes in climate could alter oyster characteristics such as growth rate and shell thickness, warmer temperatures will result in large increases in oyster drill feeding and alter the predator-prey relationship between these species.

Acknowledgments

Thanks to Jeremy Calini and Rachel Perry for assistance with data collection and experiment setup. Support provided by Connecticut Sea Grant and the University of Connecticut Outstanding Scholar Program.

References

Avery, R., Mynott, A., 1990. The effects of temperature on prey handling time in the common lizard, *Lacerta vivipara*. Amphibia-Reptilia 11, 111–122.

Blake, J., 1960. Oxygen consumption of bivalve prey and their attractiveness to the gastropod, *Urosalpinx cinerea*. Limnol. Oceanogr. 5, 273–280.

Brookes, J.I., Rochette, R., 2007. Mechanism of a plastic phenotypic response: predator-induced shell thickening in the intertidal gastropod *Littorina obtusata*. J. Evol. Biol. 20, 1015–27.

Buhle, E., Ruesink, J. 2009. Impacts of invasive oyster drills on Olympia oyster (*Ostrea lurida* Carpenter 1864) recovery in Willapa Bay, Washington, United States. J. Shell. Res. 28, 87–96.

Carlton, J., 1992. Introduced marine and estuarine mollusks of North America: an end-of-the-20th-century perspective. J. Shell. Res. 11, 489–505.

Carriker, M., 1955. Critical review of biology and control of oyster drills, *Urosalpinx* and *Eupleura*. 150pp.

Carriker, M., 1969. Excavation of boreholes by the gastropod, *Urosalpinx*: an analysis by light and scanning electron microscopy. Am. Zool. 9, 917–933.

Coen, L., Brumbaugh, R., Bushek, D., Grizzle, R., Luckenbach, M., Posey, M., Powers, S., Tolley, S., 2007. Ecosystem services related to oyster restoration. Mar. Ecol. Prog. Ser. 341, 303–307.

Dame, R, 1972. The ecological energies of growth, respiration and assimilation in the intertidal American oyster *Crassostrea virginica*. Mar. Biol. 17, 243–250.

Díaz, J., Carrascal, L., 1993. Variation in the effect of profitability on prey size selection by the lacertid lizard *Psammodromus algirus*. Oecologia 94, 23-29.

Edwards, D., Huebner, J., 1977. Feeding and growth rates of *Polinices duplicatus* preying on *Mya arenaria* at Barnstable Harbor, Massachusetts. Ecology 58, 1218–1236.

Elliott, J.K., Leggett, W.C., 1996. The effect of temperature on predation rates of a fish (*Gasterosteus aculeatus*) and a jellyfish (*Aurelia aurita*) on larval capelin (*Mallotus villosus*). Can. J. Fish. Aquat. Sci. 53, 1393–1402.

Eno, N., Clark, R., Sanderson, W., 1997. Non-native marine species in British waters: a review and directory.

Faasse, M., Ligthart, M. 2007. The American oyster drill, *Urosalpinx cinerea* (Say, 1822), introduced to The Netherlands – increased risks after ban on TBT? Aq. Inv. 2, 402–406.

Federighi, H., 1931. Salinity death-points of the oyster drill snail, *Urosalpinx cinerea* Say. Ecology 12, 346–353.

Franz, D., 1971. Population age structure, growth and longevity of the marine gastropod *Urosalpinx cinerea* Say. Biol. Bull. 140, 63–72.

Garton, D., Stickle, W., 1980. Effects of salinity and temperature on the predation rate of *Thais haemastoma* on *Crassostrea virginica* spat. Biol. Bull. 158, 49–57.

Gilman, S.E., Urban, M.C., Tewksbury, J., Gilchrist, G.W., Holt, R.D., 2010. A framework for community interactions under climate change. Trends Ecol. Evol. 25, 325–31.

Hanks, J., 1957. The rate of feeding of the common oyster drill, *Urosalpinx cinerea* (Say), at controlled water temperatures. Biol. Bull. 112, 330–335.

Helmuth, B., Harley, C.D.G., Halpin, P.M., O'Donnell, M., Hofmann, G.E., Blanchette, C.A., 2002. Climate change and latitudinal patterns of intertidal thermal stress. Science 298, 1015–7.

Irie, T., 2005. Geographical variation of shell morphology in *Cypraea annulus* (Gastropoda: Cypraeidae). J. Moll. Stud. 72, 31–38.

IPCC, 2007. Climate Change 2007: Synthesis Report. 52pp.

Johnson, K.D., Smee, D.L., 2012. Size matters for risk assessment and resource allocation in bivalves. Mar. Ecol. Prog. Ser. 462, 103–110.

Kanazawa, T., Sato, S., 2007. Environmental and physiological controls on shell microgrowth pattern of *Ruditapes philippinarum* (Bivalvia: Veneridae) from Japan. J. Moll. Stud. 74, 89–95.

Kawai, K., 2008. Shell growth, reproduction and mortality of *Ovula ovum* in southern Kyushu, Japan. J. Moll. Stud. 75, 35–40.

Kordas, R.L., Harley, C.D.G., O'Connor, M.I., 2011. Community ecology in a warming world: The influence of temperature on interspecific interactions in marine systems. J. Exp. Mar. Biol. Ecol. 400, 218–226.

Loiterton, B., Sundbom, M., Vrede, T., 2004. Separating physical and physiological effects of temperature on zooplankton feeding rate. Aquat. Sci. 66, 123–129.

Lord, J.P., Whitlatch, R.B., 2012. Inducible defenses in the eastern oyster *Crassostrea virginica* Gmelin in response to the presence of the predatory oyster drill *Urosalpinx cinerea* Say in Long Island Sound. Mar. Biol. 159, 1177–1182.

Mahdian, K., Vantornhout, I., Tirry, L., De Clercq, P., 2006. Effects of temperature on predation by the stinkbugs *Picromerus bidens* and *Podisus maculiventris* (Heteroptera: Pentatomidae) on noctuid caterpillars. Bull. Entomol. Res. 96, 489–96.

Manzi, J., 1970. Combined effects of salinity and temperature on the feeding, reproductive, and survival rates of *Eupleura caudata* (Say) and *Urosalpinx cinerea* (Say) (Prosobranchia: Muricidae). Biol. Bull. 138, 35–46.

Morse, J.W., Mackenzie, F.T., 1990. Geochemistry of Sedimentary Carbonates. Elsevier B.V., New York. 707pp.

Newell, R.I.E., Kennedy, V.S., Shaw, K.S., 2007. Comparative vulnerability to predators, and induced defense responses, of eastern oysters *Crassostrea virginica* and non-native *Crassostrea ariakensis* oysters in Chesapeake Bay. Mar. Biol. 152, 449–460.

Ordzie, C., Garofalo, G., 1980. Predation, attack success, and attraction to the bay scallop, *Argopecten irradians* (Lamarck) by the oyster drill, *Urosalpinx cinerea* (Say). J. Exp. Mar. Biol. Ecol. 47, 95–100.

Palmer, A., 1982. Growth in marine gastropods. A non-destructive technique for independently measuring shell and body weight. Malacologia 23:63-73.

Pincebourde, S., Sanford, E., Helmuth, B., 2008. Body temperature during low tide alters the feeding performance of a top intertidal predator. Limnol. Oceanogr. 53, 1562–1573.

Sanchez-Salazar, M., Griffiths, C., Seed, R., 1987. The effect of size and temperature on the predation of cockles *Cerastoderma edule* (L.) by the shore crab *Carcinus maenas*(L.). J. Exp. Mar. Biol. Ecol. 111, 1987.

Sanford, E., 1999. Regulation of keystone predation by small changes in ocean temperature. Science 283, 2095–7.

Schöne, B., Rodland, D., Fiebig, J., 2006. Reliability of multitaxon, multiproxy reconstructions of environmental conditions from accretionary biogenic skeletons. J. Geol. 114, 267–285.

Stachowicz, J., Fried, H., Osman, R., Whitlatch, R., 2002. Biodiversity, invasion resistance, and marine ecosystem function: reconciling pattern and process. Ecology 83, 2575–2590.

Traniello, J., Fujita, M., Bowen, R., 1984. Ant foraging behavior: ambient temperature influences prey selection. Behav. Ecol. Sociobiol. 15, 65–68.

Trussell, G., Ewanchuk, P., Bertness, M., 2003. Trait-mediated effects in rocky intertidal food chains: predator risk cues alter prey feeding rates. Ecology 84, 629–640.

Waldbusser, G.G., Voigt, E.P., Bergschneider, H., Green, M.A., Newell, R.I.E., 2010. Biocalcification in the eastern oyster (*Crassostrea virginica*) in relation to long-term trends in Chesapeake Bay pH. Estuar. Coast. 34, 221–231.

Werner, E., Hall, D., 1974. Optimal foraging and the size selection of prey by the bluegill sunfish (Lepomis macrochirus). Ecology 55, 1042–1052.

Figure Captions

Figure 2.1. Oyster size selection of oyster drills in prey size experiment. As temperature increased above 10°C, drills switched from preying solely on small oysters to both small and large, then eventually to solely large oysters. Asterisks indicate significant differences at $p = 0.05$ based on paired T-tests for that date (±SE bars).

Figure 2.2. Annual pattern of *U. cinerea* feeding, with monthly averages in terms of grams of prey tissue per week. Feeding rate increases sharply with temperature, to a maximum in summer months. The line indicates a two-month moving average of feeding rates, while bars show monthly averages (±SE bars).

Figure 2.3. Oyster drill feeding rates (grams of prey tissue per week) in control and elevated temperature treatments for each month from May 2012 to January 2013. There is no significant difference in feeding rate between treatments during spring and early summer, but *U. cinerea* at elevated temperature treatments in September, October and December fed significantly more than controls. The line shows ambient seawater temperatures (control) over the course of the experiment (±SE bars).

Figure 2.4. Linear relationship between *U. cinerea* feeding rate and water temperature, with data from annual feeding experiments. Feeding rates were very low below 10°C but then increased linearly with temperature.

Table 2.1. Measured and potential feeding rates of *U. cinerea* at different temperatures. Based on temperature experiment results shown in figure 3, weekly feeding rates and annual feeding rates (total number of oysters) were calculated at 0, 2 and 4 °C above current ambient seawater temperatures in Long Island Sound.

Temperature	Experimental Feeding		Potential Consumption (per year)			Percent
(above ambient)	Rate (g/wk)	Annual (yr)	Spat (1g)	3g oyster	5g oyster	Increase
Current (0 ℃)	0.38	19.9 oysters	106	35	21	N/A
2 ℃	0.50	25.8 oysters	137	46	27	29.9%
4 ℃	0.61	31.7 oysters	169	56	34	59.8%

Figure 2.1 (see caption on p. 53)

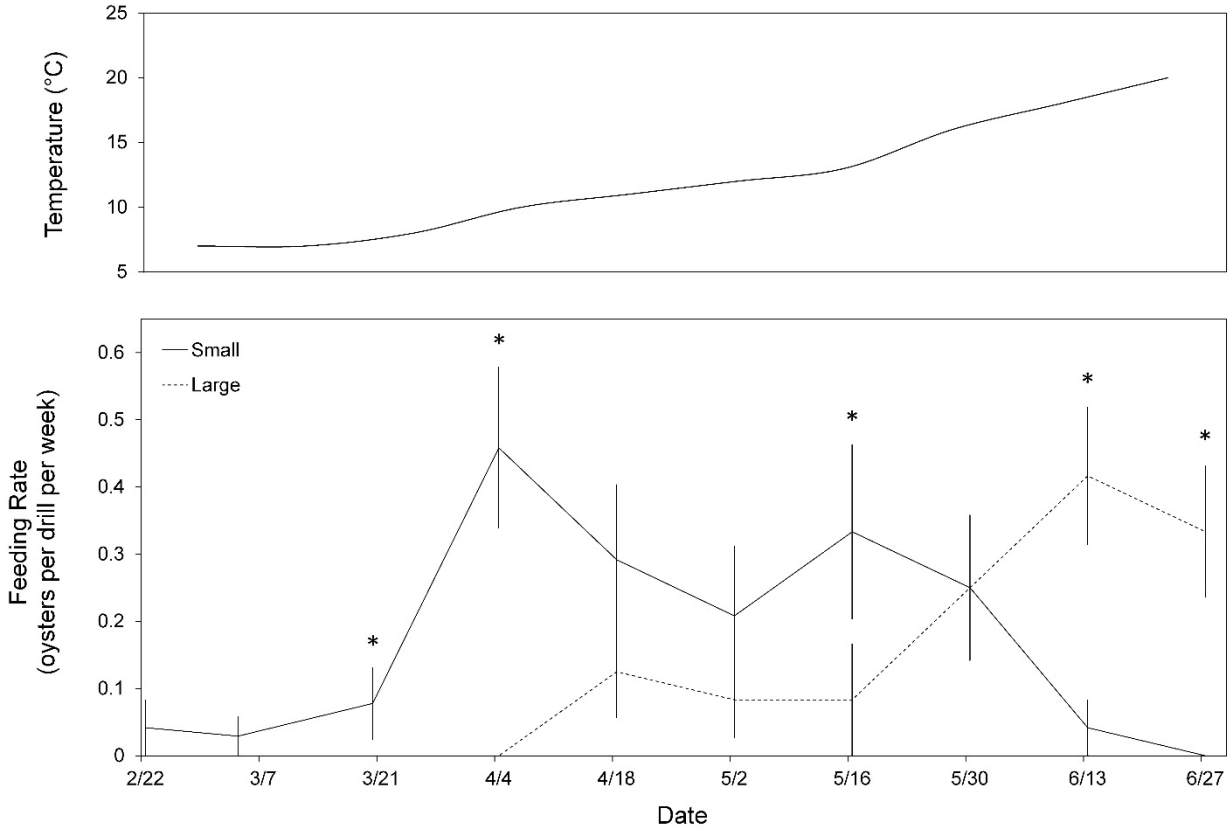


Figure 2.2 (see caption on p. 53)

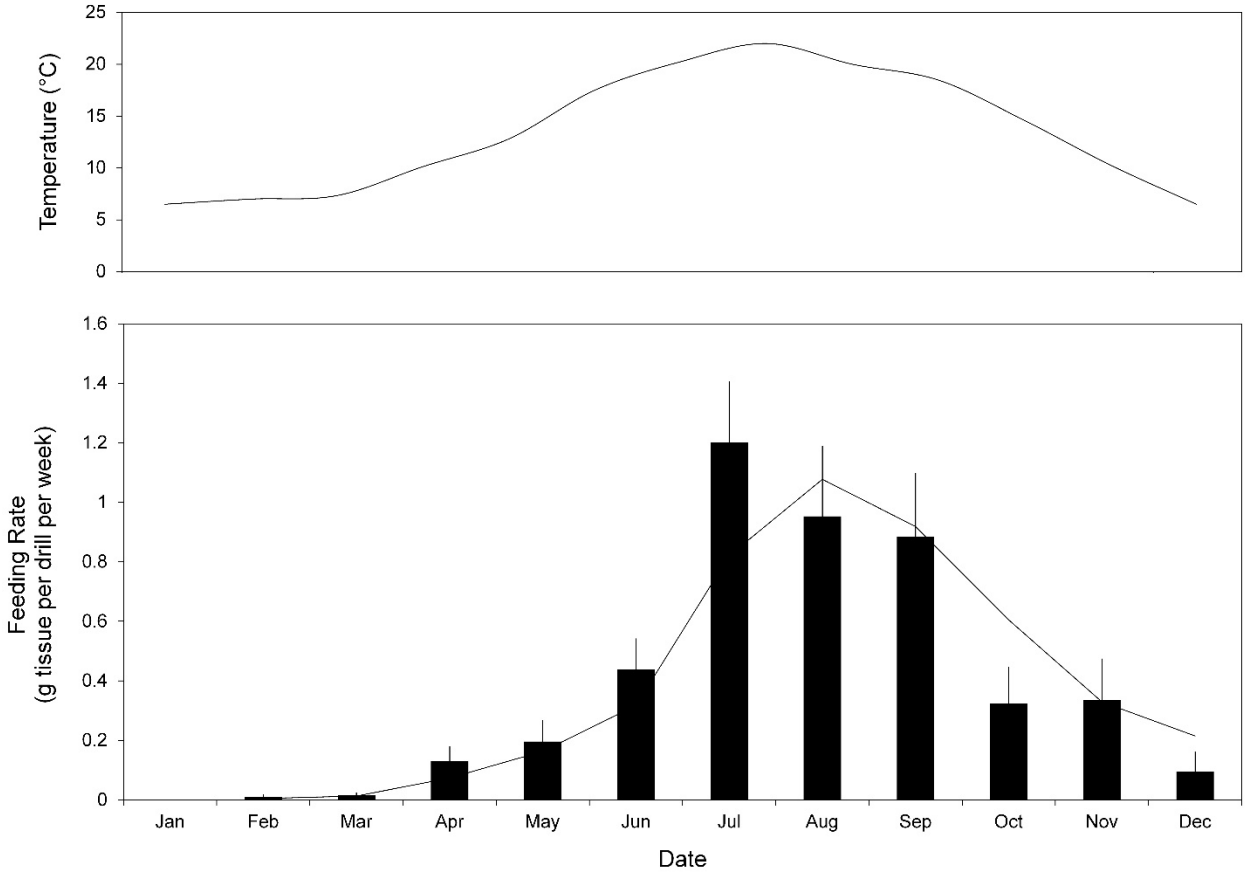


Figure 2.3 (see caption on p. 53)

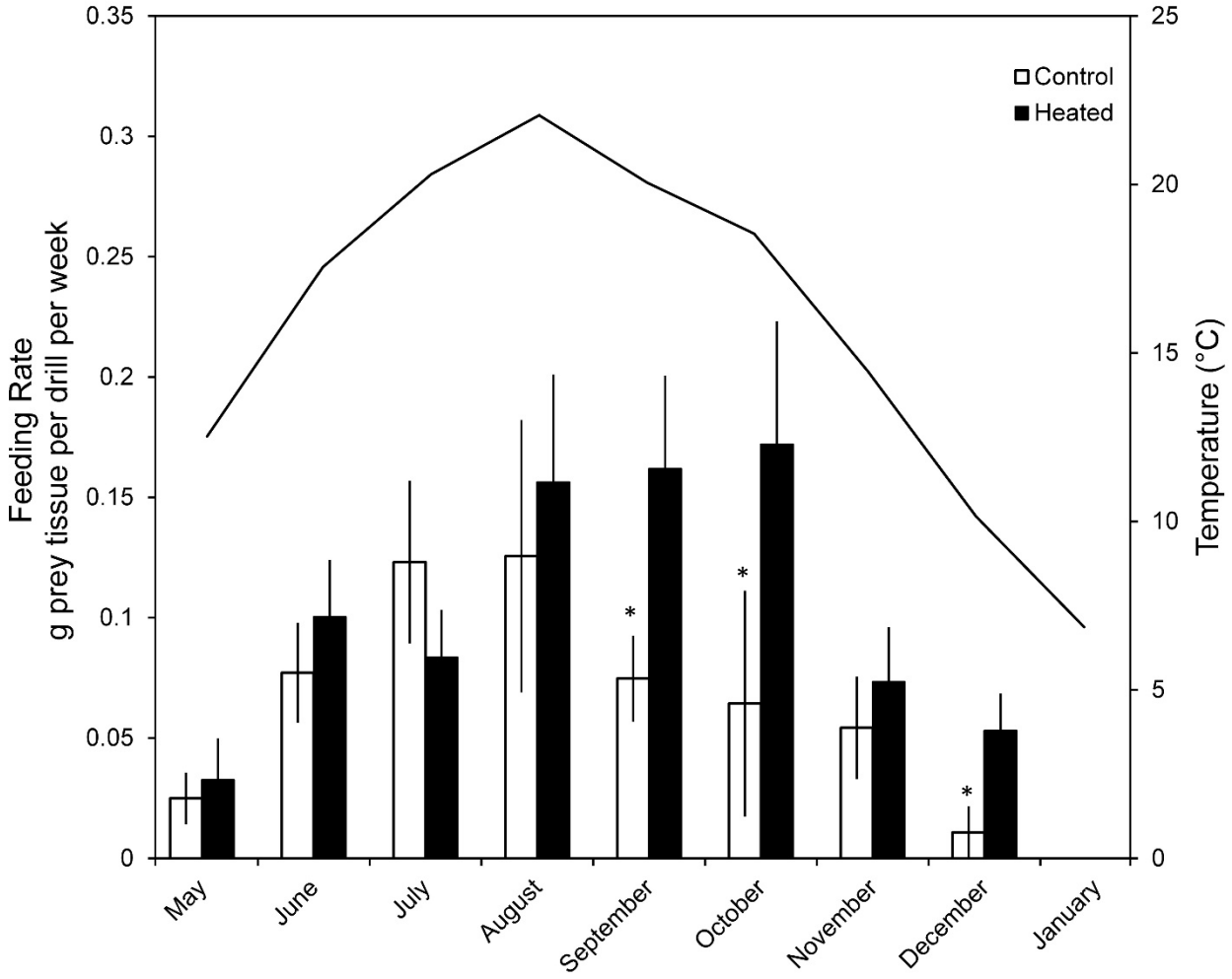
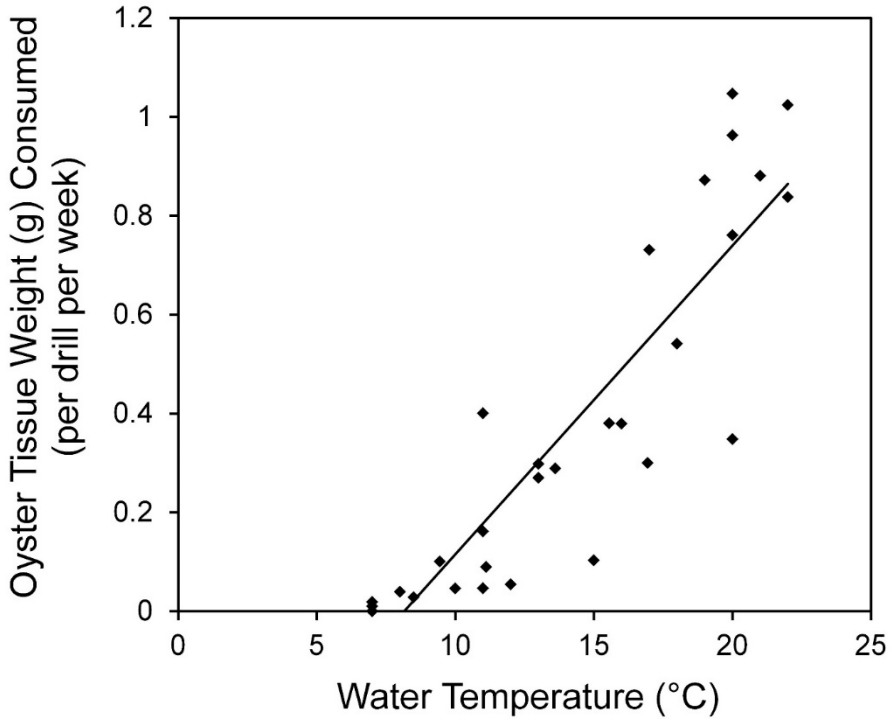


Figure 2.4 (see caption on p. 53)



Chapter 3

Latitudinal patterns of shell thickness and metabolism in the eastern oyster *Crassostrea virginica* along the east coast of North America[3]

Abstract

The goal of this study was to quantify growth and metabolic responses of oysters to increased temperatures like those that will occur due to global warming. Impact of temperature on eastern oyster (*Crassostrea virginica*) shell growth and metabolism was investigated by sampling 24 sites along the eastern North American seaboard ranging from New Brunswick, Canada, to Florida, USA, in March and August 2013. There was a positive correlation between oyster shell thickness and site temperature. At southern sites, shells were up to 65% thicker than at the northernmost site, likely due to higher precipitation of $CaCO_3$ in warmer water. This was supported by laboratory experiments showing that thicker shells were produced in response to temperatures 2, 4, and 6°C above ambient seawater temperatures (8-14°C) in Connecticut, USA. Field experiments with oyster respiration were conducted during winter and summer at 13 sites to compare responses to thermal stress with latitude. Respiration rates were much higher during summer than winter, but the combination of summer and winter data fell along the same exponential curve with respect to temperature. At all sites, temperature-specific metabolic rates

[3]Lord J. & Whitlatch R. (2014) Latitudinal patterns of shell thickness and metabolism in the eastern oyster Crassostrea virginica along the east coast of North America. *Marine Biology,* in press.

at elevated temperatures were lower than predicted, indicating significant seasonal acclimatization by *C. virginica*.

Keywords: oyster; shell growth; metabolism; respiration; temperature; shell thickness

Introduction

To predict impacts of climate change on individual species, it is vital to understand how the link between temperature and physiology changes on a seasonal and latitudinal basis. In species with large geographic ranges, there are often morphological and physiological differences among populations that can lead to differential responses to elevated temperatures (Dittman et al. 1998). Variability among populations over a large geographic range can thus complicate attempts to predict species-specific metabolic responses to climate change (Jansen et al. 2007; Cherkasov et al. 2009). Morphological changes in response to temperature over a latitudinal range are especially complex in estuarine habitats, where changes in water temperature on a latitudinal and seasonal basis are accompanied by variation in salinity, food availability and other environmental factors (Brown and Hartwick 1988; Irie 2005; Schöne et al. 2006; Waldbusser et al. 2011). Calcifying organisms display a particularly complex reaction to environmental conditions since calcification rates change based on factors such as temperature, salinity, pH, nutrients, and calcium carbonate (calcite and aragonite) saturation states (Morse and Mackenzie 1990; Dissard et al. 2010). For example, in *Mytilus edulis*, a temperature increase from 4 to 20℃ more than doubles calcification rates (Malone and Dodd 1967). It is therefore necessary to integrate experimental data with large-scale geographic patterns in order to assess

the impact of latitudinal temperature gradients on metabolism and morphology and to predict the influence of climate change (Jansen et al. 2007).

In marine ectotherms, such as oysters, metabolic rate is positively correlated with temperature (e.g. Newell et al. 1977; Shumway and Koehn 1982; Haure et al. 1998; Jihong et al. 2004; Mao et al. 2006; Pernet et al 2008). Temperature varies with latitude and season, so oysters and other species with broad distributions have intraspecific variations in metabolic rates on a spatial and temporal basis. Previous studies relating temperature to metabolism have measured responses to changes in temperature at one site (Shumway and Koehn 1982; Boucher and Boucher-Rodoni 1988; Mao et al. 2006; Martin et al. 2006) or measured in situ metabolic rates along a geographical temperature gradient (Dittman et al. 1998; Jansen et al. 2007). Some species, such as the bivalves *Mytilus edulis* and *Macoma balthica*, reduce their metabolism in summer due to temperatures above their breakpoint temperature (maximum respiration rate), while other mollusks, such as *Crassostrea gigas* and *Crepidula fornicata,* display their highest metabolic rates during the summer (Boucher and Boucher-Rodoni 1988; Mao et al. 2006; Martin et al. 2006).

For calcifying organisms, shell growth and calcification patterns are also affected by seawater carbonate chemistry, which is dependent on temperature and salinity (Morse and Mackenzie 1990; Ferguson et al. 2008; Waldbusser et al. 2011). Shell growth varies with temperature seasonally (Kanazawa and Sato 2008; Waldbusser et al. 2011), latitudinally (Irie 2005), and on a climatological scale (Schone et al. 2006; Miyaji 2010). Temperature can impact the defensive abilities of calcifying organisms such as mollusks by altering shell strength or shell thickness, affecting vulnerability to crushing (crabs) or drilling predators (gastropods) (Nicol 1967; Palmer 1992; Trussell and Etter 2001). Large-scale patterns of shell thickness can also be

affected by biological factors such as predator density, which can induce shell-thickening in intertidal snails including *Nucella lapillus, Littorina obtusata* and *L. littorea* (Trussell and Etter 2001; Trussell et al. 2003), clams including *Mercenaria mercenaria* (Nakaoka 2000), and oysters including *Crassostrea virginica* and *C. ariakensis* (Newell et al. 2007; Lord and Whitlatch 2012).

The eastern oyster, *Crassostrea virginica*, increases calcification rates and shell growth rates at higher temperatures (Dame 1972; Waldbusser et al. 2011). The metabolism of *C. virginica* from Long Island Sound, USA is also strongly temperature dependent (Shumway and Koehn 1982). Studies of metabolic rates of marine invertebrates typically use oxygen consumption (VO_2) as a proxy (Jansen et al. 2007). Species such as *C. virginica* that have temperature-dependent metabolism and shell growth will be affected by predicted 2–4 °C increases in global ocean temperature by 2100 (Pachauri and Reisinger 2007).

Changes in growth and metabolism of eastern oyster populations may have large ecological and economic impacts, since oysters are a commercially important species throughout their range and are ecosystem engineers (Coen et al. 2007). Oysters not only filter estuarine water and play a large role in benthic-pelagic coupling, but oyster reefs provide feeding habitat and refuge from predation for several benthic and pelagic species (e.g. Grabowski et al. 2005; Coen et al. 2007; Stunz et al. 2010). In order to predict future climate-induced changes in oyster populations and in oyster distribution it is necessary to understand the way that oyster shell characteristics and metabolism respond to increased temperatures along a latitudinal gradient.

The goals of this study were to determine the effect of temperature on *C. virginica* metabolism and shell growth by testing the responses of oysters to experimentally elevated temperatures in the laboratory, and in the field at sites spanning a wide latitudinal range. We

tested metabolic responses and measured shell thickness on both local and latitudinal scales because experimentally-determined thermal effects on shell growth and metabolism are often generalized for an entire species, despite large variability among populations and along a latitudinal gradient (Dame 1972; Singh and Zouros 1978; Shumway and Koehn 1982; Dittman et al. 1998; Jansen et al. 2007; Waldbusser et al. 2011). We tested three main hypotheses in this study: (1) oysters from the northeastern US would show increased growth rates when grown in warmer laboratory conditions since they live far from the southern range limit of the species, (2) shell thickness would increase from north to south because warmer temperatures result in lower solubility of calcium carbonate, which facilitates shell deposition and (3) southern *C. virginica* populations would be more stressed by elevated temperatures during the summer than northern populations since they are closer to the upper temperature limit for the species, i.e. ~36°C (Galtsoff 1964). We also quantified the density of a major oyster predator, the oyster drill *Urosalpinx cinerea,* in order to test if predator density affects shell thickness over a large geographic range. We conducted field surveys and experiments at 24 sites over a large latitudinal gradient (26–47 ° N) along the east coast of North America, enabling us to assess the role of temperature in describing and predicting large scale patterns in *C. virginica* metabolism and morphology.

Materials and methods

Laboratory experiments

All laboratory experiments were conducted in the J. S. Rankin Jr. Seawater Research Laboratory at the University of Connecticut, Avery Point. Oysters were provided by Fisher's Island Oyster Farm, where they were grown in brackish Ocean Pond and then in an open ocean

environment on Fisher's Island, New York. They were tagged with Hallprint® shellfish tags super-glued to the left valve (cupped side of the shell). Oysters were kept in 20, 25-L flow-through seawater tanks, each connected to a heat exchanger that controlled the temperature of the flowing seawater. Each tank was fed by a separate seawater hose that coiled through a heat exchanger, so all water sources were independent and supplied seawater at approximately 1 L min^{-1}. Aqua Logic® digital temperature controllers with temperature probes in the experimental tanks turned the heaters on and off in order to regulate the temperature of the heat exchangers and produce the desired temperature outflow in each experimental tank. Temperature controllers were precise to within 1°C and were used to create four temperature treatments of five tanks each: control (ambient seawater temperature), +2°C above ambient, +4°C above ambient, and +6°C above ambient. Temperatures in the tanks were further monitored by HOBO® data loggers which recorded water temperature every hour for the three month duration of the experiment. Ambient seawater temperatures increased from 8°C at the start of the experiment to 14°C by the end and elevated temperature treatments went up accordingly.

Each of the five tanks in the control treatment contained 10 small (1 cm long) and 10 large (6 cm long) oysters (total 50 per size class), with no significant differences in size among treatments at the start of the experiment. Each of the elevated temperature treatment tanks contained 5 large and 5 small oysters (5 tanks per treatment = 25 per size class per treatment). Twice as many oysters were used in the control treatment to account for the possibility of food limitation; under food-limited conditions control oysters should display thicker shells than in other treatments (Brown and Hartwick 1988). Oysters were obtained in December 2011, stored in flowing ambient seawater temperature tanks for one month, and then transferred to the experimental tanks for three months from January to April 2012. Prior to the start, and at the end

of the experiment, all oysters were weighed in air (wet weight) and weighed in water (immersed weight) as in Palmer (1982) in order to estimate the shell weight of live oysters. The immersed weight correlates linearly with shell weight (Fig. 3.1). A regression was then determined by dissecting and weighing the shells of 20 non-experimental oysters, and used to convert immersed weight to shell weight (Palmer 1982). All oysters were also photographed before and after the experiment and the area of the left valve was measured with the image analysis software ImageJ (NIH). These data were used to determine growth rates in terms of shell weight, shell thickness, shell area, and tissue weight for *C. virginica* at different temperatures over the three month experimental period. In order to control for variability in the initial size of the oysters, all growth rates were calculated as new growth divided by initial size in the unit being measured. Shell growth differences were quantified with ANOVA tests between treatments, with Tukey-tests for pairwise comparisons.

Latitudinal patterns in shell thickness

Oyster shells were collected from natural populations in 24 different intertidal locations along the east coast of North America, from the northernmost end of the range of *C. virginica* (New Brunswick, Canada) to south Florida (Fig. 3.2, Table 3.1). To assess changes in shell morphology with latitude, shell thickness was determined to be the most useful measure of morphological differences, since growth rates could not be determined over short sampling intervals and shell lengths and weights tend to be highly variable (Palmer and Carriker 1979). Shell thickness was inferred from shell weights (from dissected oysters) divided by surface area of the left valve (measured via photograph analysis). This was compared with actual maximum shell thickness, determined by measuring shell thickness of longitudinal cross sections with

digital calipers for 171 oysters (~10 from each site sampled in March) to the nearest 0.1mm. There was a strong correlation between inferred thickness (g cm^{-2}) and measured shell thickness (mm) indicating that this inferred thickness estimates actual shell thickness (Regression ANOVA $F_{1,169} = 323.37$, $r^2 = 0.66$, $p < 0.001$) (Fig. 3.3). While shell thickness was not measured directly, the same method was used for all 24 sites and for laboratory experiments and was an accurate measure of the amount of shell material deposited by oysters per square centimeter of left valve area. Differences in inferred shell thickness between sites were analyzed by comparing residuals from a common regression in order to account for size-based differences in shell weights. A regression between left valve shell area (from photographs and image analysis) and shell weight was created from a pooled dataset including all oysters from all 24 sites. Residuals were then calculated as the difference between each actual oyster shell weight and the regression-predicted shell weight for that oyster. This method reduced variability in inferred thickness comparisons and eliminated concerns that between-site variability could be dependent on the size of *C. virginica* sampled at each site.

At each site, 17 *C. virginica* were collected haphazardly along a 25-m transect, weighed on an electronic balance accurate to 0.01g, and photographed for measurement of shell area. Oysters were then dissected, with wet tissue and wet shell weights measured separately on an electronic balance in order to determine the relative amounts of shell and tissue. Some oysters at each site were excluded from analysis if they had broken or damaged shells that would influence measurements of area or weight, but at least 12 oysters were dissected and measured at all sites.

Several other abiotic and biotic measurements were made at each site to determine possible correlations with oyster shell morphology. Water temperature and dissolved oxygen levels were measured with a YSI® Ecosense DO200A dissolved oxygen and temperature meter,

and salinity was measured with a refractometer. Average annual seawater temperature estimates for each region were obtained from the NOAA National Oceanographic Data Center, which provides monthly temperature data for the USA coast. Surveys for oyster drills (*Urosalpinx cinerea* and *Eupleura caudata*) were conducted in order to assess correlations between predator density and shell growth patterns. At each site one 15-m transect was run parallel to the shoreline through the center of the oyster bed in the area from which oysters were collected. Oyster drills within 0.5 m on either side of this transect (1-m wide swath) were counted; loose oysters and rocks were turned over to search for drills, but surveys were otherwise non-destructive.

Latitudinal patterns in respiration

Respiration experiments were conducted at 11 sites in March 2012 and 13 sites in August 2012, (Table 3.1, Fig. 3.2). At each site, 30 *C. virginica* were collected and placed (left valve downward) in haphazard order into closed 355-mL glass jars with in- and out-flowing air tubing. These closed chamber respirometers were placed in coolers filled with seawater; 15 chambers in a cooler at ambient seawater temperature, and 15 chambers in a cooler at seawater temperature elevated 3 °C above ambient. Chambers were aerated for 45 min so oysters could acclimate before the experiment, after which the air tubes were capped, sealing the chamber. Using closed chambers to measure bivalve respiration is common and effective (Buxton et al. 1981; Shumway and Koehn 1982; Vedpathak et al. 2011), and laboratory experiments confirmed a strong correlation between field respirometer and Strath-Kelvin respirometer results (regression ANOVA, $F_{1,28} = 578.9$, $p < 0.0001$, $r^2 = 0.954$) (Fig. 3.4).

Experiments were conducted for two hours during the winter and 30 minutes during the summer due to dramatically increased respiration rates during warmer months. At the end of the

experimental period, the lids of the respirometer chambers were removed and dissolved oxygen levels in each chamber measured with a YSI® Ecosense DO200A dissolved oxygen (DO) meter in ml L^{-1}. Each treatment had two control chambers that went through the same aeration and experimental process but did not contain oysters, so oxygen consumption (VO_2) for each oyster was measured as control DO level minus experiment DO level in ml L^{-1}. Each oyster was weighed on a digital balance and tissue weight was estimated from regressions between wet weight and tissue weight that were created from dissected oysters at each site. To compare respiration between oysters of different sizes, VO_2 for each oyster was corrected to mean dry tissue weight (0.88 g) using the allometric equation $VO_2 = mass^{0.8}$ (from Bougrier et al. 1995) as in Lannig et al. (2006). Therefore, all respiration measurements are in units of ml O_2 L^{-1} h^{-1} 0.88 g^{-1} tissue. Statistical analyses were conducted using SAS® and Minitab®.

Results

Shell growth and metabolic patterns of *Crassostrea virginica* varied with temperature in both laboratory and field experiments. Strong latitudinal gradients were found in shell thickness, while respiration rate varied with both latitude and season.

Laboratory experiments

Differences within treatments were not significant ($p > 0.4$), so data points (small and large oysters) within each treatment were pooled (Underwood 1997). Shell weight of large oysters was significantly greater in +4°C treatments than in control or +2°C treatments (Tukey test, $p < 0.05$). Shell growth in the +6°C treatment was significantly higher than in all other treatments. (Tukey test, $p < 0.05$: ANOVA for difference between treatments, $F_{(3,111)} = 21.07$, $p <$

0.001) (Fig. 3.5A). Small oysters showed a similar pattern of shell weight growth, with significantly more shell production at 4°C and 6°C above ambient temperature (ANOVA, $F_{3,116}$=6.90, $p < 0.001$, Tukey test for treatment comparisons, $p < 0.05$) (Fig. 3.5C). Inferred shell thickness showed a comparable pattern for both large (ANOVA, $F_{(3,107)} = 8.63$, $p < 0.001$) and small (ANOVA, $F_{(3,115}$=5.23, $p < 0.01$) oysters, with individuals in +4°C and +6°C treatments producing significantly thicker shell than those in ambient and +2°C treatments for both size classes (Tukey tests, $p < 0.05$) (Fig. 3.5B, D). Control oysters did not produce thicker shells than other treatments despite having twice the number of oysters per tank, suggesting that food limitation was not the mechanism driving observed shell growth patterns.

Both large and small oysters showed significant but non-directional differences in tissue growth between treatments (ANOVA, small $F_{(3,117)} = 10.4$, $p < 0.001$, larger $F_{(3,110)} = 9.21$, $p < 0.001$). Mean tissue weights for small oysters in each temperature elevation treatment were: control, 0.13; +2°C, 0.06; +4°C, -0.05; +6°C, -0.02, and for large oysters were: control, 0.01; +2°C, 0.17; +4°C, 0.02; +6°C, -0.11. Differences in large and small oyster lateral shell growth (shell area) were not significantly different between cooler (control, 2°C) and warmer (+4, 6°C) temperature treatments (small cool $\bar{x}$ ± SE = 0.39 ± 0.45 cm^2; small warm 0.38 ± 0.27 cm^2; larger cool 1.36 ± 0.48 cm^2; larger warm 1.18 ± 0.51 cm^2).

Latitudinal patterns in shell thickness

Inferred shell thickness increased significantly with increasing average annual temperature (regression ANOVA, $F_{(1,22)} = 22.9$, $r^2 = 0.51$, $p < 0.001$) (Fig. 3.6, Table 3.2). The difference between minimum and maximum shell thickness values was 64.7%, with higher shell thickness at warmer sites to the south. Because latitude displayed strong collinearity with site

temperature, it was not included as a second variable in a multiple regression. Site salinity did not add significantly (ANOVA, variables added-in-order test, $F = 0.97$, $p > 0.3$) to the regression model that included temperature, and was not considered to be a major driver of shell thickness patterns. There was also no correlation ($p > 0.3$) between shell thickness and oyster drill (*U. cinerea* + *E. caudata*) density at each site. While the effect of temperature cannot be separated from that of latitude, temperature is likely to be a major driver underlying oyster shell growth patterns.

Latitudinal patterns in metabolism

Respiration rates were strongly correlated with temperature and not with any other environmental factor measured. Respiration rates showed an exponential increase with water temperature for both March and August experiments (regression ANOVA on log-transformed data, $F_{(1,22)} = 59.4$, $r^2 = 0.73$, $p < 0.001$) (Fig. 3.7, Table 3.2). There was an approximately five-fold increase in respiration rates from March to August at all latitudes and temperatures, indicating a strong seasonal difference in metabolism superimposed on latitudinal differences (Fig. 3.7). Dissolved oxygen (DO) levels were at 100% prior to all experiments due to air supply during 45-min acclimate period, and even in the warmest treatment (heated, MacArthur Beach, Florida) dropped to an average of 73%. Few chambers at any site had DO levels drop below 60%, the minimum level used in previous studies (Mao et al. 2006).

While oysters displayed higher respiration rates at sites with higher temperatures and at experimentally elevated temperatures within sites (Fig. 3.7, 5), responses to elevated temperatures were lower than expected based on the exponential temperature-respiration curve established in control treatments (Fig. 3.8). The elevated temperature treatments tested

acclimation to local water temperatures by exposing oysters to temperatures 3°C above their local level. Respiration rates in the elevated treatment were lower than predicted by the control function at all latitudes and during both summer and winter as shown by the significant difference in control and elevated regressions with temperature (General linear model with log-transformed data, group difference $F_{1,44} = 4.61$, $p = 0.037$) (Fig. 3.8, Table 3.2).

Discussion

Several temperature-related patterns emerged from both laboratory-controlled and latitudinal thermal gradient studies, highlighting the role of temperature as a driver of large-scale patterns in oyster growth and metabolism. Our hypothesis that oysters from Long Island Sound would display increased growth rates at experimentally elevated temperatures was supported by growth data from a three-month experiment exposing *C. virginica* to flowing seawater temperature 2°C, 4°C, and 6°C above ambient temperatures. This is not novel in itself and has been shown by other researchers (e.g. Dame 1972), but the added shell thickness component in this experiment is valuable. The observed increase in shell thickness and weight growth rates at +4°C and +6°C was likely a result of the lower solubility of calcium carbonate, specifically calcite, at higher temperatures which facilitates shell deposition (Burton and Walter 1987; Morse and Mackenzie 1990; Gazeau 2010). This pattern has been observed in several species of calcifying organisms including oysters, cowries, and barnacles (Schone et al. 2006; Kawai 2009; Waldbusser et al. 2011). Higher shell growth rates at warmer temperatures could also be due to increased metabolic rates, which could accelerate the processes involved in shell deposition, but this scenario would be accompanied by higher somatic tissue growth, which was not observed. Shell and tissue growth rates could have been limited by food availability in the tanks due to the

high filtration rates of oysters, but this effect would be the same for all of the treatments and would not explain the patterns found.

Oysters can filter large volumes of water (5 L h^{-1} g^{-1} dry mass) (Newell 1988) so it is possible that *C. virginica* in flowing seawater tanks were food-limited, especially in the control treatment that had twice the number of oysters. While oysters often produce thicker shells at low food concentration, clearance rates estimated by Riisgard (1988) at 28°C suggest that the highest total clearance rate in any treatment should be 20 L h^{-1} and water was supplied at 60 L h^{-1}(Brown and Hartwick 1988). Our shell growth experiments were conducted in winter at temperatures of 8-20°C, depending on treatment, which would cause even lower feeding rates. In addition, there were significant differences in shell deposition between +2°C, +4°C, and +6°C treatments, which each had the same number of oysters, making it unlikely that the number of control oysters influenced results. Increased shell growth at warmer temperatures indicates that shell deposition rates are higher under these conditions, irrespective of the allocation to shell thickness or lateral growth. These results show a strong effect of temperature changes on shell growth patterns for oysters from a single population and suggest that temperature could be a mechanism behind latitudinal patterns in shell morphology.

Previous studies on calcifying species (Nicol 1967; Pytkowicz 1969; Graus 1974; Trussell and Etter 2001; Waldbusser et al. 2011) support our finding that oyster shell thickness is greater at lower latitudes, with a 65% increase from north to south. Inferred shell thickness (correlated with actual thickness) showed a strong negative correlation with latitude and a positive correlation with temperature (Table 3.2, Fig. 3.6). The 65% increase in shell thickness from north to south shows a large latitudinal gradient that is likely due in part to temperature-induced increases in shell deposition rate. Oysters do increase calcification rates (Waldbusser et

al. 2011) and shell growth rates (Fig. 3.5) at experimentally elevated temperatures, supporting the hypothesis that these latitudinal patterns are temperature related. Increased shell thickness at warmer latitudes has also been reported for littorine snails in the Northwest Atlantic and cowries in the Northwest Pacific (Trussell and Etter 2001; Irie 2005). These studies hypothesize that the impact of water temperature on shell deposition is driving latitudinal thickness patterns, an idea that is supported by laboratory results for *C. virginica* (Fig. 3.5) (Waldbusser et al. 2011).

Shell deposition rates are inferred from shell thickness patterns but cannot be definitively established without growth data at all sites, which would require either long-term experiments or aging of specimens -- both beyond the scope of this study. Latitudinal shell thickness patterns corresponded well with laboratory temperature experiments, but collinearity between latitude and temperature makes it difficult to establish causation on a large geographic scale. Increasing predation intensity toward the equator has been proposed as a mechanism behind latitudinal patterns in shell morphology for some mollusks and cannot be ruled out for *C. virginica* (Vermeij and Veil 1978). Eastern oysters can increase shell thickness as an inducible defense in response to crab (*Rhithropanopeus harrisii* and *Callinectes sapidus*) and oyster drill (*Urosalpinx cinerea*) predators (Newell et al. 2007; Johnson and Smee 2012; Lord and Whitlatch 2012). Whereas surveys in the present study ruled out *U. cinerea* and *E. caudata* density as a factor impacting large-scale shell thickness patterns, predators such as crabs or other species of oyster drills (e.g. *Stramonita* spp.) tend to increase in abundance to the south and could impact *C. virginica* morphology.

The final hypothesis that populations of southern *C. virginica* would be more stressed at experimentally elevated temperatures during the summer than northern oysters was not supported by experimental respiration data, as oysters showed similar thermal responses at

warmer and cooler sites during summer and winter (Fig. 3.8). The oxygen consumption measurements in the present study were brief, and provide snapshots of respiration responses to thermal stress at two different times of year across latitudes. As such, there may be longer-term differences in acclimation capacity between seasons that were not captured by these experiments. Respiration rates (proxy for metabolism) were strongly and exponentially related to temperature during both March and August sampling seasons, as expected based on previous work on thermal responses of oysters (Shumway and Koehn 1982) (Fig. 3.7). As a result, respiration rates were up to five-fold higher during summer than winter, indicating large seasonal swings in metabolic rate of *C. virginica*. Since oysters play a large and active role in estuaries in terms of filtration and benthic-pelagic coupling, high seasonal variability in metabolic rate indicates seasonal shifts in the ecological influence of oyster reefs (Coen et al. 2007). Seasonal acclimatization plays a role as well, as evidenced by lower-than-expected respiration rates at elevated temperatures at all sites (Fig. 3.8).

While respiration rates in elevated temperature treatments were generally higher than the ambient temperature controls, they fell consistently below the regression between control temperature and respiration rate. This means that the temperature-specific respiration rates were higher for control than elevated treatments at all latitudes, suggesting relatively finely tuned acclimatization in *C. virginica*. Seasonal acclimatization, or the adjustment to natural seasonal changes in temperature, may allow oysters to gradually adjust their metabolic rate based on seasonally shifting water temperatures (Gatten et al. 1988). This would explain the higher temperature-specific respiration rates in control treatments, as oysters were acclimatized to that specific temperature and could not fully acclimate in elevated temperature treatments (Fig. 3.8). The fact that oysters from all latitudes responded similarly to temperatures elevated 3°C above

their local seasonal maximum suggests a high level of local adaptation or acclimatization. The large degree of seasonal and latitudinal acclimatization displayed by *C. virginica* suggests that studies conducted at one location or point in time (e.g. Dame 1972; Buxton et al. 1981; Shumway and Koehn 1982; Hummel et al. 2000; Mao et al. 2006) should take a cautious approach when attempting to draw broad conclusions about the metabolic response of a species.

Adaptation or even acclimation was not directly measured during this study since it took place over a short period of time, but a few conclusions can be drawn about the latitudinal metabolic patterns. The latitudinal compensation hypothesis described by Levinton (1983) suggests that populations could evolutionarily shift their temperature-metabolism curve to optimize performance under local environmental conditions. This does not appear to be the case for *C. virginica*, as oxygen consumption at all sites fell along a similar exponential curve related to site temperature (Fig. 3.7). In addition, populations over a latitudinal gradient responded similarly to experimental temperature increases (Fig. 3.8), suggesting a low degree of adaptation to local temperatures. Metabolic rates increased concomitantly with site temperature as expected from previous studies (Shumway and Koehn 1982), so there also does not appear to be any type of counter-gradient variation in metabolic rate (Levins 1969). The overall pattern of oxygen consumption with latitude appeared to be well-constrained by site temperature alone, which explained over 60% of the variability in temperature between sites. However, there are several factors that can influence oyster feeding and metabolism (reviewed by Cranford et al. 2011), so temperature is likely to be just one of several factors (e.g. food availability) that influence respiration rate.

In conclusion, we demonstrated that temperature can exhibit strong control on oyster shell growth and deposition in a controlled setting. Ocean temperature may also be a major

driver of latitudinal patterns in shell thickness, as we observed a strong correlation between temperature and inferred shell thickness at 24 sites over 21 degrees of latitude on the east coast of North America. Latitudinal differences in metabolic rates were highly correlated with site temperature, but oysters showed a significant level of seasonal acclimatization to local water temperatures. Growth experiments and shell thickness surveys suggest that warmer temperatures resulting from climate change will likely cause increases in both oyster shell growth and shell thickness across latitudes. The strong positive correlation between seawater temperature and shell thickness may be caused by decreased solubility of calcium carbonate in warmer water, facilitating shell deposition with global warming. We illustrate that oyster metabolic rates, acclimatization, and shell deposition vary widely with latitude and season and that differences in temperature may be one of the major mechanisms underlying this variation.

Acknowledgements

Jeremy Calini was instrumental in August 2012 sampling at all sites. Additional thanks to Dr. Evan Ward and Dr. Dane Frank for providing valuable input into the analysis of the data and preparation of the manuscript. Marine labs that provided lodging and facilities included Virginia Institute of Marine Science, VIMS Eastern Shore Lab, UNC-Institute of Marine Sciences, Duke Marine Lab, Skidaway Institute of Oceanography, USC Belle Baruch Field Station, and Whitney Marine Lab. Collecting permits were granted by the Florida Fish and Wildlife Conservation Commission (SAL 12-1368-SR and SAL 12-1401-SR), Florida DER Division of Recreation and Parks (Permit # 07171210), MacArthur Beach State Park (Permit # 5-12-26), Georgia DNR, South Carolina DNR and North Carolina Estuarine Research Reserve (Permit # 3-2012).

Funding provided by University of Connecticut pre-doctoral fellowship and Connecticut Sea Grant College Program.

References

Boucher G, Boucher-Rodoni R (1988) In situ measurement of respiratory metabolism and nitrogen fluxes at the interface of oyster beds. Mar Ecol Prog Ser 44:229–238

Bougrier S, Geairon P, Deslous-Paoli J, Bacher C, Jonquieres G (1995) Allometric relationships and effects of temperature on clearance and oxygen consumption rates of *Crassostrea gigas* (Thunberg). Aquaculture 134:143–154

Brown J, Hartwick E (1988) Influences of temperature, salinity and available food upon suspended culture of the Pacific oyster, *Crassostrea gigas*: I. Absolute and allometric growth. Aquaculture 70:231–251

Burton EA, Walter LM (1987) Relative precipitation rates of aragonite and Mg calcite from seawater: Temperature or carbonate ion control? Geology 15:111–114

Buxton C, Newell R, Field J (1981) Response-surface analysis of the combined effects of exposure and acclimation temperatures on filtration, oxygen consumption and scope for growth in the oyster *Ostrea edulis*. Mar Ecol Prog Ser 6:73–82

Cherkasov A, Taylor C, Sokolova IM (2010) Seasonal variation in mitochondrial responses to cadmium and temperature in eastern oysters *Crassostrea virginica* (Gmelin) from different latitudes. Aquat Toxicol (Amsterdam, Netherlands) 97:68–78

Coen L, Brumbaugh R, Bushek D, Grizzle R, Luckenbach M, Posey M, Powers S, Tolley S (2007) Ecosystem services related to oyster restoration. Mar Ecol Prog Ser 341:303–307

Cranford P, Ward JE, Shumway S (2011) Bivalve filter feeding: variability and limits of the aquaculture biofilter. In: Shumway S (ed) Shellfish aquaculture and the environment. John Wiley and Sons, Hoboken, pp 81–124

Dame R (1972) The ecological energies of growth, respiration and assimilation in the intertidal American oyster *Crassostrea virginica*. Mar Biol 17:243–250

Dissard D, Nehrke G, Reichart GJ, Bijma J (2010) The impact of salinity on the Mg/Ca and Sr/Ca ratio in the benthic foraminifera *Ammonia tepida*: Results from culture experiments. Geochim Cosmochim Acta 74:928–940

Dittman D, Ford S, Haskin H (1998) Growth patterns in oysters, *Crassostrea virginica*, from different estuaries. Mar Biol 132:461–469

Ferguson JE, Henderson GM, Kucera M, Rickaby REM (2008) Systematic change of foraminiferal Mg/Ca ratios across a strong salinity gradient. Earth Planet Sci Lett 265:153–166

Galtsoff PS (1964) The American oyster, *Crassostrea virginica* (Gmelin). Fishery Bull Fish Wildl Serv US 64:1–480

Gatten Jr R, Echternacht A, Wilson M (1988) Acclimatization versus acclimation of activity metabolism in a lizard. Physiol Zool 61:322–329

Gazeau F, Gattuso J, Dawber C (2010) Effect of ocean acidification on the early life stages of the blue mussel *Mytilus edulis*. Biogeosciences 7:2927–2947

Grabowski J, Hughes A, Kimbro D, Dolan M (2005) How habitat setting influences restored oyster reef communities. Ecology 86:1926–1935

Graus R (1974) Latitudinal trends in the shell characteristics of marine gastropods. Lethaia 7:303–314

Haure J, Penisson C, Bougrier S, Baud J (1998) Influence of temperature on clearance and oxygen consumption rates of the flat oyster *Ostrea edulis*: determination of allometric coefficients. Aquaculture 169:211–224

Hummel H, Bogaards R, Bachelet G, Caron F, Sola J, Amiard-Triquet C (2000) The respiratory performance and survival of the bivalve *Macoma balthica* (L.) at the southern limit of its distribution area: a translocation experiment. J Exp Mar Biol Ecol 251:85–102

Irie T (2005) Geographical variation of shell morphology in *Cypraea* a*nnulus* (Gastropoda: Cypraeidae). J Moll Stud 72:31–38

Jansen JM, Pronker AE, Kube S, Sokolowski A, Sola JC, Marquiegui MA, Schiedek D, Wendelaar Bonga S, Wolowicz M, Hummel H (2007) Geographic and seasonal patterns and limits on the adaptive response to temperature of European *Mytilus* spp. and *Macoma balthica* populations. Oecologia 154:23–34

Jihong Z, Fang J, Hawkins A, Pascoe P (2004) The effect of temperature on clearance rate and oxygen consumption of scallops, *Chlamys farreri*. J Shell Res 23:715–721

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

Kanazawa T, Sato S (2007) Environmental and physiological controls on shell microgrowth pattern of *Ruditapes philippinarum* (Bivalvia: Veneridae) from Japan. J Moll Stud 74:89–95

Lannig G, Flores JF, Sokolova IM (2006) Temperature-dependent stress response in oysters, *Crassostrea virginica*: pollution reduces temperature tolerance in oysters. Aquat Toxicol 79:278–87

Levins R (1968) Thermal acclimation and heat resistance in *Drosophila* species. Am Nat 103:483–499

Levinton J (1983) The latitudinal compensation hypothesis: growth data and a model of latitudinal growth differentiation based upon energy budgets. I. Interspecific comparison of *Ophryotrocha* (Polychaeta: Dorvilleidae). Biol Bull 165:686–698

Lord JP, Whitlatch RB (2012) Inducible defenses in the eastern oyster *Crassostrea virginica* Gmelin in response to the presence of the predatory oyster drill *Urosalpinx cinerea* Say in Long Island Sound. Mar Biol 159:1177–1182

Malone P, Dodd J (1967) Temperature and salinity effects on calcification rate in *Mytilus edulis* and its paleoecological implications. Limnol Oceanogr 12:432–436

Mao Y, Zhou Y, Yang H, Wang R (2006) Seasonal variation in metabolism of cultured Pacific oyster, *Crassostrea gigas*, in Sanggou Bay, China. Aquaculture 253:322–333

Martin S, Thouzeau G, Chauvaud L, Jean F, Guérin L, Clavier J, Thouzeau G, Jean F, Guerin L (2006) Respiration, calcification, and excretion of the invasive slipper limpet, *Crepidula fornicata* L.: Implications for carbon, carbonate, and nitrogen fluxes in affected areas. Limnol Oceanogr 51:1996–2207

Miyaji T, Tanabe K, Matsushima Y, Sato S, Yokoyama Y, Matsuzaki H (2010) Response of daily and annual shell growth patterns of the intertidal bivalve *Phacosoma japonicum* to Holocene coastal climate change in Japan. Palaeogeogr, Palaeoclimatol, Palaeoecol 286:107–120

Morse JW, Mackenzie FT (1990) Geochemistry of sedimentary carbonates. Elsevier, New York

Nakaoka M (2000) Nonlethal effects of predators on prey populations: predator-mediated change in bivalve growth. Ecology 81:1031–1045

Newell RIE (1988) Ecological changes in Chesapeake Bay: Are they the result of overharvesting the American oyster, *Crassostrea virginica*? Understanding the estuary. Adv Chesapeake Bay Res 129:536–546

Newell RIE, Johson L, Kofoed L (1977) Adjustment of the components of energy balance in response to temperature change in *Ostrea edulis*. Oecologia 30:97–110

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

Nicol D (1967) Some characteristics of cold-water marine pelecypods. J Paleontol 41:1330–1340

Pachauri RK, Reisinger A (2007) Climate change 2007. Synthesis report. Contribution of working groups I, II, and III to the fourth assessment report. Intergovernmental Panel on Climate Change, Geneva, Switzerland

Palmer A (1982) Growth in marine gastropods. A non-destructive technique for independently measuring shell and body weight. Malacologia 23:63–73

Palmer A (1992) Calcification in marine molluscs: how costly is it? Proc Nat Acad Sci 89:1379–1382

Palmer RE, Carriker MR (1979) Effects of cultural conditions on morphology of the shell of the oyster *Crassostrea virginica*. Proc Nat Shellfish Ass 69:58–72

Pernet F, Tremblay R, Redjah I, Sévigny JM, Gionet C (2008) Physiological and biochemical traits correlate with differences in growth rate and temperature adaptation among groups of the eastern oyster *Crassostrea virginica*. J Exp Biol 211:969–77

Pytkowicz R (1969) Chemical solution of calcium carbonate in sea water. Am Zool 9:673–679

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

Schöne B, Rodland D, Fiebig J (2006) Reliability of multitaxon, multiproxy reconstructions of environmental conditions from accretionary biogenic skeletons. J Geol 114:267–285

Shumway S, Koehn R (1982) Oxygen consumption in the American oyster *Crassostrea virginica*. Mar Ecol Prog Ser 9:59–68

Singh S, Zouros E (1978) Genetic variation associated with growth rate in the American oyster (*Crassostrea virginica*). Evolution 32:342–353

Stunz G, Minello T, Rozas L (2010) Relative value of oyster reef as habitat for estuarine nekton in Galveston Bay, Texas. Mar Ecol Prog Ser 406:147–159

Trussell GC, Etter RJ (2001) Integrating genetic and environmental forces that shape the evolution of geographic variation in a marine snail. Genetica 112-113:321–37

Trussell GC, Ewanchuk P, Bertness M (2003) Trait-mediated effects in rocky intertidal food chains: predator risk cues alter prey feeding rates. Ecology 84:629–640

Underwood, AJ (1997) Experiments in ecology: their logical design and interpretation using analysis of variance. Cambridge University Press, Cambridge, UK

Vedpathak A, Muley R, Tangade D (2011) Variations in the rate of oxygen consumption, ammonia excretion and O:N ratio of freshwater bivalve, *Indonaia caeruleus* (Prashad, 1918) in relation to body size during summer. Bioscan 6:315–318

Vermeij G, Veil J (1978) A latitudinal pattern in bivalve shell gaping. Malacologia 17: 57–61

Waldbusser GG, Voigt EP, Bergschneider H, Green MA, Newell RIE (2010) Biocalcification in the Eastern Oyster (*Crassostrea virginica*) in relation to long-term trends in Chesapeake Bay pH. Est Coast 34:221–231

Figure Captions

Figure 3.1 Linear regression between actual measured shell weight and immersed weight of live *C. virginica*.

Figure 3.2 Sampling locations for *C. virginica* studies in March and August 2012. Open diamonds represent sites sampled only in March; open circles, in August; filled diamonds both March and August. See Table 3.1. Latitude onright margin.

Figure 3.3 Linear regression between inferred *C. virginica* shell thickness (log) and measured shell thickness (log).

Figure 3.4 Linear regression between *C. virginica* oxygen consumption measured in closed chamber and Strath-Kelvin respirometers.

Figure 3.5 Laboratory *C. virginica* growth results (control, +2, +4, and +6 °C treatments). Large oyster growth measured in terms of wet shell weight (A) and inferred shell thickness (B). Small juvenile oysters showed similar shell weight (C) and thickness (D) patterns (±SE bars). Letters indicate significant differences between groups.

Figure 3.6 Differences in *C. virginica* shell thickness with average annual temperature (n = 24); annual temperature data from NOAA NODC. Shell thickness displayed as residual calculated from a regression between shell weight and left shell area.

Figure 3.7 Metabolism of *C. virginica* at all sites during both March (n = 11) and August (n = 13) experiments at ambient seawater temperatures. Respiration rate is exponentially related to site temperature (Table 3.2).

Figure 3.8 Respiration responses of *C. virginica* exposed to ambient and +3°C elevated seawater temperatures at each site. Examples of site pairs (ambient and heated from same site) highlighted by letters: (a) Damariscotta River in winter, (b) Fort Pierce in summer, and (c) Tybee Island in summer.

Table 3.1. Site details for all 24 sites sampled in March (winter) or August (summer) 2012. Winter and summer temperatures refer to measurements taken at each site; if a site was not sampled in that season, then temperatures are taken from average local SST for that month (asterisk next to satellite data) (from www.nodc.noaa.gov). Site labels refer to site locations shown in Figure 1. Sites at which metabolism experiments were conducted are indicated along with experiment timing in the metabolism experiment column.

Site Name	Site Label	Metab Expt	Latitude N to S	Salinity	Temperature (°C)	
					Winter	Summer
Miramichi, NB	NB	Winter	47.22	30	*-1.0	*15.0
North Point, PEI	PEI		47.05	30	*0.0	*16.0
Damariscotta River, ME	DR	Both	44.03	27	7.9	17.0
Sippiwissett Marsh, MA	SM	Winter	41.57	25	6.0	*22.3
Quissett Harbor, MA	QH	Summer	41.57	24	*11.9	24.3
Newport, RI	RI	Summer	41.47	25	7.5	21.0
Groton, CT	CT	Winter	41.33	27	6.0	*22.0
Eastern Shore 1, VA	ES1	Both	37.62	26	16.7	30.7
Eastern Shore 2, VA	ES2		37.61	24	15.6	30.8
Chesapeake Bay, VA	CB	Summer	37.46	24	*9.0	25.0
Rachel Carson 1, NC	RC1		34.71	27	16.9	26.5
Rachel Carson 2, NC	RC2		34.71	25	17.1	26.5
Rachel Carson 3, NC	RC3	Both	34.71	25	17.1	26.5
Oyster Landing, SC	OL	Summer	33.35	25	18.6	34.2
Clambank Landing, SC	CL		33.34	27	18.2	32.7
Folly Beach, SC	FB	Both	32.66	20	17.9	30.0
Tybee Island, GA	TI	Both	31.99	24	16.8	30.7
West Tybee, GA	WT		31.99	22	*16.8	30.7
Marshes of Glynn, GA	MG		31.15	23	21.0	27.2
Pumpkin Hill, FL	PH	Both	30.48	16	19.7	29.7
Butler Park, FL	BP		29.79	24	20.6	27.2
Crescent Beach, FL	HB	Both	29.76	27	20.9	26.7
Fort Pierce, FL	FP	Summer	27.46	27	*23.0	29.4
MacArthur Beach, FL	MB	Both	26.83	25	24.6	31.2

Table 3.2. Summary of statistical results for regressions between shell morphology or metabolism and site temperatures or locations. All F and *P* values are from regression ANOVA tables calculated in Minitab® and indicate significant correlations between all variables shown. Coefficients of determination (R^2) show that these relationships explain a considerable amount of data variability, though much geographic variability is evidently due to other unknown factors.

X	**Y**	**Function**	**R^2**	***N***	**F**	***P***
Latitude	Shell Thickness (resid)	Y = 0.18 – 0.0052X	0.48	24	20.5	< 0.001
Site Temperature	Shell Thickness (resid)	Y = -0.10 + 0.006X	0.51	24	22.8	< 0.001
Site Temperature	Log Respiration	Y = -1.01 + 0.040X	0.73	24	59.4	< 0.001
Elevated Temperature	Log Respiration (elev)	Y = -1.46 + 0.049X	0.72	24	55.6	< 0.001

Figure 3.1 (see caption on p. 83)

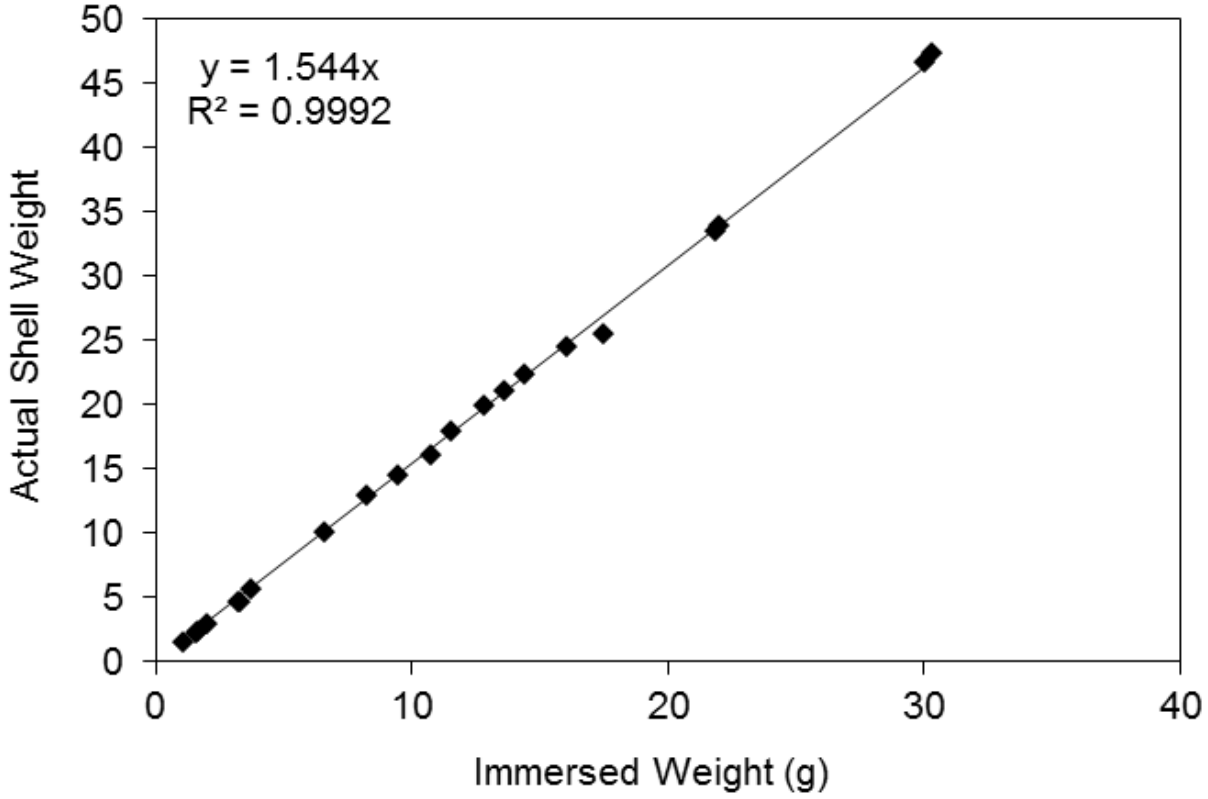


Figure 3.2 (see caption on p. 83)

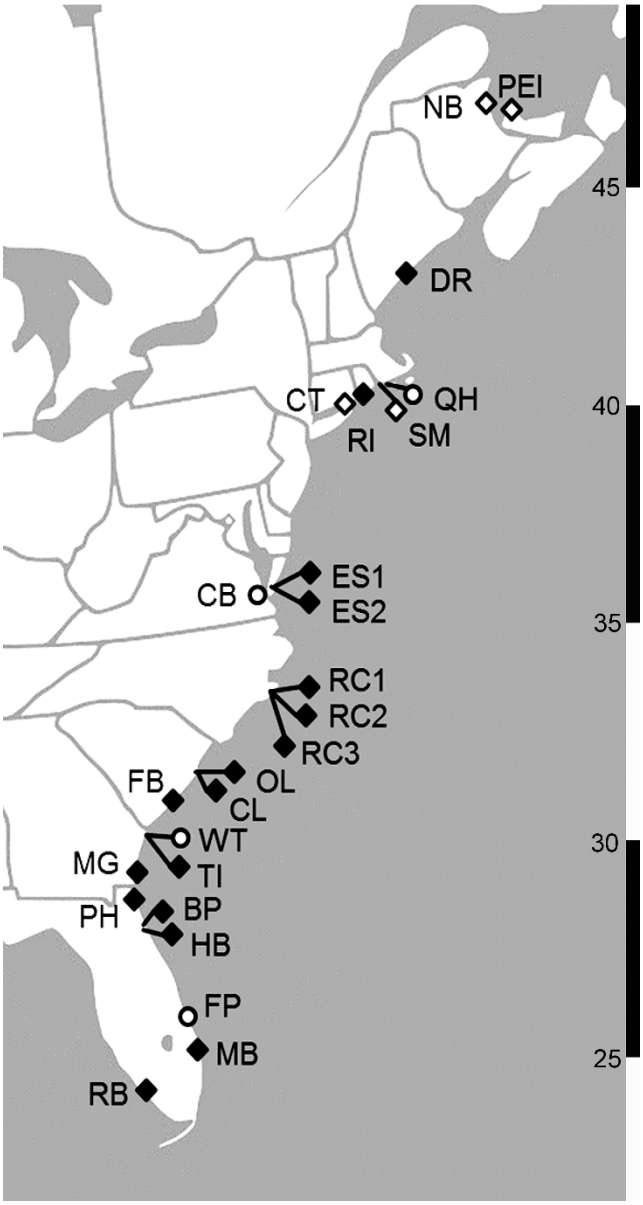


Figure 3.3 (see caption on p. 83)

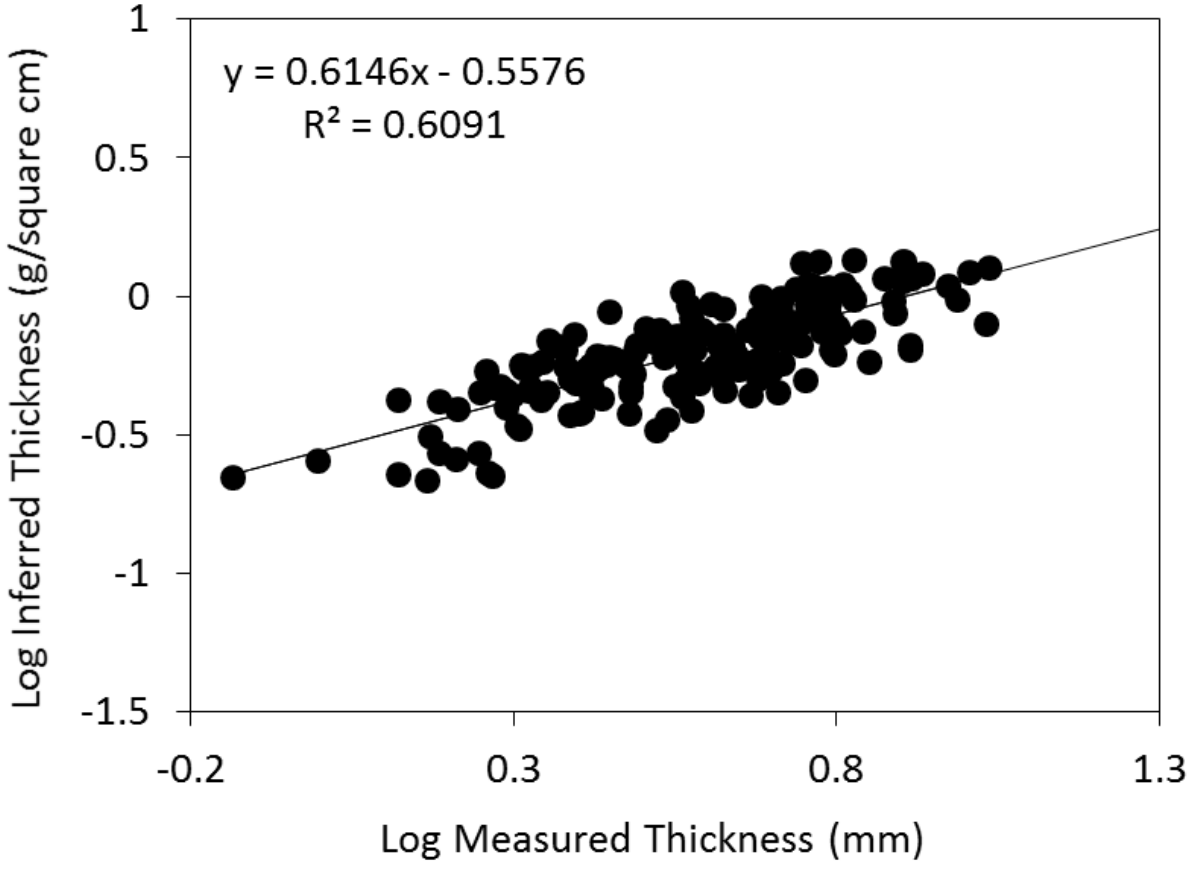


Figure 3.4 (see caption on p. 83)

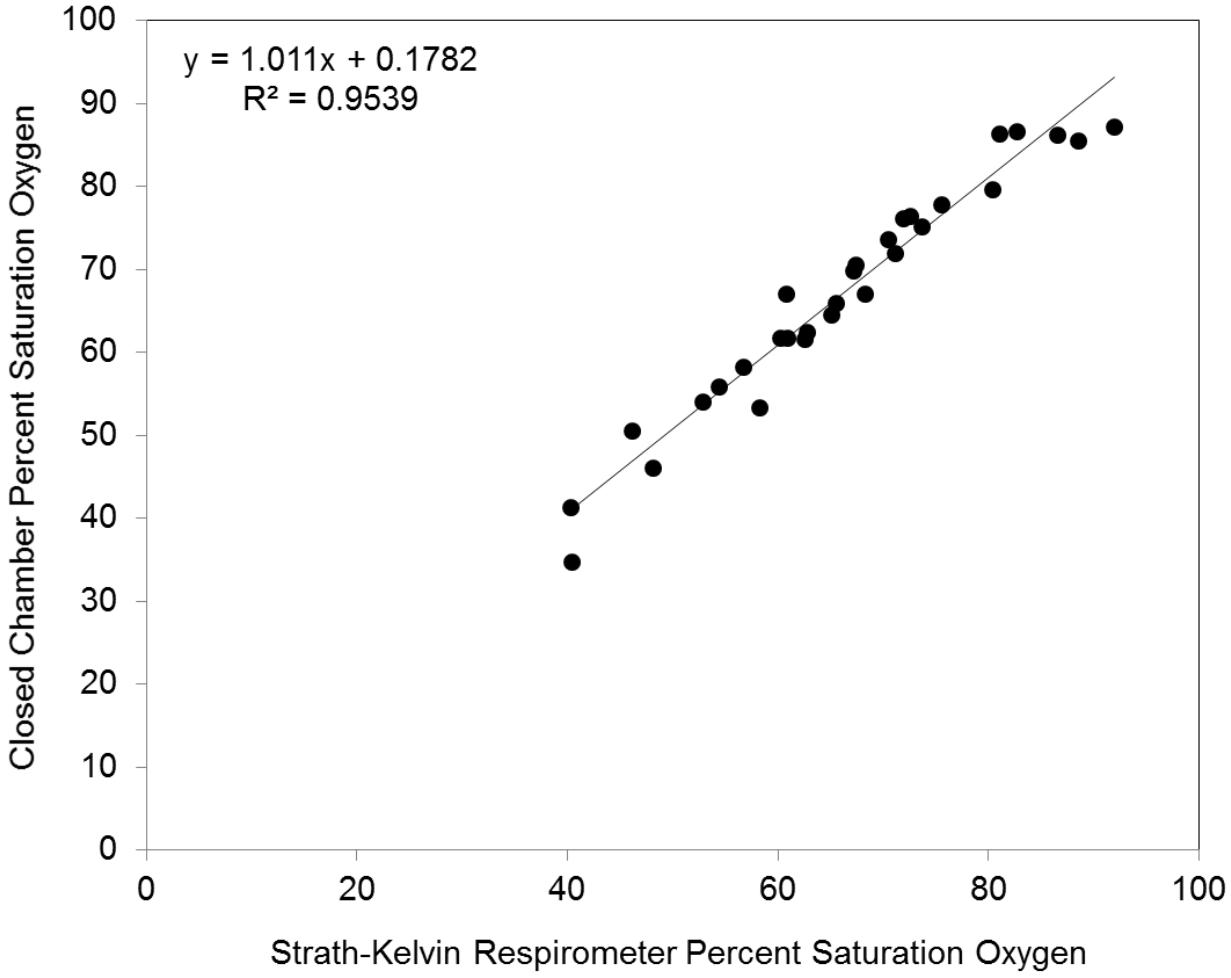


Figure 3.5 (see caption on p. 83)

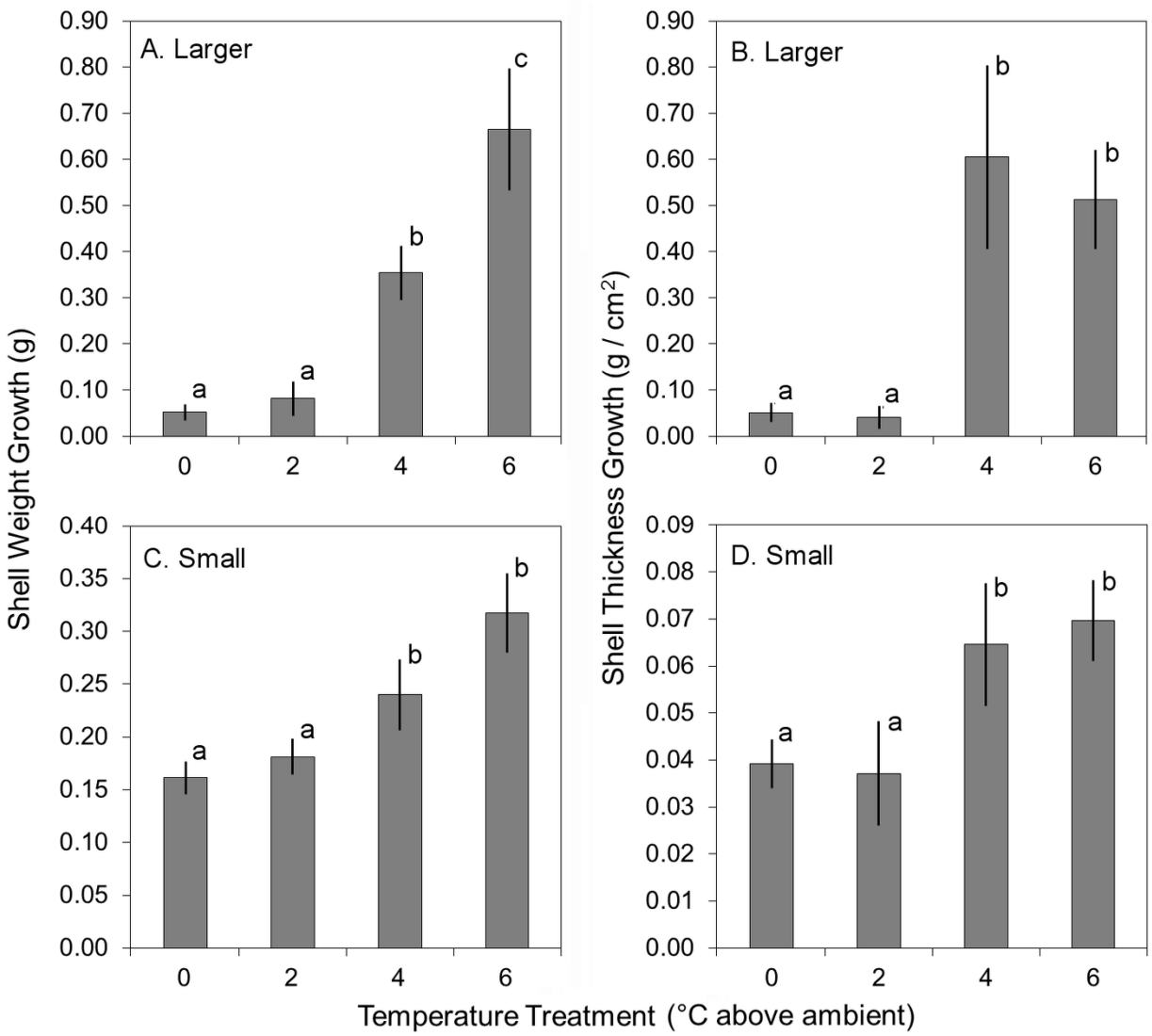


Figure 3.6 (see caption on p. 83)

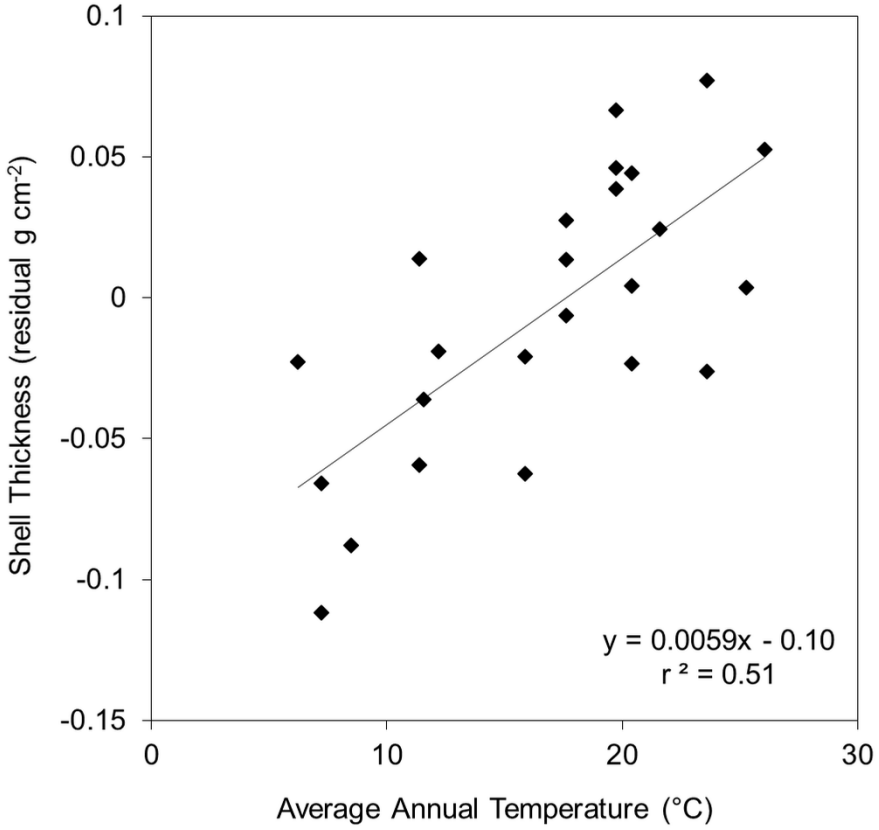


Figure 3.7 (see caption on p. 83)

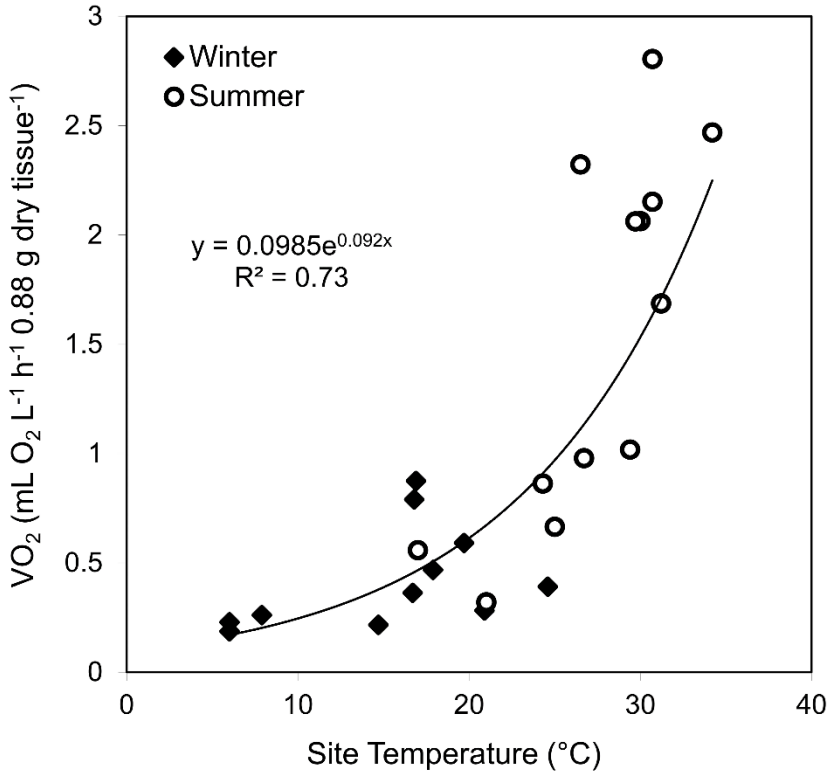
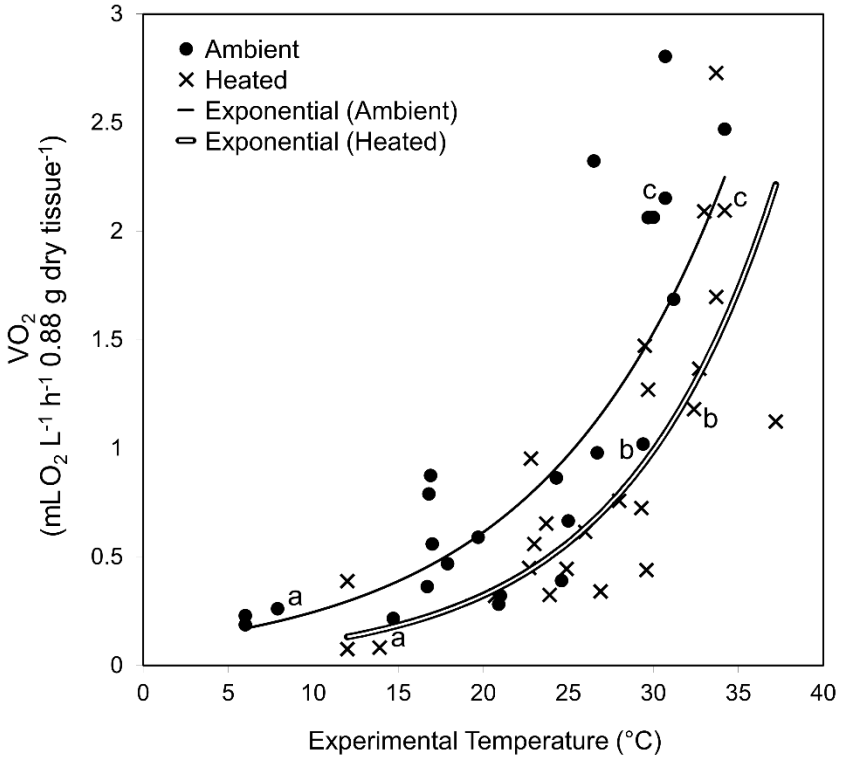


Figure 3.8 (see caption on p. 83)



Chapter 4

Predicting competitive shifts and responses to climate change based on latitudinal distributions of species assemblages[4]

Abstract

Many terrestrial plant and marine benthic communities involve intense competition for space as a means to survive and reproduce. Superior competitors can overwhelm other species numerically with high reproductive rates, indirectly with high growth rates that facilitate space acquisition, or directly with competitive overgrowth. To assess how climate change could affect competitive interactions, we examined latitudinal patterns in species growth rates and overgrowth competition via field surveys and experiments with marine epibenthic communities. Epibenthic fouling communities are dominated by invasive tunicates, bryozoans and other species that grow on docks, boats and other artificial structures. Fouling communities are space-limited, so growth rate and overgrowth competition play an important role in shaping abundance patterns. We experimentally assessed temperature-dependent growth rates of several tunicates and bryozoans in eight regions spanning the US east and west coasts. Several species displayed positive growth responses to warmer temperature in the northern portions of their latitudinal ranges, and vice versa. We used photo surveys of floating docks at 20 harbors in each region to compare communities and overgrowth competition. There was a strong correlation across species

[4]Lord J. & Whitlatch R. Predicting competitive shifts and responses to climate change based on latitudinal distributions of species assemblages. *Ecology*, in review.

and regions between growth rate and competitive ability, indicating that growth plays an important role in competitive outcomes. Because growth rates are typically temperature-dependent for organisms that compete for space, including terrestrial plants, fungi, algae, bacteria, and sessile benthic organisms, global warming could affect competitive outcomes. Our results suggest that these competitive shifts can be predicted by species' relative growth rates and latitudinal ranges.

Keywords: competition, growth, climate change, fouling, benthic ecology, invasive species, tunicates, bryozoans

Introduction

Global warming is likely to alter community composition in many terrestrial and marine systems because of range shifts, differential responses to thermal stress, and changes in competitive interactions between species. Alterations of community structure are difficult to test experimentally because of the complexity of most systems and because of the number of competitive and predator-prey interactions between species. As a result, most predictions of responses to global warming test thermal tolerance of individual species (e.g. Chamaille-Jammes 2006, McCarthy et al. 2007, Koopmans & Wijffels 2008, Zani 2008, Epelbaum et al. 2009) either through laboratory experiments (e.g. Koskela et al. 1997, Amui-Vedal et al. 2007, Stoner et al. 2010, Lord & Whitlatch 2013) or by sampling along a latitudinal temperature gradient (e.g. Lonsdale & Levinton 1985, Houde 1989, Doorslaer & Stoks 2005, Parra et al. 2009). These studies provide valuable information about species-specific responses to warmer temperatures

but do not address interspecific interactions that will also play a large role in determining community-level responses.

Several publications on impacts of global warming emphasize the need for studies that focus on species groups (Gilman et al. 2010), interspecific interactions (Davis et al. 1998, Harley et al. 2006, Sorte & Stachowicz 2011) and that incorporate large-scale thermal experiments (Somero 2012). Relationships between species are not the only thing complicating the assessment of community responses to global warming, as growth rates within species vary not only with climate but also with season and latitude (Tanabe & Oba 1988, Houde et al. 1989, Parra et al. 2009). Species can respond differently to increased temperature in disparate parts of their latitudinal range, often displaying either latitudinal compensation (i.e., higher maximum growth or metabolic rate in northern portion of their distribution) or local adaptation (i.e., shifts in optimum temperatures) (Levinton 1983, Conover & Present 1980, Yamahira & Conover 2002, Doorslaer & Stoks 2005). Competition between species also does not remain static with changing conditions, as competitive outcomes can be influenced by altitude (Schob et al. 2013), salinity (Pennings et al. 2003), nutrient limitation (Tilman 1976), temperature (Chu et al. 1978, Nedwell & Rutter 1994, Milazzo et al. 2013) or other stressors.

We set out to test latitudinal changes in competition and thermal responses because changes in growth with temperature (thermal performance curves) can be used to predict competitive shifts (Widden 1984, Wilson 1984, Deutsch et al. 2008, Clusella-Trullas et al. 2011, Milazzo et al. 2013). Differential growth responses to temperature have been cited as a common mechanism driving changes in competition between species in several groups of organisms including bacteria (Nedwell & Rutter 1994), plants (Chu et al. 1978), and corals (Johannes et al. 1983). Growth rate is particularly relevant to competition in systems in which space is limiting

and there are few dominant species, so thermal effects on growth would be expected to alter competitive interactions in these systems. The impact of growth on competition is commonly studied in terrestrial plants where fast growth rates allow individuals or clones to outcompete other species for space and light (e.g. Chu et al. 1978, Goldberg & Landa 1991, Clay et al. 1993, Loehle 1998, Aerts 1999). In the marine realm, sessile epibenthic invertebrate communities compete in a similar fashion to terrestrial plants, as they are space-limited and compete at the edges of clones or colonies (Sebens 1982).

Marine epibenthic fouling communities are primarily composed of sessile marine invertebrates such as sponges, tunicates, and bryozoans, many of which have encrusting colonies that compete for space via overgrowth competition (Sebens 1986, Nandakumar et al. 1993, Bell & Barnes 2003, Lindeyer & Gittenberger 2011). Because fouling community species have short lifespans, these communities do not have a climax stage, as in terrestrial plant communities (Sutherland & Karlson 1977). Nevertheless, they serve as effective study systems for growth and competitive effects because they are largely two-dimensional and are space limited (Sorte & Stachowicz 2011). These communities can be influenced by predation (Whitlatch & Osman 2009), wave exposure (Janiak et al. 2013), flow (Maughan & Barnes 2000) and other factors, but on a latitudinal scale, temperature is the overriding factor driving community composition (Engle & Summers 1999). Fouling communities are often dominated by non-native species, so differences in thermal responses between natives and non-natives could cause a shift in the range, abundance, and competitive dominance of non-native fouling species (Sorte & Stachowicz 2011).

Growth rates at multiple temperatures within one site have been established for several fouling species (Bell & Barnes 2003, McCarthy et al. 2007, Bullard & Whitlatch 2009, Mercado-

Molina & Yoshioka 2009, Saunders & Metaxas 2009, Janiak et al. 2013). While these data provide useful thermal performance curves they do not incorporate latitudinal changes in thermal responses for a given species. Other studies on marine epibenthic communities catalogue changes in community composition over time then correlate this with corresponding increases in temperature over the same period (Sagarin et al. 1999, Sorte & Stachowicz 2011, Trowbridge et al. 2013). Data on range shifts and species distributions over time do not show causation without physiological experiments that establish the mechanism behind shifts in species assemblages (Somero 2012). Water temperatures are expected to increase 2-4 °C in the northwestern Atlantic and 1.5-3°C in the northeastern Pacific by 2100, so changes in temperature-dependent growth rates and competitive abilities will strongly influence changes in community composition (Hansen et al. 2006, IPCC 2007).

The goal of this study was to determine how responses to warmer temperatures vary within and between species and locations by testing thermal growth responses of several fouling species at several locations over a large latitudinal range. In addition to thermal experiments, we conducted extensive surveys of abundance and competitive overgrowth at 160 sites on the east and west coasts of North America in order to correlate differential growth responses to temperature with latitudinal changes in competitive interactions. We included several interacting species from the fouling community in these experiments and surveys because the impact of global warming on interspecific interactions is important to understand when predicting community responses to warming (Davis et al. 1998, Gilman et al. 2010, Sorte & Stachowicz 2011). Because a goal of the project was to explore the possibility that temperature-dependent growth rates were the mechanism behind changes in community composition along a latitudinal

gradient, the thermal response experiments were necessary to indicate causation for temperature or factors correlated with it (Somero 2012).

We tested two hypotheses with these thermal experiments and field surveys of fouling communities. First, we hypothesized that individual species would respond positively (higher growth rate) to experimentally elevated temperatures in the northern portion of their latitudinal ranges and negatively in the southern parts of their ranges. Most species have thermal performance curves that have a peak or optimum temperature (T_{opt}) somewhere near the middle of their latitudinal range, so performance (growth) should increase with elevated temperature below T_{opt} and decrease above T_{opt} (Deutsch et al. 2008, Clusella-Trullas et al. 2011). Second, we hypothesized that changes in proportionate growth rates (ratio of growth rates between species pairs) with latitude would result in competitive shifts towards faster growing species. This could then be used to predict community responses to climate change or to new invasions based on the growth and competitive changes with temperature for each species.

Methods

To test the thermal growth responses of fouling species in different parts of their range, we conducted experiments and surveys in 8 regions, 4 on each coast of the United States. The regions from north to south on the east coast were Maine (ME), Connecticut (CT), North Carolina (NC), and Florida (FL), and on the west coast were Alaska (AK), Oregon (OR), San Francisco (SF) and San Diego (SD) (Fig. 4.1). Regions were chosen to include broad ranges of seawater temperatures on each coast, with sites over a wider geographic range on the west coast of North America because of its shallower latitudinal thermal gradient (Hickey & Banas 2003, Shearman & Lentz 2010). Differences in mean summer seawater temperature were obtained

from the NOAA NODC Coastal Water Temperature Guide (http://www.nodc.noaa.gov/dsdt/cwtg/) and were approximately 10°C between Maine and Florida and 9°C between Alaska and San Diego. Both thermal experiments and surveys were conducted in all of these regions.

Thermal Experiments

Thermal experiments were conducted in flow-through seawater systems at laboratories in 8 different locations, 4 on each coast of the US during summer 2013 (Table 4.1, Fig. 4.1). Dates were chosen to generally coincide with maximum seawater temperature at each site while still conducting all experiments within one summer (dates and seawater temperatures are listed in Table 4.1). On the east coast, facilities included Darling Marine Center (Walpole, ME), University of Connecticut Avery Point (Groton, CT), Duke University Marine Laboratory (Beaufort, NC), and Smithsonian Marine Station (Fort Pierce, FL). On the west coast, experiments were run at the Sitka Sound Science Center (Sitka, AK), Oregon Institute of Marine Biology (Charleston, OR), Bodega Bay Marine Laboratory (Bodega Bay, CA), and Scripps Institution of Oceanography (La Jolla, CA).

Collaborators at each location suspended sets of PVC settlement plates (10cm x 10cm) 0.5m below the sea surface from floating docks 4 weeks (±2 days) before the start of an experiment (Table 4.1). These panels are commonly used to collect new sponge, bryozoan, barnacle and ascidian recruits (e.g. Bullard & Whitlatch 2009, Sorte & Stachowicz 2011). Settlement panels at each location were populated with whatever larvae recruited and grew during the 4 week collection period. At the start of each experiment, panels were collected in the field, placed in seawater-filled coolers, and 20 panels with visually similar communities were

selected. Upright colonial and solitary species and barnacles were removed in order to focus on encrusting colonial species, and each panel was photographed before the three week experimental period. Percent cover at the start of the experiment varied from 5-50% with region, but did not differ between treatments. Changes in colony size were due solely to growth, not competition with other colonies because of the ample open space available at the start of the experiment and short experimental period. The few space-limited colonies were excluded from the analyses.

At each location, treatments consisted of control containers kept at ambient seawater temperature conditions and heated containers experimentally elevated 3°C above ambient conditions for 3 week periods. The experimental setup consisted of a flowing seawater reservoir that was supplied by the flowing seawater system and maintained constant head pressure for 10 flexible vinyl hoses in order to stabilize flow rates. Each hose supplied a tall rectangular tank (11cm x 11cm x 25cm tall) which contained two PVC settlement panels populated by epifaunal colonies obtained from the field. Tanks in the heated treatment (5) were mostly submerged in a warm water bath heated by a Process Technologies Smartone® 1000 watt aquarium heater. Water temperatures were maintained at 3°C above ambient by a temperature controller that had a temperature probe in one of the heated experimental tanks. Temperature was monitored with Hobo® pendant data loggers in the control and heated treatments, showing that heated tanks stayed within ±1°C of the intended 3°C difference between treatments. Control tanks were not submerged in a water bath, so they maintained the ambient seawater temperature of the flowing seawater system. The reservoir and hose system supplied each tank with flow of approximately one liter per minute, so each tank had a water residence time (complete flushing) of 3 minutes.

The relatively high flow rates and raw seawater supply ensured adequate food conditions for the fouling organisms during the course of the experiment.

Panels were removed from tanks and photographed after 3 weeks. The area of each colony was measured at the start and end of each experiment using ImageJ® (http://imagej.nih.gov/ij/). Individual colony growth rates were calculated as proportional increase in size (final area / initial area) in three weeks. To compare the responses to increased temperature between species and sites, growth rates at elevated temperatures were divided by control growth rates at each site for each species. To compare the thermal growth responses of species with different latitudinal ranges, all sites in the latitudinal range of each species were provided with a value from 0 to 1, with 0 being the southern tip of the distribution and 1 being the northern tip. Latitudinal range positions were calculated as: [(site latitude – minimum range latitude) / (maximum range latitude – minimum range latitude)]. Latitudinal range data were mostly compiled from the Smithsonian NEMESIS database, with additional data obtained from Karlson and Osman (2012) and Stanford SeaNet (http://seanet.stanford.edu/). This latitudinal range position ratio allowed for a comparison of species' responses to experimentally elevated temperatures in the same relative positions within their ranges.

This same experimental method was used in experiments in Connecticut at the University of Connecticut Avery Point during February, April, and June 2013. Exposing fouling species to a wide array of temperatures (from 8°C in winter to 22°C in summer) over the course of the year allowed for a comparison of responses to increased temperature on a seasonal basis. Thermal performance curves from these experiments were then compared with those from latitudinal experiments in order to assess similarities between seasonal and latitudinal differences in temperature.

Competition Surveys

To assess interspecific competitive interactions along a latitudinal gradient, we conducted photo surveys, with an underwater digital camera, of fouling communities growing on floating docks at 155 marinas. Marinas that had floating docks and were within 200 km north and south of each experiment were selected from satellite photos, with at least 16 survey sites in each region (Table 4.1). Temperature and salinity were measured at each site, and sites with salinities above 25 were classified as marine locations (Table 4.1). These marine sites were used for interspecific competitive analysis. At each marina survey site, at least 30 photos were taken over the side of the floating dock at haphazard intervals; the communities were not visible until the researcher was lying face down on the dock, so the locations of the photos were unbiased by species present. Photos were taken 0.25m away from the substrate (sides of the dock), so photos contained approximately the same area. All surveys were conducted within 3 weeks after the experiment start dates in each region (Table 4.1).

Images were compiled with the image cataloguing program Daminion® and each image was digitally tagged with the species present. At least 400 photos were analyzed within each region, with competitive interactions pooled for each region. In each photograph, each instance of one species clearly overgrowing another was classified as a 'win' for the overgrowing species and a 'loss' for the species being overgrown (as in Barnes & Clarke 1998, Bell & Barnes 2003). Wins were given two points, losses 0, and apparent stand-offs were classified as 'ties' and each species was given one point. Competitive scores could then be calculated for each species against all other species or against any subgroup of its competitors.

Because species assemblages, and therefore pools of potential competitors, were different in each region, competitive scoring was broken down into species pairs. All competitive interactions within each region pooled together in order to make comparisons of species pairs. For example, the percent of interactions in which the tunicate *Diplosoma listerianum* overgrew (competitive win) the tunicate *Botrylloides violaceus* (competitive loss) was compared between different regions. Latitudinal position ratios were calculated for each species in each region in the same manner as for the growth experiments. Competitive scores or winning percentages for pairs of species were plotted against relative growth rates of the paired species, using growth rates from thermal experiments in each region. This provided a measure of the impact of growth rate on competition and allowed for comparison of potential competitive shifts with changes in proportionate growth rates for each pair of species.

Results

Thermal Experiments

Growth rates were established at local ambient temperatures and at experimentally elevated temperatures at all 8 sites for a total of 19 species. Growth rates in control and heated temperature treatments were established in at least 3 regions for four species of tunicates: *Botrylloides violaceus* (6 sites), *Botryllus schlosseri* (5 sites), *Diplosoma listerianum* (4 sites), and *Didemnum vexillum* (3 sites). Increased growth rate was characterized as a positive response to elevated temperature while decreased growth was considered a negative response. The magnitude of growth rate change differed between species and regions; for example, *B. violaceus* nearly doubled its growth rate in heated treatments in Alaska (Fig. 4.2D), while *D. listerianum*

displayed a much weaker response in Maine, the northern edge of its range (Fig. 4.2G). However, species generally responded positively to temperature increases (3°C) in the northern portions of their latitudinal range and negatively towards the southern portion of their ranges (Fig. 4.2).

For all species and sites combined, there was a strong positive correlation between latitudinal position ratio and the percent change in growth rate in heated treatments (Pearson correlation coefficient (r) = 0.796, $F_{1,17} = 29.4$, $p < 0.001$) (Fig. 4.3). Species in the southern, warmer portions of their ranges (latitudinal position ratio near 0) showed a negative growth response to experimentally elevated temperatures, while in the northern part of their ranges, species displayed a distinctly positive response (Fig. 4.3). This means that in northern portions of their ranges, species grew faster at warmer temperatures (compared to control), and towards southern edges they grew slower at warmer temperatures. Because of the short length of the experiments, mortality at all sites was low (< 5%) and did not differ by treatment, so experimental temperatures altered growth rate but did not increase to lethal levels.

Thermal performance curves (TPCs) were created for all 4 species that settled on panels in at least 3 regions and thus were included in those thermal experiments (*Botryllus schlosseri, Botrylloides violaceus, Diplosoma listerianum, Didemnum vexillum*) (Table 4.2, Fig. 4.4). Species showed similar responses to seasonal and latitudinal changes in temperature, based on the latitudinal experiments in 8 regions and the 4 seasonal sampling periods in Connecticut. Therefore, TPCs based on growth rate were created from combined seasonal and latitudinal datasets for each species, fit with Gaussian curves in Sigmaplot®, and are shown in figure 4. The species with the southernmost range was *D. listerianum*, which had the TPC shifted furthest to the right (higher temperature) (Fig. 4.4). The species with the northernmost range was *B.*

violaceus, which had the TPC shifted furthest to the left (colder) out of the four species (see Table 4.2 for equations, fit statistics, and thermal optima) (Fig. 4.4).

Competition Surveys

Using competitive interactions between pairs of species in different regions minimized bias based on varying species assemblages between regions. There were large differences in competitive outcomes within species pairs based on region. For example, the tunicate *Botryllus schlosseri* (BS) has a more southerly distribution than *Botrylloides violaceus* (BV), and the overgrowth percentage for BS over BV in Maine was 59%, rising up to 70% in the warmer waters of Connecticut (Table 4.3). The sub-tropical species *Diplosoma listerianum* (DL) 'won' 59% of interactions with BV in Maine, and 83% in Connecticut, an increase of 24% from north to south (Table 4.3). Of the 8 species pairs for which at least 15 competitive interactions were scored at multiple sites, 7 showed a competitive shift toward the warmer-water species at the more southern site. The only exception was a 3% decrease in competitive score between *D. listerianum* and *B. schlosseri* in Maine and Connecticut, although these species have only slightly different latitudinal ranges on the east coast of the US.

To determine the relationship between growth and competitive outcomes, proportionate growth rates were calculated as the growth rate of one species divided by the growth rate of another species at the same site. Using all sites and species pairings for which growth and competition data were available, there was a strong correlation between proportionate growth rate (natural log) and competitive score or overgrowth proportion (Pearson correlation coefficient (r) = 0.836, $F_{1,11} = 25.5$, $p < 0.001$) (Fig. 4.5). A species with a higher growth rate at a given site was more likely to overgrowth the slower growing species (Fig. 4.5).

Discussion

This study focused on the potential effect of global warming on growth and competition in marine epibenthic fouling communities along a latitudinal gradient on the east and west coasts of the United States. Significant differences in thermal responses between species and within species along a latitudinal thermal gradient were found. Temperature appears to be the primary factor driving changes in growth rate over a seasonal and latitudinal gradient for these species in these space-limited habitats. Changes in growth rate affected competitive outcomes and suggests that warming climates could cause shifts in competition and community composition in fouling communities and other marine and terrestrial systems where growth rates strongly influence competition and percent abundance patterns (e.g. bacteria (Nedwell & Rutter 1994), plants (Goldberg & Landa 1991), and coral (Johannes et al 1983)).

The hypothesis that species would show positive growth responses to elevated temperatures in the northern parts of their latitudinal ranges and negative responses in the southern portions of their ranges was supported by the results from the large-scale thermal experiments (Figs. 4.2, 4.3). These results are consistent with previous studies of ectotherm thermal performance curves, demonstrating increasing growth with temperature until a thermal optimum and decreasing growth above this temperature (Deutsch et al. 2008, Clusella-Trullas et al. 2011) (Table 4.2, Fig. 4.4). There was a strong linear relationship between latitudinal range positions (i.e. northern or southern portions of a species' latitudinal range) and responses to experimentally elevated temperatures (3°C) across all species studied. Species near their southern range limits displayed a maximum of over 60% reduction in growth with 3°C temperature increase, while species near their northern range limits showed up to an 80% increase in growth rate with a 3°C increase in temperature (Fig. 4.3). While there was a

consistent and predictable growth response with respect to latitudinal position, responses to elevated temperatures varied widely across sites and between species within sites. These differential responses to warmer temperatures are what could drive changes in competitive outcomes and alter community composition with global warming.

The second hypothesis that competitive outcomes would shift along with proportionate growth rates (ratio of growth rates between two competing species) was also supported by data from the competition surveys and thermal experiments. Faster growing species (relative to their paired competitor) displayed drastically higher rates of competitive overgrowth (Fig. 4.5), which is the major mechanism of competition in the fouling community (Sebens 1986, Nandakumar et al. 1993). This is similar to competition for light in terrestrial plant communities, where growth rate is often a strong driver of competitive outcomes, though nutrients play a large role as well (Chu et al. 1978, Goldberg & Landa 1991, Wilson & Tilman 1991). Not only did faster growing species outcompete slower growers (Fig. 4.5), competition within species pairs also shifted with latitude based on ranges of the two species (Table 4.3). Competitive outcomes favored warmer water species along the latitudinal temperature cline from colder to warmer sites (Table 4.3). Most of the species included in this study were non-natives, as fouling communities in most locations are dominated by non-native species (Lambert & Lambert 1997). The prevalence of non-natives in these habitats suggests that they are superior spatial competitors to native species, but not enough native species were present at sites included in this study to make direct comparisons. Nonetheless, thermal growth experiments show regions in which specific non-native species benefit from increased temperatures (Fig. 4.2), so species such as *B. violaceus* will likely increase in abundance in the northern portion of their range as seawater temperatures rise.

With ocean temperatures expected to increase 2-4°C along the east coast and 1.5-3°C along the west coast of the US by 2100, the 3°C experimental increase in temperature provides insight into future responses of species to global warming (IPCC 2007). While these short-term experiments did not take long-term adaptation to warmer temperature into account, the use of a latitudinal cline in temperature allowed for experimentation with populations that could be adapted to local thermal conditions. While some tunicates can show genetic differentiation even on the scale of meters because of limited larval dispersal (Yund & O'Neal 2000), seasonal and latitudinal experiments showed a similar response to temperature within species for all seasons and latitudes (Fig. 4.4). There was no apparent local adaptation (shift of TPCs) or latitudinal compensation (higher maximum growth rate at colder sites) within any of the 4 species that were included in experiments in at least 3 locations (Fig. 4.4) (Levinton 1983, Lonsdale & Levinton 1985, Doorslaer & Stoks 2005). One possible explanation for this could be the primary dispersal mechanism for many fouling species; they are often transported between harbors on ship hulls which could act as a significant source of gene flow and lead to homogenized populations (Stoner et al. 2002).

By including a suite of interacting species as suggested by several publications on global warming (Harley et al. 2006, Gilman et al. 2010, Sorte & Stachowicz et al. 2011), this study provides information about differential responses of species assemblages to warmer temperatures at multiple sites (Figs. 4.2, 4.3). The strong linkage between growth rates and competitive outcomes over a latitudinal gradient (Fig. 4.5) suggests that changes in temperature will cause shifts in competitive interactions as temperatures increase and that altered growth rates will be the primary mechanism behind these shifts. The robust correlation between latitudinal position within a species' range and its response to warmer temperatures (Fig. 4.3) should be able to

provide greater predictive power as to the direction of competitive shifts with global warming. In addition, the linkage between latitudinal range position and response to temperature (Fig. 4.3) could allow for predictions of the competitive abilities of newly introduced species under present and future ocean temperatures. By incorporating multi-species thermal experiments into an examination of competition over a large geographic range, this study quantified the thermal growth mechanism behind competitive shifts and thus facilitated prediction of changes in community composition with latitudinal and climate-related increases in temperature.

Acknowledgments

Thanks to all of the individuals that deployed settlement panels for me and allowed me to use lab space, as well as to the institutions that provided me with space in offices and flowing seawater systems. These include but are not limited to: Taylor White and Lon Garrison at the Sitka Sound Science Center, Laurel Hiebert and Dr. Richard Emlet at the Oregon Institute of Marine Biology, Matt Whalen and Karl Menard at Bodega Marine Laboratory, Brianna Tracy (USD) and Dr. Jennifer Smith at Scripps Institution of Oceanography, Tim Miller and Linda Healy at Darling Marine Center, Jeremy Calini and Charlie Woods at University of Connecticut, Dr. Dan Rittschof at Duke Marine Laboratory, and Hugh Reichardt and Sherry Reed at Smithsonian Marine Station. In addition, this survey work could not have been completed without voluntary cooperation from over 160 marinas on both coasts of the United States. This manuscript was greatly improved with input from Dr. Rick Osman. Funding provided by Connecticut Sea Grant Project Number PD-12-17 and by the University of Connecticut Department of Marine Science Pre-doctoral Fellowship.

References

Aerts, R. 1999. Interspecific competition in natural plant communities: mechanisms, trade-offs and plant-soil feedbacks. Journal of Experimental Botany 50:29–37.

Amui-Vedel, A.-M., P. J. Hayward, and J. S. Porter. 2007. Zooid size and growth rate of the bryozoan *Cryptosula pallasiana* Moll in relation to temperature, in culture and in its natural environment. Journal of Experimental Marine Biology and Ecology 353:1–12.

Barnes, D., and A. Clarke. 1998. The ecology of an assemblage dominant: the encrusting bryozoan *Fenestrulina rugula*. Invertebrate Biology 117:331–340.

Bell, J., and D. Barnes. 2003. The importance of competitor identity, morphology and ranking methodology to outcomes in interference competition between sponges. Marine Biology 143:415–426.

Bullard, S., and R. B. Whitlatch. 2009. In situ growth of the colonial ascidian *Didemnum vexillum* under different environmental conditions. Aquatic Invasions 4:275–278.

Chamaille-Jammes, S., M. Massot, P. Aragon, and J. Clobert. 2006. Global warming and positive fitness response in mountain populations of common lizards *Lacerta vivipara*. Global Change Biology 12:392–402.

Chu, C., P. Ludford, J. Ozbun, and R. Sweet. 1978. Effects of temperature and competition on the establishment and growth of redroot pigweed and common lambsquarters. Crop Science 18:308–310.

Clay, K., S. Marks, and G. Cheplick. 1993. Effects of insect herbivory and fungal endophyte infection on competitive interactions among grasses. Ecology 74:1767–1777.

Clusella-Trullas, S., T. M. Blackburn, and S. L. Chown. 2011. Climatic predictors of temperature performance curve parameters in ectotherms imply complex responses to climate change. American Naturalist 177:738–51.

Conover, D., and T. Present. 1990. Countergradient variation in growth rate: compensation for length of the growing season among Atlantic silversides from different latitudes. Oecologia 83:316–324.

Davis, A., L. Jenkinson, and J. Lawton. 1998. Making mistakes when predicting shifts in species range in response to global warming. Nature 391:783–786.

Deutsch, C., and J. Tewksbury. 2008. Impacts of climate warming on terrestrial ectotherms across latitude. Proceedings of the National Academy of Science 105:6668–6672.

Doorslaer, W. Van, and R. Stoks. 2005. Growth rate plasticity to temperature in two damselfly species differing in latitude: contributions of behaviour and physiology. Oikos 111:599–605.

Engle, V., and J. Summers. 1999. Latitudinal gradients in benthic community composition in Western Atlantic estuaries. Journal of Biogeography 26:1007–1023.

Epelbaum, A., L. M. Herborg, T. W. Therriault, and C. M. Pearce. 2009. Temperature and salinity effects on growth, survival, reproduction, and potential distribution of two non-indigenous botryllid ascidians in British Columbia. Journal of Experimental Marine Biology and Ecology 369:43–52.

Gilman, S. E., M. C. Urban, J. Tewksbury, G. W. Gilchrist, and R. D. Holt. 2010. A framework for community interactions under climate change. Trends in Ecology & Evolution 25:325–31.

Goldberg, D., and K. Landa. 1991. Competitive effect and response: hierarchies and correlated traits in the early stages of competition. Journal of Ecology 79:1013–1030.

Hansen, J., M. Sato, R. Ruedy, K. Lo, D. W. Lea, and M. Medina-Elizade. 2006. Global temperature change. Proceedings of the National Academy of Sciences of the United States of America 103:14288–14293.

Harley, C. D., R.A. Hughes, K. M. Hultgren, B. G. Miner, C. J. Sorte, C. S. Thornber, L. F. Rodriguez, L. Tomanek, and S. L. Williams. 2006. The impacts of climate change in coastal marine systems. Ecology Letters 9:228–41.

Hickey, B. M., and N. S. Banas. 2003. Oceanography of the U.S. Pacific Northwest Coastal Ocean and estuaries with application to coastal ecology. Estuaries 26:1010–1031.

Houde, E. 1989. Comparative growth, mortality, and energetics of marine fish larvae: temperature and implied latitudinal effects. Fishery Bulletin 87:471–495.

IPCC, 2007. Climate Change 2007: Synthesis Report (52 pp.).

Janiak, D. S., R. W. Osman, and R. B. Whitlatch. 2013. The role of species richness and spatial resources in the invasion success of the colonial ascidian *Didemnum vexillum* Kott, 2002 in eastern Long Island Sound. Journal of Experimental Marine Biology and Ecology 443:12–20.

Johannes, R., W. Wiebe, C. Crossland, D. Rimmer, and S. Smith. 1983. Latitudinal limits of coral reef growth. Marine Ecology Progress Series 11:105–111.

Karlson, R., and R. Osman. 2012. Species composition and geographic distribution of invertebrates in fouling communities along the east coast of the USA: a regional perspective. Marine Ecology Progress Series 458:255–268.

Koopmans, M., and R. H. Wijffels. 2008. Seasonal growth rate of the sponge *Haliclona oculata* (Demospongiae: Haplosclerida). Marine Biotechnology 10:502–10.

Koskela, J., J. Pirhonen, and M. Jobling. 1997. Feed intake, growth rate and body composition of juvenile Baltic salmon exposed to different constant temperatures. Aquaculture International 5:351–360.

Lambert, C., and G. Lambert. 1998. Non-indigenous ascidians in southern California harbors and marinas. Marine Biology:675–688.

Levinton, J. 1983. The latitudinal compensation hypothesis: growth data and a model of latitudinal growth differentiation based upon energy budgets. I. Interspecific comparison of *Ophryotrocha* (Polychaeta: Dorvilleidae). Biological Bulletin:686–698.

Lindeyer, F., and A. Gittenberger. 2011. Ascidians in the succession of marine fouling communities. Aquatic Invasions 6:26–28.

Loehle, C. 1998. Height growth rate tradeoffs determine northern and southern range limits for trees. Journal of Biogeography 25:735–742.

Lonsdale, D., and J. Levinton. 1985. Latitudinal differentiation in copepod growth: an adaptation to temperature. Ecology 66:1397–1407.

Lord, J., and R. Whitlatch. 2013. Impact of temperature and prey shell thickness on feeding of the oyster drill *Urosalpinx cinerea* Say. Journal of Experimental Marine Biology and Ecology 448:321–326.

Maughan, B. C., and D. K. A. Barnes. 2000. Seasonality of competition in early development of subtidal encrusting communities. Marine Ecology 21:205–220.

McCarthy, A., R. W. Osman, and R. B. Whitlatch. 2007. Effects of temperature on growth rates of colonial ascidians: A comparison of *Didemnum* sp. to *Botryllus schlosseri* and *Botrylloides violaceus*. Journal of Experimental Marine Biology and Ecology 342:172–174.

Mercado-Molina, A. E., and P. M. Yoshioka. 2009. Relationships between water motion and size-specific survivorship and growth of the demosponge *Amphimedon compressa*. Journal of Experimental Marine Biology and Ecology 375:51–56.

Milazzo, M., S. Mirto, P. Domenici, M. Gristina, and M. Genner. 2012. Climate change exacerbates interspecific interactions in sympatric coastal fishes. Journal of Animal Ecology 82:468–477.

Nandakumar, K., M. Tanaka, and T. Kikuchi. 1993. Interspecific competition among fouling organisms in Tomioka Bay, Japan. Marine Ecology Progress Series 94:43–50.

Nedwell, D., and M. Rutter. 1994. Influence of temperature on growth rate and competition between two psychrotolerant Antarctic bacteria: low temperature diminishes affinity for substrate uptake. Applied and Environmental Microbiology 60:1984–1992.

Parra, I., A. Almodóvar, G. G. Nicola, and B. Elvira. 2009. Latitudinal and altitudinal growth patterns of brown trout *Salmo trutta* at different spatial scales. Journal of Fish Biology 74:2355–73.

Pennings, S., E. Selig, L. Houser, and M. Bertness. 2003. Geographic variation in positive and negative interactions among salt marsh plants. Ecology 84:1527–1538.

Sagarin, R., J. Barry, S. Gilman, and C. Baxter. 1999. Climate-related change in an intertidal community over short and long time scales. Ecological Monographs 69:465–490.

Saunders, M. I., and A. Metaxas. 2009. Effects of temperature, size, and food on the growth of *Membranipora membranacea* in laboratory and field studies. Marine Biology 156:2267–2276.

Schöb, C., C. Armas, M. Guler, I. Prieto, and F. I. Pugnaire. 2013. Variability in functional traits mediates plant interactions along stress gradients. Journal of Ecology 101:753–762.

Sebens, K. 1982. Competition for space: growth rate, reproductive output, and escape in size. American Naturalist 120:189–197.

Sebens, K. 1986. Spatial relationships among encrusting marine organisms in the New England subtidal zone. Ecological Monographs 56:73–96.

Shearman, R. K., and S. J. Lentz. 2010. Long-Term Sea Surface Temperature Variability along the U.S. East Coast. Journal of Physical Oceanography 40:1004–1017.

Somero, G. N. 2012. The Physiology of Global Change: Linking Patterns to Mechanisms. Annual Review of Marine Science 4:39–61.

Sorte, C. J., and J. Stachowicz. 2011. Patterns and processes of compositional change in a California epibenthic community. Marine Ecology Progress Series 435:63–74.

Stoner, A. W., M. L. Ottmar, and L. a. Copeman. 2010. Temperature effects on the molting, growth, and lipid composition of newly-settled red king crab. Journal of Experimental Marine Biology and Ecology 393:138–147.

Sutherland, J., and R. Karlson. 1977. Development and stability of the fouling community at Beaufort, North Carolina. Ecological Monographs 47:425–446.

Tanabe, K., and T. Oba. 1988. Latitudinal variation in shell growth patterns of *Phacosoma japonicum* (Bivalvia: Veneridae) from the Japanese coast. Marine Ecology Progress Series 47:75–82.

Tilman, D. 1976. Ecological competition between algae: experimental confirmation of resource-based competition theory. Science 192:463–465.

Trowbridge, C. D., C. Little, B. Dlouhy-Massengale, P. Stirling, and G. M. Pilling. 2013. Changes in brown seaweed distributions in Lough Hyne, SW Ireland: a long-term perspective. Botanica Marina 56:323–338.

Whitlatch, R. B., and R. W. Osman. 2009. Post-settlement predation on ascidian recruits: predator responses to changing prey density. Aquatic Invasions 4:121–131.

Widden, P. 1984. The effects of temperature on competition for spruce needles among sympatric species of Trichoderma. Mycologia 76:873–883.

Wilson, D. 1984. Species packing and temperature dependent competition among burying beetles (Silphidae, Nicrophorus). Ecological Entomology:205–216.

Wilson, S., and D. Tilman. 1991. Component of plant competition along an experimental gradient of nitrogen availability. Ecology 72:1050–1065.

Yamahira, K., and D. Conover. 2002. Intra-vs. interspecific latitudinal variation in growth: adaptation to temperature or seasonality? Ecology 83:1252–1262.

Yund, P. O., and P. G. O'Neil. 2000. Microgeographic genetic differentiation in a colonial ascidian (*Botryllus schlosseri*) population. Marine Biology 137:583–588.

Zani, P. A. 2008. Climate change trade-offs in the side-blotched lizard (*Uta stansburiana*): effects of growing-season length and mild temperatures on winter survival. Physiological and Biochemical Zoology 81:797–809.

Figure Captions

Figure 4.1. Map of North America showing 8 regions in which competition surveys and thermal experiments were conducted. Triangles are individual survey sites, with at least 15 survey sites in each region.

Figure 4.2. Fouling species growth rates under control and heated conditions in different parts of their ranges; (A) *Botryllus schlosseri* on west coast of the United States, (B) *B. schlosseri* on the east coast, (C) *Hydroides* spp. on the east coast, (D) *Botrylloides violaceus* on the west coast, (E) *B. violaceus* on the east coast, (F) *Distaplia occidentalis* on the west coast, (G) *Diplosoma listerianum* on the east coast, (H) *Didemnum vexillum* on the east coast, (I) *Watersipora subtorquata* on the west coast. Growth rates are expressed as percent increase in colony size over 3 week experimental period (+/- SE). Letters indicate significant differences within plots.

Figure 4.3. Correlation between latitudinal position ratio and growth rate changes in response to experimental temperature elevation (3°C). Latitudinal position ratio describes where each species is in its latitudinal range, with 0 being the southern edge of the range and 1 the northern edge. Species displayed negative growth responses to increased temperature in the southern part of their ranges and positive responses in the northern part of their ranges. Included data are just for encrusting tunicates and bryozoans for which control and heated growth rates were established.

Figure 4.4. Thermal performance curves for the 4 tunicate species that were used in growth experiments in at least 3 sites: (A) *Botrylloides violaceus*, (B) *Botryllus schlosseri*, (C)

Didemnum vexillum, (D) *Diplosoma listerianum*. All curves are shown on the same plot in (E) in order to highlight differences in changes in growth rate with temperature. Peaks for *D. vexillum* and *D. listerianum* are approximately 10°C above *B. schlosseri* and *B. violaceus*, with *D. listerianum* warmest overall and *B. violaceus* coolest.

Figure 4.5. Correlation between competitive score ('winning percentage') and natural log of proportionate growth rate, or the ratio between the growth rates of two competing species. Each point represents average outcomes ('winning percentage') for a pair of species at an individual site. Species with high proportionate growth rates were very successful competitors compared to species with low growth rates. Labels indicate paired sites and correspond to detailed site and species information shown in Table 4.3. Species pairs 7 and 8 do not show two sites because growth rate data was not available for both species.

Table 4.1. List of regions in which thermal experiments and competitive surveys were conducted during May-August 2013. Experiment start dates, control and heated temperatures for experiments in each region, and the number of survey sites. The number of survey sites with salinities >25 are listed in parentheses.

Location	Experiment Start Date	State	Control (ambient) (℃)	Heated (℃)	# Survey Sites (high salinity)
Darling Marine Center	August 9	ME	18.5	21.5	20 (19)
University of Connecticut Avery Point	August 6	CT	21.8	24.8	18 (10)
Duke University Marine Lab	May 22	NC	23.7	26.7	22 (13)
Smithsonian Marine Station	May 20	FL	29.3	32.3	21 (10)
Sitka Sound Science Center	July 15	AK	12.0	15.0	16 (16)
Oregon Institute of Marine Biology	July 11	OR	13.0	16.0	19 (16)
Bodega Marine Lab	June 18	CA	14.5	17.5	20 (18)
Scripps Institution of Oceanography	June 14	CA	18.3	21.3	19 (19)

Table 4.2. Equations and fit statistics for Gaussian curves fit to growth responses to temperature for four tunicate species shown in Figure 4.

Species	Gaussian Curve (y = growth rate, x = °C)	Peak (°C)	R^2
Botryllus schlosseri	$y = 2.27 * (e \wedge \{ [-(x-15.50)^2] / (2*6.49^2)\})$	14.9	0.60
Botrylloides violaceus	$y = 2.28 * (e \wedge \{ [-(x-14.83)^2] / (2*4.82^2)\})$	14.9	0.72
Diplosoma listerianum	$y = 12.3 * (e \wedge \{ [-(x-22.93)^2] / (2*4.23^2)\})$	21.8	0.93
Didemnum vexillum	$y = 3.93 * (e \wedge \{ [-(x-22.37)^2] / (2*9.39^2)\})$	22.0	0.80

Table 4.3. Competitive scores ('winning percentage') for pairs of species at multiple sites shown in Figure 5. Southerly species had higher competitive scores in warmer regions within species pairings than more northerly species. (ME=Maine, CT=Connecticut, OR=Oregon, SF=San Francisco, SD=San Diego).

Species 1 (warmer range)	**Species 2** (colder range)	Pair #	Colder Site A	Warmer Site B	Sp. 1 win %		Δ
					A	B	
Botryllus schlosseri	*Botrylloides violaceus*	1	ME	CT	0.59	0.71	0.12
Botryllus schlosseri	*Diplosoma listerianum*	2	ME	CT	0.11	0.08	-0.03
Botryllus schlosseri	*Watersipora subtorquata*	3	SF	SD	0.96	0.81	0.15
Didemnum vexillum	*Botrylloides violaceus*	4	ME	CT	0.69	0.81	0.12
Didemnum vexillum	*Diplosoma listerianum*	5	ME	CT	0.33	0.17	0.16
Diplosoma listerianum	*Botrylloides violaceus*	6	ME	CT	0.59	0.83	0.24
Botrylloides violaceus	*Watersipora subtorquata*	7	SF	SD	0.92	0.65	0.27
Botrylloides violaceus	*Watersipora subtorquata*	8	OR	SD	0.91	0.65	0.26

Figure 4.1 (see caption on p. 117)

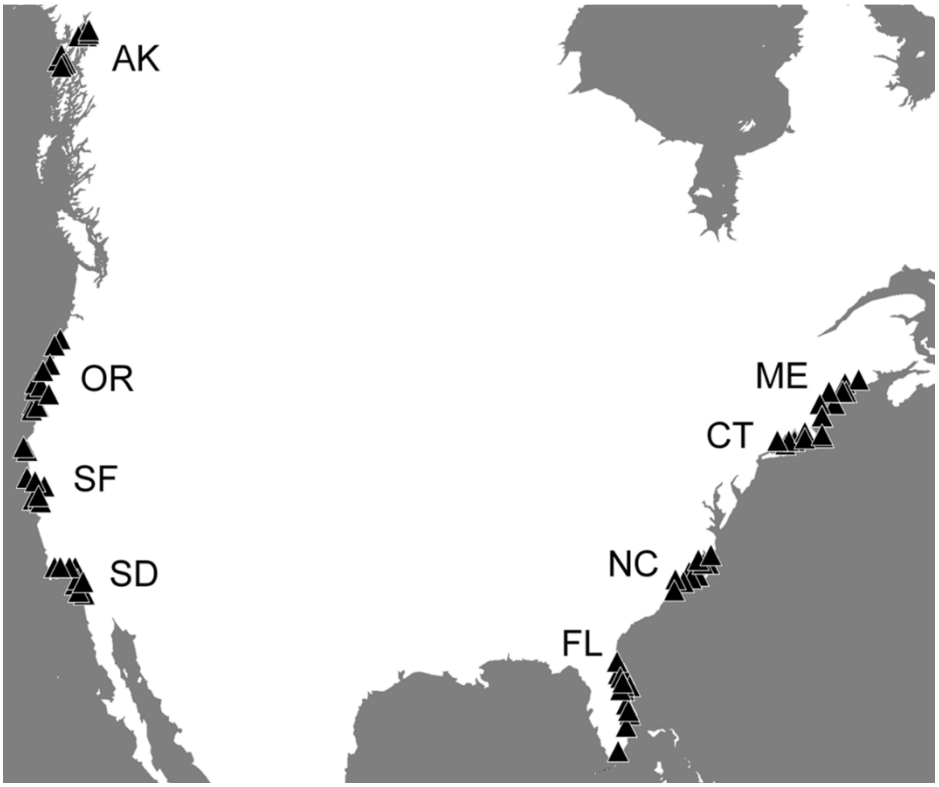


Figure 4.2 (see caption on p. 117)

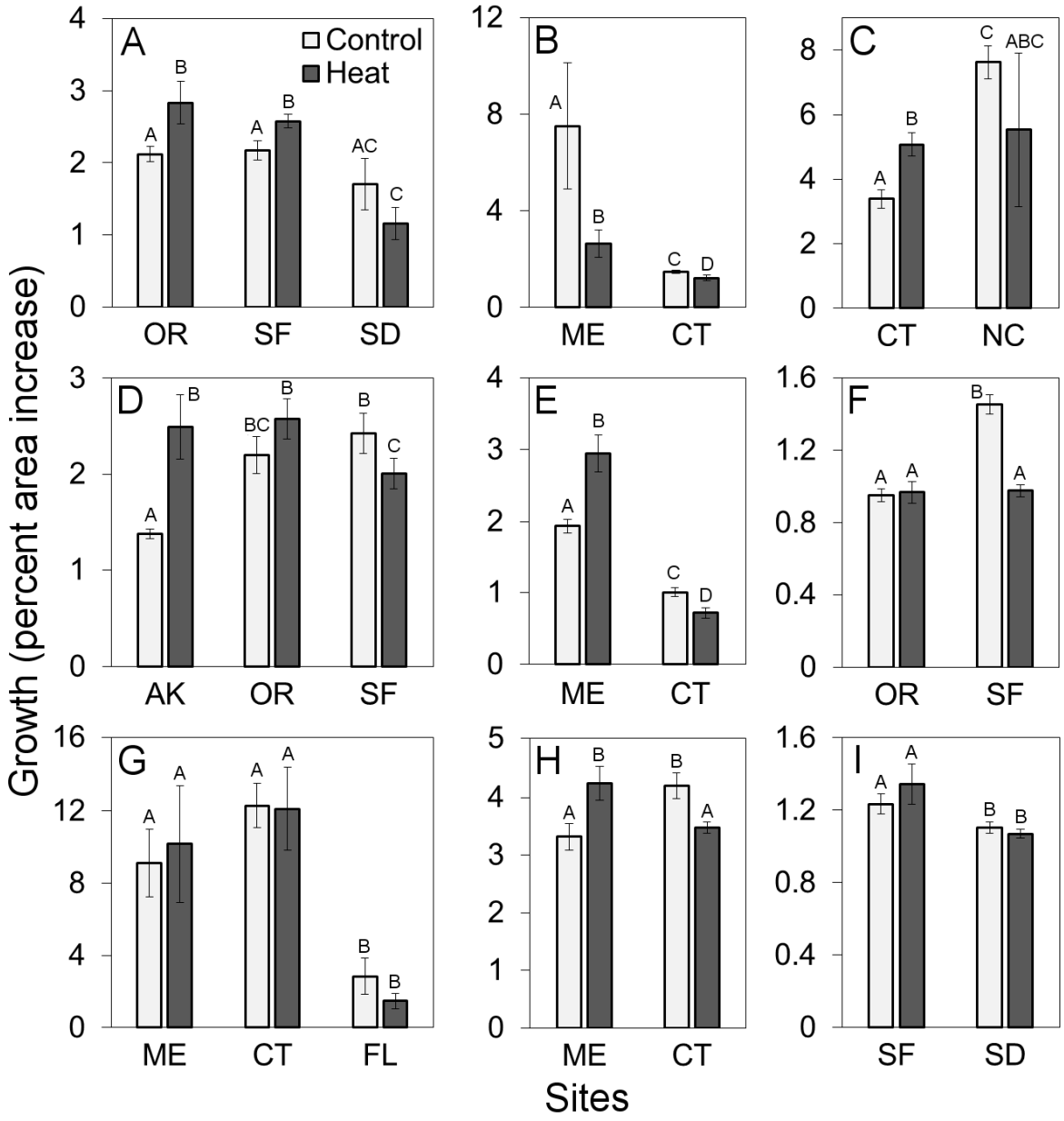


Figure 4.3 (see caption on p. 117)

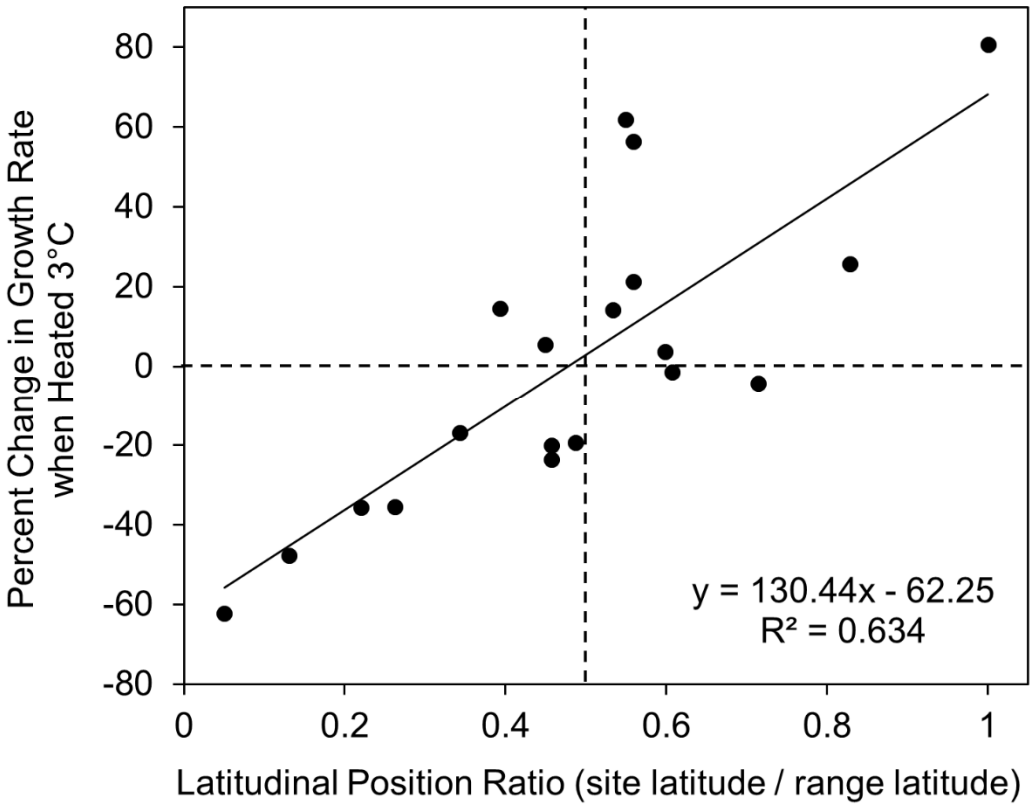


Figure 4.4 (see caption on p. 117)

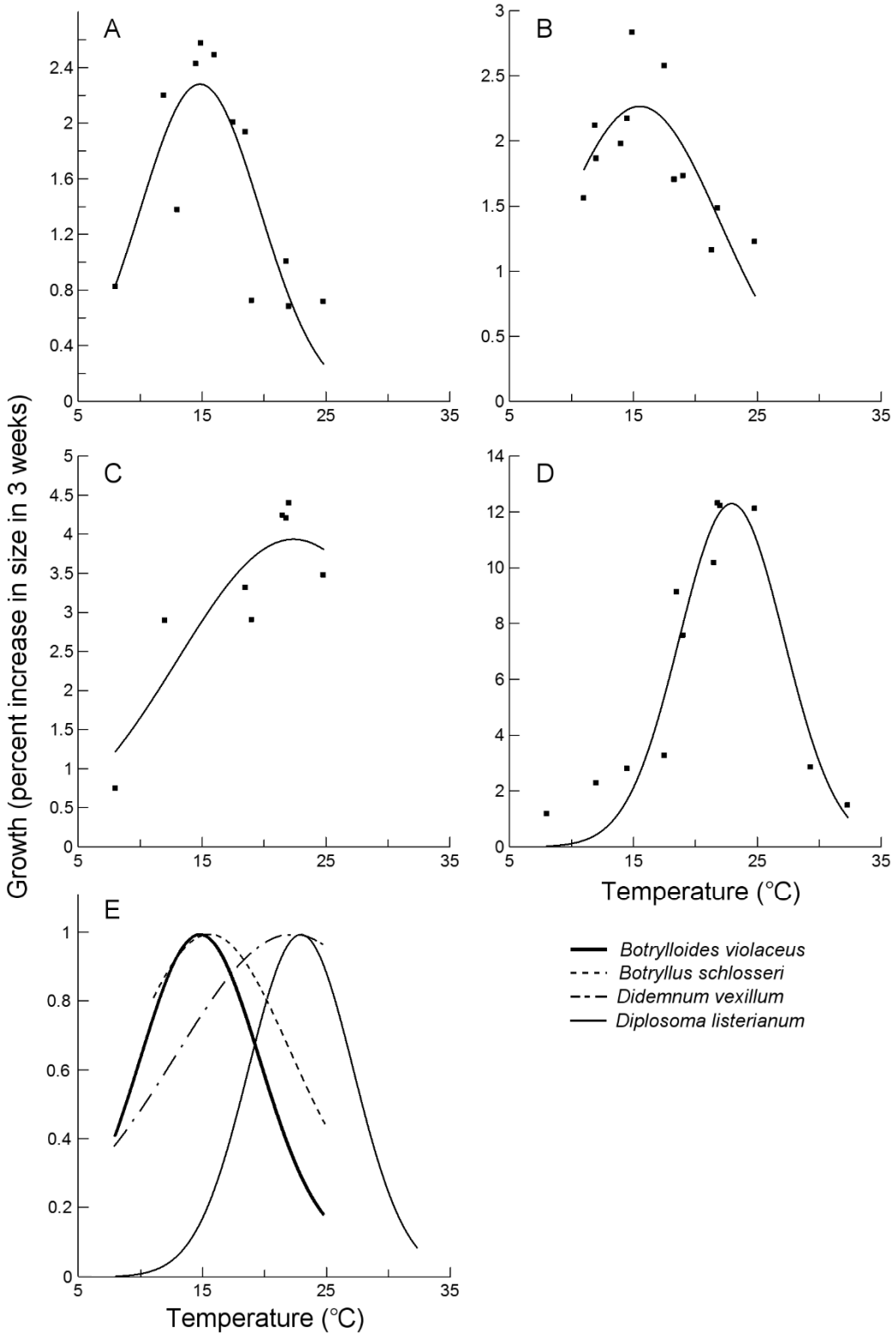
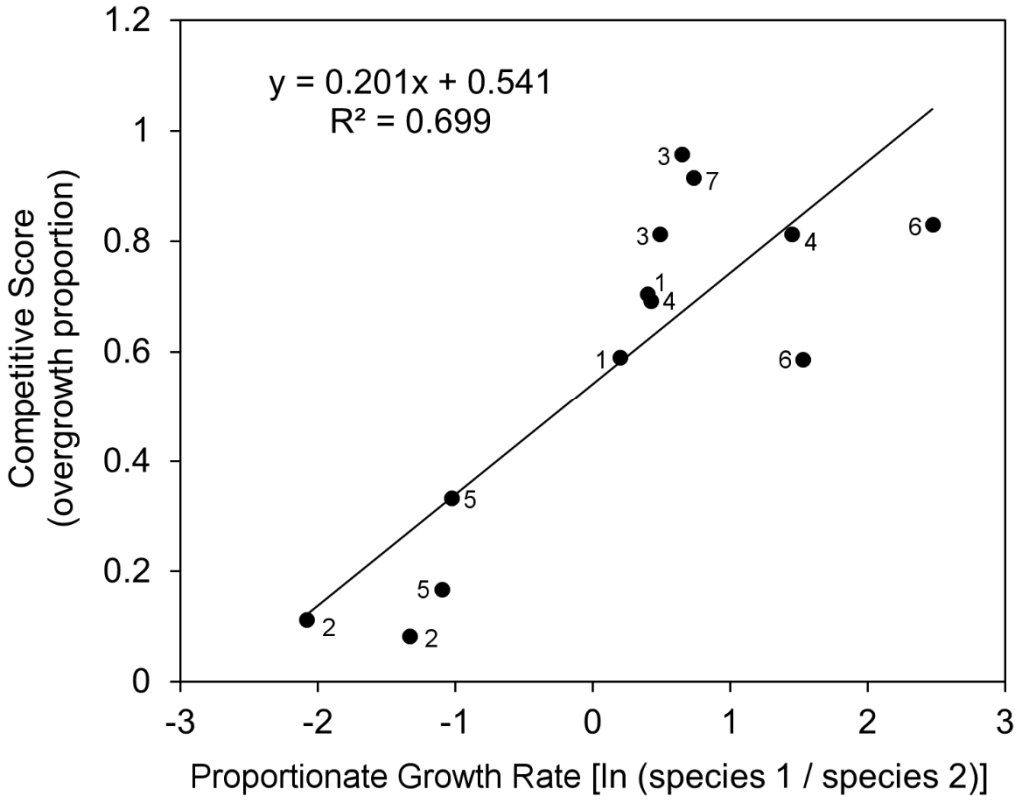


Figure 4.5 (see caption on p. 117)



Chapter 5

Influence of seawater temperature and shipping on the spread and establishment of invasive marine fouling species[5]

Abstract

We quantified the extent to which seawater temperature and shipping control introduction, establishment, and spread of invasive fouling species on both local and continental scales. Photo surveys of epibenthic fouling communities were conducted at 160 marinas over a broad geographic range on the east and west coasts of the US. All native and introduced species in each photo were identified, and percent cover of all abundant species was calculated for each site. Similarity was computed between paired sites to quantify the spread of introduced species between harbors and understand the mechanisms by which these invasions occur. Native species richness was positively correlated with mean summer seawater temperature, whereas introduced richness displayed a parabolic relationship with a peak at 20°C. Temperature and commercial shipping traffic explained 66% of variability in the number of introduced species in 16 sub-regions on Atlantic and Pacific coasts of the US. Similarity of species assemblages was not correlated with distance between sites. Shipping apparently drives species introductions but temperature controls which species can become established. Many fouling invasions are still in progress, as recent invaders (<25 yrs) are still spreading within their present geographic range in

[5]Lord J. & Whitlatch R. Influence of seawater temperature and shipping on the spread and establishment of invasive marine fouling species. *Diversity and Distributions*, in review.

a dispersal process not linked to climate. Even established introduced species did not exclude native species and did not homogenize species pools, which appear to be stochastic and unrelated to distance between sites.

Keywords: Fouling, invasive species, temperature, climate change, connectivity, diversity

Introduction

Invasive species can impact diversity and ecosystem function in many types of marine and terrestrial environments (Dukes & Mooney 1999, Stachowicz et al. 1999, Hejda et al. 2009, Vance et al. 2009). They can reduce diversity of native assemblages because introduced species are often competitively dominant and environmentally tolerant, which provides them with an advantage in heavily disturbed or dynamic environments (Dukes & Mooney 1999). While dominant species that can reduce diversity are often introduced, native species with similar characteristics can also cause reductions in species diversity (Houlahan & Findlay 2008). Both competitive dominance and biological invasions can be affected by climate change, which can make communities more susceptible to the influence of introduced species (Dukes & Mooney 1999, Sorte & Stachowicz 2011). Introduced species are likely to become more prevalent with global warming due to higher environmental tolerances; characteristics that make species successful invaders (tolerance of wide array of environmental conditions, high growth rate) also make them less susceptible to global warming (Helmuth et al. 2002).

Climate change is expected to increase coastal seawater temperatures by 2-4°C in the northwest Atlantic Ocean and 1.5-3°C in the northeast Pacific by 2100 (IPCC 2007), which

could cause shifts in benthic marine communities. Seawater temperature is the primary mechanism underlying latitudinal changes in benthic community composition, and is a proposed cause for latitudinal patterns in marine and terrestrial diversity (Pianka 1966, Rohde 1992, Engle & Summers 1999, Tittensor et al. 2010). Climate change has been linked to range expansion and range shifts with latitude in several groups of organisms including terrestrial plants and benthic marine communities (Sagarin 1999, Pauli et al. 2007, Dijkstra et al. 2011, Sorte & Stachowicz 2011). These climate-induced range shifts are particularly relevant to introduced species, because it allows new dominant, thermally tolerant species to gain a foothold in new environments, where they are exposed to novel assemblages and can negatively impact biodiversity and ecosystem function (Vitousek et al. 1996).

Marine epibenthic fouling communities are mostly composed of sessile marine invertebrates (tunicates, bryozoans, sponges) that typically proliferate on artificial hard substrates such as docks and aquaculture equipment but can live on natural hard substrates as well. Fouling communities are highly invaded systems that are space-limited and are controlled by competition between species, making them ideal systems to study the spread and impact of introduced species (Sebens 1986, Altman & Whitlatch 2007, Sorte & Stachowicz 2011, Karlson & Osman 2012). Both disturbance (physical or biological) and increases in temperature can facilitate the establishment and increased abundance of introduced fouling species (Altman & Whitlatch 2007, Sorte & Stachowicz 2011). Geographic ranges are established for many fouling species (Karlson & Osman 2012), and range expansion in benthic communities is often used to measure community responses to increased seawater temperatures (Sagarin 1999, Dijkstra et al. 2011, Sorte & Stachowicz 2011). In terms of growth rates, individual fouling species respond positively to increased temperature in the northern portion of their latitudinal ranges and

negatively in the south (Lord & Whitlatch in review). This suggests that responses to global warming will be context-dependent, even within species, and provides a potential mechanism behind climate-induced shifts in community composition.

Mechanisms behind range shifts and spread of introduced species are not well understood, in part because introduced species are not ubiquitous even within their current geographic ranges. Therefore, attempts to link increased abundance of introduced fouling species to long-term increases in temperature are complicated by factors including range shifts, new introductions, and the spread of introduced species within their current geographic range (Dijkstra et al. 2011, Sorte & Stachowicz 2011). In order to understand and mitigate the spread of introduced fouling species, it is important to determine the factors influencing the spread and establishment of these species, not just in terms of range expansion but also within the limits of their range. Establishing which species are likely invaders and quantifying the importance of human-mediated and natural modes of dispersal is vital to limiting the impact of these species (Hulme et al. 2009).

The overall goal of this project was to gain insight into the mechanisms behind not just introduction, but also spread and resulting abundance of introduced species on local and continental scales. International shipping and aquaculture are typically the main vectors for marine introduced species transport, leading to introductions in novel environments, but less is known about what enables introduced species to persist and spread within their invaded range (Molnar et al. 2008). We conducted surveys of marine epibenthic fouling communities living on docks at 160 marinas along the east and west coasts of the United States and compared similarity of species assemblages between sites and regions in order to assess the potential degree of population connectivity and dispersal on small (< 100 km) and large (1000+km) scales. Since

temperature is a proposed cause for higher diversity and warmer temperatures can facilitate invasion (Pianka 1966, Rohde 1992, Tittensor et al. 2010, Sorte & Stachowicz 2011), we hypothesized that the warmest regions would have the highest species richness and most introduced species. Because commercial shipping vessels are a major vector for marine invasions (Molnar et al. 2008), especially for fouling species, we also hypothesized that sites with the highest commercial shipping traffic would have higher diversity of introduced species than lower shipping traffic sites at similar seawater temperature regimes. Determining the drivers of native and introduced species diversity and assessing similarity between species assemblages at different sites facilitates an understanding of the mechanisms behind the introduction and spread of marine introduced species across different geographic scales.

Methods

Photo surveys were conducted on floating docks at 160 marinas to assess relative abundance, percent cover, and species richness for native and introduced fouling species. Surveys were conducted in 8 regions on Atlantic and Pacific coasts of the US and were centered around marine laboratories that served as bases of operations. Regions included Maine (Darling Marine Center), Connecticut (University of Connecticut Avery Point), North Carolina (Duke Marine Laboratory), Florida (Smithsonian Marine Station), Alaska (Sitka Sound Science Center), Oregon (Oregon Institute of Marine Biology), central California (Bodega Marine Laboratory), and southern California (Scripps Institution of Oceanography). Surveys were conducted at marinas with floating docks within 100 km north and south of each laboratory, and individual sites were selected by harbors or marinas that both had floating docks and allowed researchers access to them. All marinas voluntarily allowed access to their docks, where

temperature was measured with a digital thermometer and salinity was measured with a refractometer. Longer-term temperature and salinity data for each site were obtained from the NOAA National Buoy Data Center (NBDC) and National Oceanographic Data Center (NODC) for the nearest recording location to each site. Photo surveys were conducted at 20 sites in each region, but only the 10 highest salinity sites in each region (80 total sites) were used for the analysis in order to minimize salinity-induced variability (Table 5.1, Fig. 5.1).

Photos of the sides of floating docks were taken with a 12 megapixel digital camera in waterproof housing, 0.25m from the side of each dock for an area of approximately $^{1}/_{16}\,m^{2}$ per photo. Photo locations were haphazardly selected and were unbiased because each photo location was chosen before lying down above the side of the dock to take the photo. As such, there was no prior knowledge of the organisms in each photograph. All photos were taken by the same researcher in order to standardize the photography method. At each site, 40 locations were selected and photographed 3 times each in order to ensure photo clarity. Photos were transferred to the computer and initially analyzed in the photo software Daminion©, with which each photograph was digitally tagged with identities of all species present. This allowed sorting by sites and regions based on the presence or absence of species in each photograph, henceforth referred to as occurrence data. All species were identified and tagged by the same researcher based on the Smithsonian NEMESIS database (http://invasions.si.edu/nemesis/index.jsp), Stanford SeaNet database (http://seanet.stanford.edu/), as well as information from local field guides and experts in each region. The majority of organisms (88%) were identified to species, while others were identified to the lowest taxonomic level based on morphological characteristics (e.g. sponges *Haliclona* spp.*, Halichondria* spp.).

Percent cover estimates were conducted using point counts for 20 randomly selected photos from each of the 80 sites. Point counts were conducted in the photo analysis program JMicroVision©, which randomly selected 75 points for identification in each photograph. Moving averages were used to ensure that photos were not under-sampled, and percent estimates levelled out by 50 points, showing that 75 points per photograph was sufficient to accurately represent the assemblage in those photographs. Similarity values were computed from percent cover data between all 8 regions ($\sum_{i=1}^{n}$(site 1_i − site 2_i), which were used to create a dendrogram in Minitab©. Species used in percent cover and occurrence analyses were classified as native or introduced based primarily on the Smithsonian NEMESIS database, supplemented by literature descriptions of native ranges for each species. Most introduced fouling species are considered pests, so they are henceforth referred to as invasive species. In order to compare differences between and within geographic regions, each region was divided geographically into two sub-regions (north and south) (Table 5.1).

Mean summer temperatures (June-August) obtained from the NOAA NDBC (http://www.ndbc.noaa.gov/) and NODC (http://www.nodc.noaa.gov/) were used for statistical analysis instead of those measured directly at each site to provide a more accurate estimate of typical summer conditions and reduce variability due to tides and short-term weather patterns. Heterogeneity or β-diversity within sites (β-site) was calculated as: (mean number of species per photograph / total species in site). β-diversity within regions (β-region) was calculated as: (mean number of species per site / total species in region). Connectivity was inferred from Jaccard's similarity index (shared / total species) and was calculated pairwise for all sites on each coast (as in de Juan et al. 2013).

Shipping data for each sub-region was calculated as the sum of commercial trade for all major ports in that sub-region, and commercial shipping data for 2012 were obtained from the US Army Corps of Engineers, Waterborne Commerce Statistics Center (http://www.iwr.usace.army.mil/About/TechnicalCenters/WCSCWaterborneCommerceStatisticsCenter.aspx). The spread or extent of each abundant (in at least 3 regions) species was calculated as the percent of sites at which each species was present within its geographic range. To make pairwise comparisons between manageable numbers of sites, each of the 10 sites within each region were ordered north to south, with the 1st, 4th, 7th, and 10th site chosen from each region. This allowed for latitudinal comparisons between 16 relatively evenly distributed sites on both the Atlantic and Pacific coasts, hereafter referred to as distributed sites.

Results

Occurrence data quantified the presence or absence of all species in each sub-region and showed a significantly positive but weak correlations between mean summer temperature (MST) and total species richness (regression ANOVA, $F_{(1,14)} = 6.92$, $r^2 = 0.33$, $p = 0.02$). Native species richness displayed a strong positive linear relationship with MST (regression ANOVA, $F_{(1,14)} = 27.39$, $r^2 = 0.66$, $p < 0.001$) (Fig. 5.2A), while invasive species richness did not (regression ANOVA, $F_{(1,14)} = 0.12$, $r^2 = 0.01$, $p = 0.74$). Invasive species richness instead showed a parabolic relationship with MST (2nd order polynomial regression ANOVA, $F_{(2,13)} = 13.00$, $r^2 = 0.56$, $p < 0.001$) (Fig. 5.2B, Table 5.2). Invasive species richness was positively correlated with commercial shipping traffic ($\log_{10}$) broken down by sub-region (regression ANOVA, $F_{(1,14)} = 13.62$, $r^2 = 0.49$, $p = 0.002$) (Fig. 5.3). The best fit for invasive species richness was a multiple linear regression incorporating both MST and commercial shipping traffic, as this explained

more variance than either factor did individually (multiple linear regression ANOVA, $F_{(2,13)}$ = 12.53, r^2 = 0.66, p = 0.001). Highest numbers of invasive species existed in sub-regions with high shipping volume and intermediate MST (e.g. sub-regions in New England, southern California), with a peak at approximately 20°C (Fig. 5.4). The proportion of invasive species (invasive richness / total richness) for each sub-region was also best explained by this model including both MST and shipping (multiple linear regression ANOVA, $F_{(2,13)}$ = 27.42, r^2 = 0.81, $p < 0.001$). Major global shipping routes and traffic levels are shown in Figure 5.5, with the highest number of routes into the United States coming from temperate locations in Europe (from Kaluza et al. 2010).

Occurrence data were used to quantify presence or absence of abundant species at all sampled sites within their latitudinal ranges, and native species occurred at an average of 53.6% (N = 10) of sites within their ranges. Invasive species introduced to the US greater than 25 years ago were present at 67.6% (N = 14) of sites within their ranges, compared to 27.9% (N = 4) for those introduced within the last 25 years (Fig. 5.6). Species introduced within the last 25 years were tunicates *Didemnum vexillum* (to the west coast), *Polyandrocarpa zorritensis, Symplegma reptans,* and bryozoan *Tricellaria inopinata*. Percent occurrence for recent invaders was significantly lower than both established invaders (T-test, T_{16} = -3.15, p = 0.006) and native species (T-test, T_{12} = -2.24, p = 0.044) (Fig. 5.6). Of 8 species that were present at over 70% of sites within their range, only 1 was native and 7 were invasive species introduced at least 25 years ago.

Beta-diversity or heterogeneity calculated at both site (between photos) and region (between sites) scales displayed a weak relationship with regional species richness, but there were no significant ($p > 0.15$) relationships between β-site or β-region and shipping, temperature,

species richness, or the number of invasive species (Table 5.3). Similarity between regions was calculated from percent cover data and the resulting dendrogram showed that sites grouped more based on temperature regime than coast, as Maine and Connecticut were most closely linked to San Francisco and San Diego (Fig. 5.7). Florida and North Carolina grouped out on a separate branch and were less than 50% similar to any other sites (Fig. 5.7).

Similarity from occurrence data at pairs of 16 distributed sites per coast displayed a large-scale negative log relationship with distance on both the east (Logistic regression ANOVA, $F_{1,118} = 117.97$, $p < 0.001$) and west coast (Logistic regression ANOVA, $F_{1,118} = 118.01$, $p < 0.001$) (Fig. 5.8A, 5.8B). On the local scale (< 100 km) there was no relationship between distance and similarity for the distributed sites on either coast ($R^2 < 0.01$, $p > 0.7$) (Fig. 5.8C, 5.8D). Similarity in percent cover data within regions was also uncorrelated with distance between sites, with a coefficient of determination (R^2) of 0.02 for all regions combined.

Discussion

Diversity of invasive marine fouling species appears to be controlled largely by mean summer temperatures (MST) and commercial shipping volume. The positive relationship between temperature and native species richness followed the general ecological pattern of higher diversity with higher temperature, possibly a result of greater seasonal stability in warmer regions or one of several other debated hypotheses (Pianka 1966, Rohde 1992, Tittensor et al. 2010) (Fig. 5.2A). Invasive species diversity did not follow this pattern and instead displayed a parabolic relationship with MST, with a peak in diversity near a mean summer temperature of ~20°C (Fig. 5.2B). The number of invasive species was also positively related to commercial shipping traffic (Fig. 5.3) and the largest shipping centers in the United States (NY/NJ, Los

Angeles) have summer temperatures of ~20°C (Fig. 5.4). Large shipping centers such as Miami, FL, had much lower numbers of invasive species, possibly because many European Atlantic ports are in temperate climates more similar to CT and ME. It is likely that long-term shipping patterns from other temperate ports are the cause of this invasion peak at 20°C, rather than a preference of invasive species for these temperatures (Fig. 5.5).

The large role of shipping traffic in fouling community invasions is expected, as it is the primary vector of marine bioinvasions (Molnar et al. 2008). Strong temperature dependence of invasive species diversity underscores the role that temperature plays in structuring communities and is especially relevant with regard to climate change (Engle & Summers 1999, Helmuth et al. 2002). Shipping appears to control the introduction of invasive species into new locations, but temperature appears to determine which species are able to survive in that location. Increased temperature due to climate change could allow warmer-water species that are already being introduced to persist in new areas. Temperature controls not only survival but growth, reproduction, and competitive ability of native and invasive fouling species (Reinhardt et al. 2012, Lord & Whitlatch in review). Therefore, increased seawater temperatures of 2-4°C by 2100 could allow a warmer-water suite of species to become established, overgrow and outcompete species that are adapter to colder water (IPCC 2007).

While warmer temperatures may lead to new introductions and increased abundance of invasive species, studies linking temperature to higher prevalence of invasive species may be confounded by the normal dispersal process of invasive species within their current geographic range. There was no significant difference between the percent occurrence of native and established invasive species at sites within their present ranges (both over 50%, Fig. 5.6), but recent invaders (< 25 years) were only found at 28% of potential sites. This is an apparent gap

between potential and presently introduced sites for recent invaders, which appear to take longer than 25 years to spread to all sites that have suitable water temperatures and other environmental conditions. Therefore, studies that cite climate as a mechanism for increased abundance of invasive fouling species should not include any recent invaders (< 25 yrs) that are dispersing within their suitable range, or they risk falsely attributing normal or invasion-related processes to global warming (Dijkstra et al. 2011, Sorte & Stachowicz 2011).

Large-scale similarity patterns were due more to shifting temperature regimes than to distance between sites, as Maine, Connecticut, San Francisco, and San Diego were most closely linked based on assemblage similarity (Fig. 5.7). While there was a negative log relationship between similarity and distance (Fig. 5.8A, 5.8B), this did not hold on intermediate or local scales, as there was no relationship between similarity and distance within regions or scales of 100 km (Fig. 5.8C, 5.8D). The differences in similarity over large (100+ km) distances was likely due to shifts in temperature regime; sites further than 100km apart can have different temperature conditions that could favor different suites of species.

The lack of a relationship between distance and similarity on local scales raises questions about local dispersal mechanisms of fouling species. If there was high amounts of mixing between sites, similarity values would be consistently close to 1, but they instead are highly variable and approximately 0.5 (Fig. 5.8C, 5.8D). This also rules out natural dispersal, which would show higher similarity for nearby sites in both the distributed sites and within regions, which did not occur. Most fouling organisms such as tunicates, bryozoans, and sponges have short-lived larvae and limited larval dispersal that can be restricted to meters (Grosberg 1987). Therefore, currents and other oceanographic conditions are unlikely to be influencing natural dispersal of these species on the 100 km scale. Sites even a few km apart appear to be reasonably

isolated, with seemingly random human-mediated "introduction" events bringing new species into each site. Local boat traffic or aquaculture activity may play a role in these smaller-scale dispersal patterns and may be responsible for many of these local transfers of species between sites.

Invasive species can reduce diversity and homogenize species pools in some systems, but there was no evidence of this in fouling communities. Invasive species make up 19-58% of the diversity in all locations studied (Table 5.2), so species richness in fouling communities has a large invasive component in all locations (Vitousek et al. 1996, 1997). There was no relationship between invasive species richness and heterogeneity, and even competitively superior fouling species rarely eliminate native species (Lord & Whitlatch in review). The lack of homogenization of species pools even in a highly invaded system like fouling communities is likely due to the lack of competitive exclusion in this system. Fouling communities are relatively ephemeral systems, with continuous replacement of the short-lived adult phase and no classical succession structure, so dominance rarely lasts more than a season (Sutherland & Karlson 1977, Sutherland 1981).

Strong temperature dependence of fouling community composition suggests that these communities are sensitive to future temperature increases due to global warming. Invasive species diversity in this system is strongly linked to both commercial shipping and site temperature, so changes in temperature could allow warmer-water species that are introduced by shipping or aquaculture to become established. However, the normal spread of invasive species within their present geographic range should not be confused with global warming-induced increases in abundance of these species, particularly for recent invaders. With a greater understanding of invasion vectors (shipping), factors allowing establishment (temperature), and

growth and competition (Lord & Whitlatch in review), it may be possible to predict future invasions and range expansions, as well as the impact and spread of these species after they become introduced.

Acknowledgements

Thanks to all of the individuals and institutions that allowed me to use office and lab space. These include but are not limited to: Lon Garrison at the Sitka Sound Science Center, Dr. Richard Emlet at the Oregon Institute of Marine Biology, Karl Menard and Dr. Brian Gaylord at Bodega Marine Laboratory, Dr. Jennifer Smith at Scripps Institution of Oceanography, Tim Miller and Linda Healy at Darling Marine Center, Charles Woods at University of Connecticut, Dr. Dan Rittschof at Duke Marine Laboratory, and Hugh Reichardt and Sherry Reed at Smithsonian Marine Station. In addition, this survey work could not have been completed without voluntary cooperation from 160 marinas on both coasts of the United States. This manuscript was greatly improved with input from Dr. Rick Osman. Funding provided by Connecticut Sea Grant Project Number PD-12-17 and by the University of Connecticut Department of Marine Science Pre-doctoral Fellowship.

References

Altman, S. & Whitlatch, R.B. (2007) Effects of small-scale disturbance on invasion success in marine communities. *Journal of Experimental Marine Biology and Ecology*, **342**, 15–29.

de Juan, S., Thrush, S.F. & Hewitt, J.E. (2013) Counting on β-diversity to safeguard the resilience of estuaries. *PloS one*, **8**, 1–11.

Dijkstra, J.A., Westerman, E.L. & Harris, L.G. (2011) The effects of climate change on species composition, succession and phenology: a case study. *Global Change Biology*, **17**, 2360–2369.

Dukes, J. & Mooney, H. (1999) Does global change increase the success of biological invaders? *Trends in Ecology & Evolution*, **14**, 135–139.

Engle, V. & Summers, J. (1999) Latitudinal gradients in benthic community composition in Western Atlantic estuaries. *Journal of Biogeography*, **26**, 1007–1023.

Grosberg, R. (1987) Limited dispersal and proximity-dependent mating success in the colonial ascidian *Botryllus schlosseri*. *Evolution*, **41**, 372–384.

Hejda, M., Pyšek, P. & Jarošík, V. (2009) Impact of invasive plants on the species richness, diversity and composition of invaded communities. *Journal of Ecology*, **97**, 393–403.

Helmuth, B., Harley, C.D.G., Halpin, P.M., O'Donnell, M., Hofmann, G.E. & Blanchette, C.A. (2002) Climate change and latitudinal patterns of intertidal thermal stress. *Science*, **298**, 1015–7.

Houlahan, J.E. & Findlay, C.S. (2004) Effect of invasive plant species on temperate wetland plant diversity. *Conservation Biology*, **18**, 1132–1138.

Hulme, P.E. (2009) Trade, transport and trouble: managing invasive species pathways in an era of globalization. *Journal of Applied Ecology*, **46**, 10–18.

IPCC (2007) *Climate Change 2007: Synthesis Report*.

Kaluza, P., Kolzsch, A., Gastner, M.T. & Blasius, B. (2010) The complex network of global cargo ship movements. *Journal of the Royal Society Interface*, **7**, 1093–1103.

Karlson, R. & Osman, R. (2012) Species composition and geographic distribution of invertebrates in fouling communities along the east coast of the USA: a regional perspective. *Marine Ecology Progress Series*, **458**, 255–268.

Molnar, J.L., Gamboa, R.L., Revenga, C. & Spalding, M.D. (2008) Assessing the global threat of invasive species to marine biodiversity. *Frontiers in Ecology and the Environment*, **6**, 485–492.

Pauli, H., Gottfried, M., Reiter, K., Klettner, C. & Grabherr, G. (2007) Signals of range expansions and contractions of vascular plants in the high Alps: observations (1994-2004) at the GLORIA* master site Schrankogel, Tyrol, Austria. *Global Change Biology*, **13**, 147–156.

Pianka, E. (1966) Latitudinal gradients in species diversity: a review of concepts. *American Naturalist*, **100**, 33–46.

Reinhardt, J.F., Osman, R. & Whitlatch, R. (2012) A phenological mid-domain analysis of non-native and native species recruitment richness. *Journal of Ecosystem & Ecography*, **02**, 4–9.

Rohde, K. (1992) Latitudinal gradients in species diversity: the search for the primary cause. *Oikos*, **65**, 514–527.

Sagarin, R., Barry, J., Gilman, S. & Baxter, C. (1999) Climate-related change in an intertidal community over short and long time scales. *Ecological Monographs*, **69**, 465–490.

Sebens, K. (1986) Spatial relationships among encrusting marine organisms in the New England subtidal zone. *Ecological Monographs*, **56**, 73–96.

Stachowicz, J., Whitlatch, R. & Osman, R. (1999) Species diversity and invasion resistance in a marine ecosystem. *Science*, **286**, 1577–1579.

Sutherland, J. & Karlson, R. (1977) Development and stability of the fouling community at Beaufort, North Carolina. *Ecological Monographs*, **47**, 425–446.

Sutherland, J. (1978) Functional roles of *Schizoporella* and *Styela* in the fouling community at Beaufort, North Carolina. *Ecology*, **59**, 257–264.

Tittensor, D.P., Mora, C., Jetz, W., Lotze, H.K., Ricard, D., Berghe, E. Vanden & Worm, B. (2010) Global patterns and predictors of marine biodiversity across taxa. *Nature*, **466**, 1098–101.

Vance, T., Lauterbach, L. & Lenz, M. (2009) Rapid invasion and ecological interactions of *Diplosoma listerianum* in the North Sea, UK. *Marine Biodiversity Records*, **2**, 1–5.

Vitousek, P., D'Antonio, C., Loope, L.L. & Westbrooks, R. (1996) Biological invasions as global environmental change. *American Scientist*, **84**, 468–478.

Vitousek, P., Mooney, H., Lubchenco, J. & Melillo, J. (1997) Human domination of Earth's ecosystems. *Science*, **277**, 494–499.

Figure Captions

Figure 5.1. Map of marina sites at which photo surveys were conducted during summer (May-August) 2013. Surveys were conducted in 4 regions on each coast, with 20 sites per region.

Figure 5.2. Relationship between mean summer seawater temperature (MST) and species richness for native (A) and invasive (B) species. Both east and west coast sites show positive linear relationship with MST for native species and a parabolic relationship for invasive species.

Figure 5.3. Positive linear relationship between invasive species richness and commercial shipping volume for both coasts of the USA.

Figure 5.4. Three-dimensional surface plot of relationship between invasive species richness (per site) and mean summer seawater temperature (MST) and commercial shipping at all 80 sites included in analysis (10 region^{-1}). Highest number of invasive species at high shipping volume and intermediate temperatures (approximately 20°C).

Figure 5.5. Map of annual global shipping traffic, with majority of traffic to northeastern USA from temperate location in Europe (Fig. from Kaluza et al. 2010; Fig. 5.1, page 2).

Figure 5.6. Proportion occurrence of native and invasive species at sites within their present geographic range. Recent invaders were present at significantly lower proportion of sites than

established invaders and native species, so will likely continue to spread with their range. Letters indicate significant differences ($p < 0.05$). Error bars ±SE.

Figure 5.7. Dendrogram of similarity between regions based on percent cover data from surveys at 10 sites within each region. ME, CT, SF, and SD were most closely linked, despite being on separate coasts.

Figure 5.8. Relationship between similarity and distance between sites, based on occurrence (presence / absence) data at 16 distributed sites per coast of the USA. Similarity displayed a negative log relationship with distance on the continental scale for both the east (A) and west coast (B). There was no relationship between similarity and distance between sites on the local scale on the east (C) or west coast (D).

Table 5.1. Sampling dates and site characteristics for all 80 sites included in the analysis.

East Coast							West Coast						
R	SR	Site	°C	S	Date	Lat	R	SR	Site	°C	S	Date	Lat
ME	1	Journeys End Marina	15.7	29.3	8/10	44.11	AK	9	Auke Harbor	14.8	15.8	7/22	58.38
ME	1	Darling Marine Center	18.5	29.3	8/10	43.93	AK	9	Andrews Marina	14.9	15.8	7/22	58.38
ME	1	Freeport Brewer Mar	18.4	28.8	8/11	43.82	AK	9	Aurora Harbor West	11.8	18.8	7/22	58.30
ME	1	Strouts Point Wharf	18.3	30.5	8/11	43.82	AK	9	Harris Harbor	11.6	18.8	7/22	58.30
ME	1	Harpswell Landing	21.8	29.8	8/12	43.83	AK	10	Harbor Drive	14.9	25.8	7/13	57.05
ME	1	Wottons Wharf	19.9	30.8	8/12	43.85	AK	10	Katlian Street	14.8	25.8	7/13	57.05
ME	2	Spring Point Marina	18.1	28.8	8/11	43.65	AK	10	Eliason Harbor	14.6	23.8	7/14	57.06
ME	2	Abner Point Seafood	18.4	30.6	8/12	43.73	AK	10	Cove Marina	15.1	21.8	7/14	57.12
ME	2	Cape Ann Marina	19.1	30.3	8/15	42.61	AK	10	Islandview Resort	15.4	29.8	7/14	57.11
ME	2	Hampton River Marina	16.5	30.8	8/15	42.90	AK	10	Lincoln Street	15.3	20.8	7/14	57.05
CT	3	Wickford Shipyard	24.7	29.3	8/20	41.57	OR	11	Hatfield Marina	12.5	31.1	7/26	44.62
CT	3	Point Judith Marina	23.6	29.8	8/20	41.39	OR	11	Joe Ney Docks	12.6	31.8	7/26	43.34
CT	3	Taylor Marina	22.0	30.8	8/24	41.75	OR	11	Charleston Inner E	11.9	30.8	7/29	43.34
CT	3	Onset Bay Marina	21.2	31.8	8/24	41.74	OR	11	Charleston Inner W	13.4	29.8	7/29	43.34
CT	4	Port Niantic	22.9	28.3	8/19	41.33	OR	11	Charleston Shipyard	15.5	31.8	7/30	43.34
CT	4	Spicers Marina	21.9	29.3	8/23	41.32	OR	11	Charleston Outer S	10.8	30.8	7/30	43.35
CT	4	Gwenmore Marina	24.1	28.3	8/23	41.35	OR	11	Charleston Outer N	10.8	30.8	7/30	43.35
CT	4	Dons Dock	22.5	30.3	8/23	41.34	OR	12	Citizens Dock S	16.3	30.3	7/25	41.75
CT	4	Shennecossett Yacht	21.8	27.8	9/5	41.32	OR	12	Citizens Dock N	16.3	30.3	7/25	41.75
CT	4	UConn Avery Point	21.8	27.8	9/5	41.32	OR	12	Crescent Public Dock	17.3	34.8	7/25	41.75
NC	5	Duke Marine Lab	23.7	33.3	5/25	34.72	SF	13	Spud Point Marina	16.2	31.1	6/17	38.33
NC	5	Spooner Creek	25.4	32.1	5/27	34.73	SF	13	SF Yacht Club	17.3	27.3	6/26	37.87
NC	5	Pivers Island	23.8	31.3	5/28	34.72	SF	13	Sausalito Yacht Harbor	17.5	27.3	6/26	37.86
NC	5	Morehead City Yacht	23.7	31.8	5/28	34.72	SF	13	SF Yacht Harbor	17.5	28.3	6/26	37.81
NC	5	Portside Marina	23.1	32.8	5/28	34.72	SF	13	Sequoia Marina	23.8	29.1	6/26	37.50
NC	5	Anchorage Marina	25.0	31.3	5/28	34.70	SF	13	Harbor Marina North	22.2	27.8	6/27	37.91
NC	6	Beach House Marina	23.5	32.8	5/27	34.43	SF	14	Breakwater Cove	14.8	30.3	6/27	36.61
NC	6	Basin Road	26.2	20.8	5/27	34.04	SF	14	Elkhorn Yacht Club	18.3	29.8	6/27	36.81
NC	6	Holden Beach Marina	25.2	30.0	5/29	33.92	SF	14	Santa Cruz Harbor	15.5	29.8	6/27	36.97
NC	6	Blue Water Point	26.6	30.4	5/29	33.92	SF	14	Pillar Point Harbor	16.9	31.0	6/27	37.50
FL	7	Harbour Isle Marina	29.7	33.5	6/3	27.46	SD	15	Long Beach Marina	21.5	30.8	7/1	33.75
FL	7	South Causeway Park	29.3	34.3	6/3	27.46	SD	15	Pacific Yacht Harbor	20.7	30.8	7/1	33.77
FL	7	Stan Blum Ramp	29.4	33.3	6/3	27.48	SD	15	Holiday Harbor	21.6	30.3	7/1	33.72
FL	7	Vero Beach Marina	30.2	25.8	6/3	27.66	SD	15	Redondo Beach	23.0	30.3	7/1	33.85
FL	7	Ocean Club	28.5	32.8	6/4	28.41	SD	15	Island Packers	20.1	30.5	7/2	34.24
FL	7	Christenson Launch	30.1	30.8	6/4	27.93	SD	15	Anacapa Isle	20.2	30.3	7/2	34.17
FL	7	Sebastian Inlet	28.4	25.3	6/5	27.87	SD	16	Seaforth Landing	19.0	33.8	6/14	32.76
FL	8	Lake Park Harbor	29.6	24.8	6/2	26.79	SD	16	Mission Bay Yacht	19.0	33.8	6/15	32.78
FL	8	Riviera Beach Marina	28.2	27.3	6/2	26.77	SD	16	Campland	23.0	33.3	6/15	32.79
FL	8	John Pennekamp Park	27.0	37.8	6/9	25.12	SD	16	Chula Vista Yacht	26.7	33.1	7/5	32.62

Table 5.2. Mean summer temperature (MST) and native and invasive species richness for each sub-region (8 / coast).

Sub-region	MST (°C)	Species Richness			Ratio (I / T)
		Total	Invasive	Native	
ME N	15.0	19	9	10	0.474
ME S	14.9	17	8	9	0.471
CT N	19.3	23	11	12	0.478
CT S	20.3	22	10	12	0.455
NC N	24.9	22	5	17	0.227
NC S	27.6	18	4	14	0.222
FL N	26.5	37	9	28	0.243
FL S	29.3	26	5	21	0.192
AK N	10.4	3	1	2	0.333
AK S	12.4	16	6	10	0.375
OR N	12.7	19	7	12	0.368
OR S	14.0	18	10	8	0.556
SF N	13.4	24	14	10	0.583
SF S	14.3	23	12	11	0.522
SD N	18.3	30	16	14	0.533
SD S	21.5	30	16	14	0.533

Table 5.3. Diversity, shipping, and species richness for each of 8 regions (4 per coast). β-site values describe heterogeneity within sites, while β-region values show heterogeneity within regions. Higher β-values indicate more connectivity (less heterogeneity).

Region	β-site	β-region	Shipping (log)	MST (°C)	Invasive Richness	Total Richness	Proportion Invasive
ME	0.465	0.425	7.488	14.93	11	18	0.611
CT	0.496	0.544	8.151	19.79	11	24	0.458
NC	0.368	0.423	6.999	26.25	3	24	0.125
FL	0.296	0.333	7.441	27.88	7	37	0.189
AK	0.390	0.350	5.894	11.39	6	15	0.400
OR	0.387	0.385	6.292	13.33	12	25	0.480
SF	0.344	0.441	7.634	13.84	16	29	0.552
SD	0.415	0.402	8.148	19.93	17	34	0.500

Figure 5.1 (see caption on p. 143)

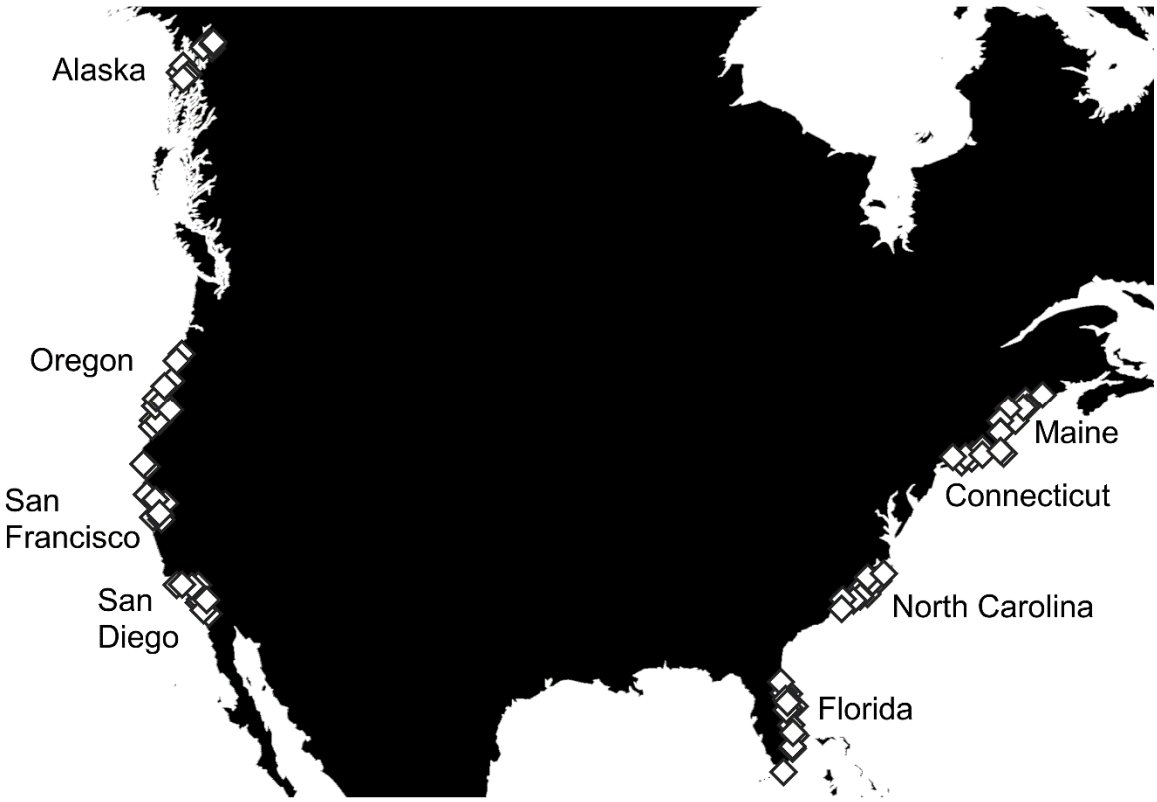


Figure 5.2 (see caption on p. 143)

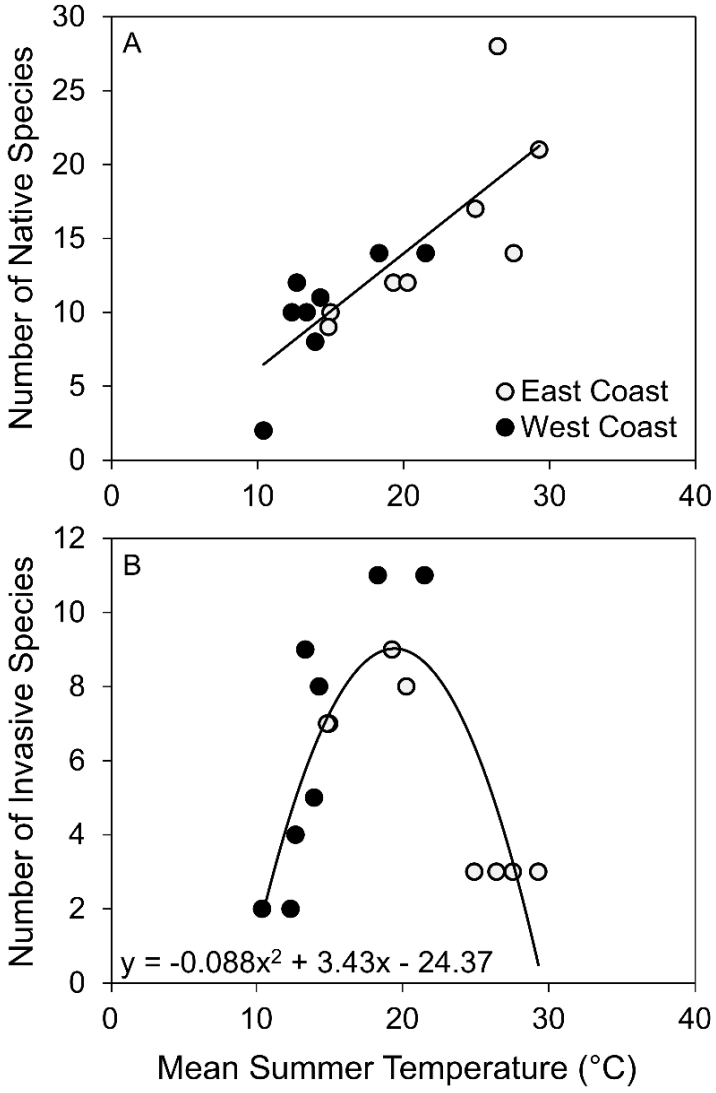


Figure 5.3 (see caption on p. 143)

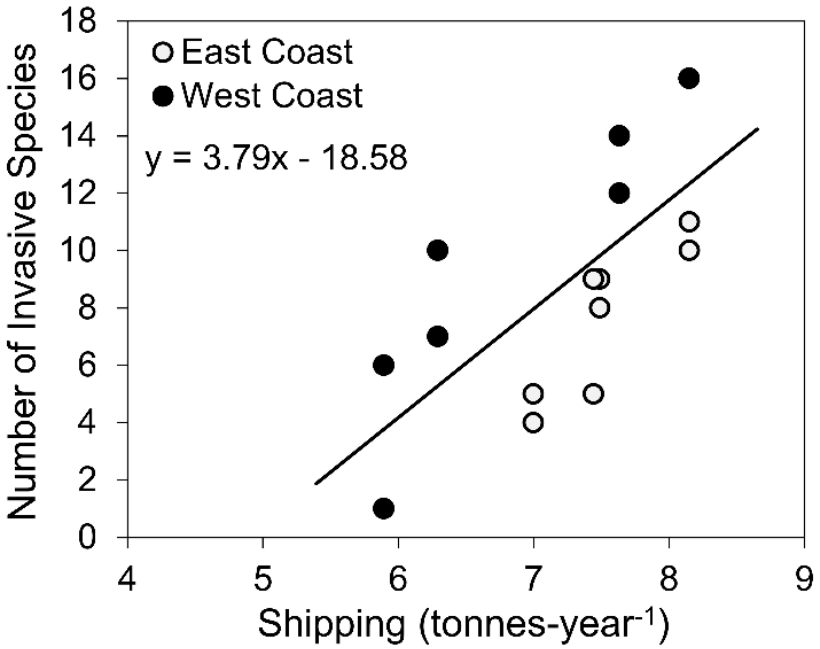


Figure 5.4 (see caption on p. 143)

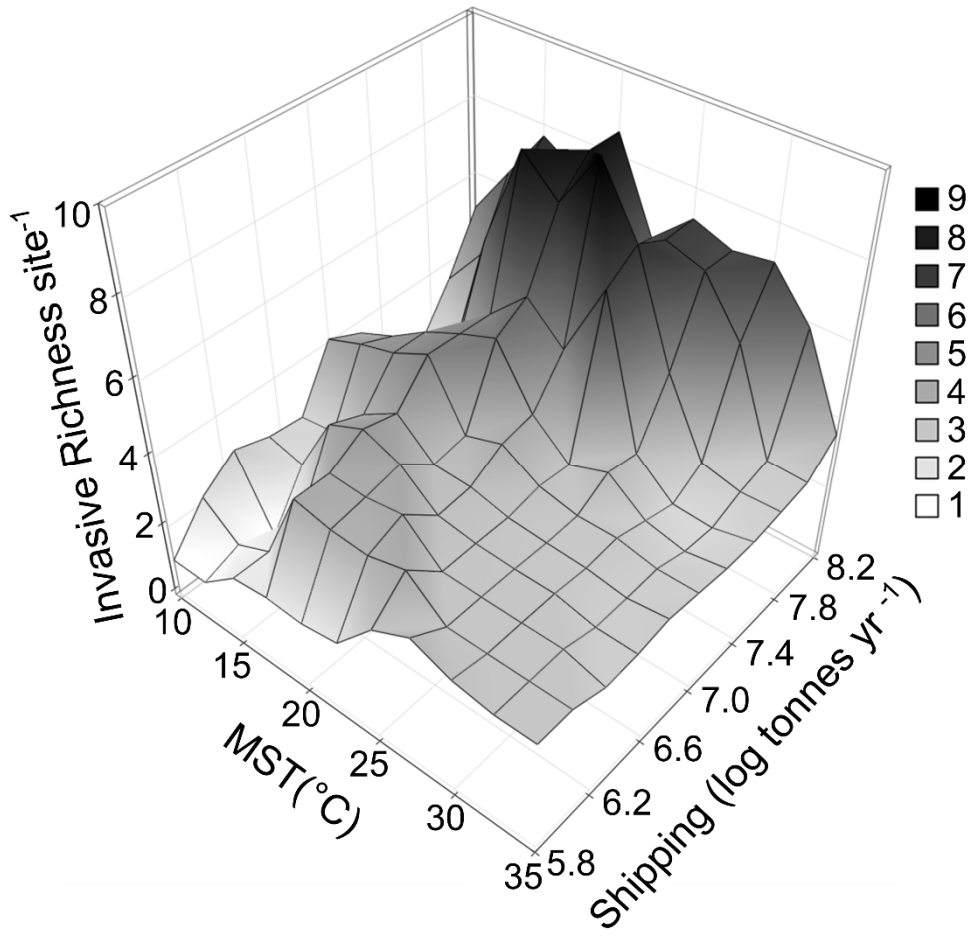


Figure 5.5 (see caption on p. 143)

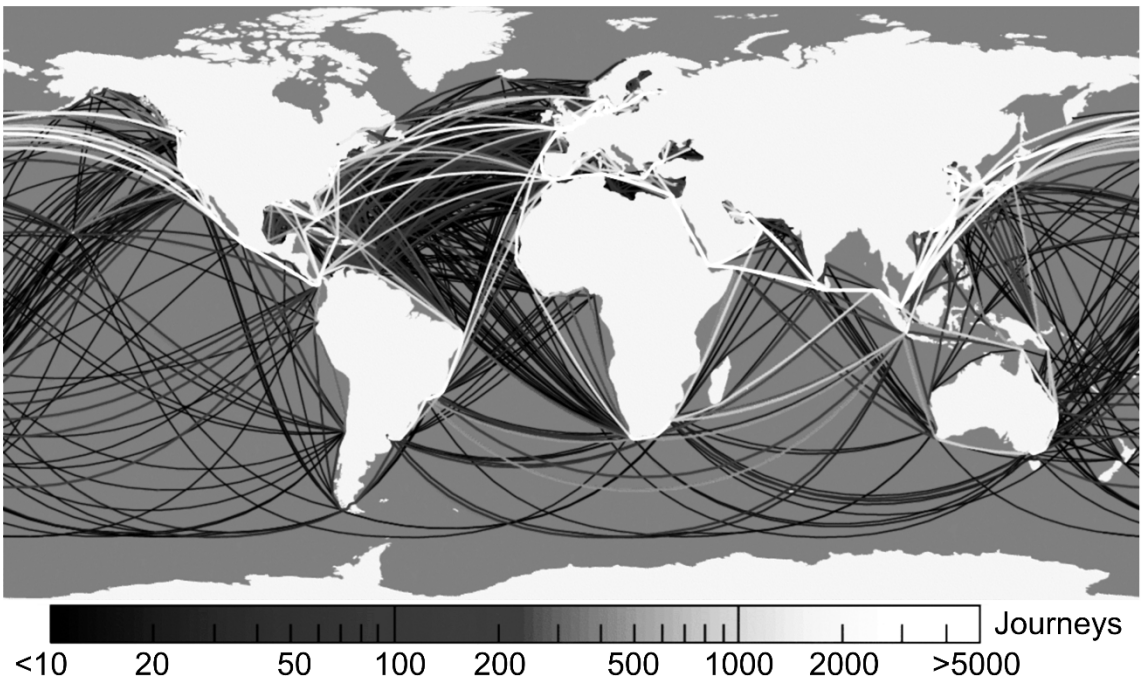


Figure 5.6 (see caption on p. 143)

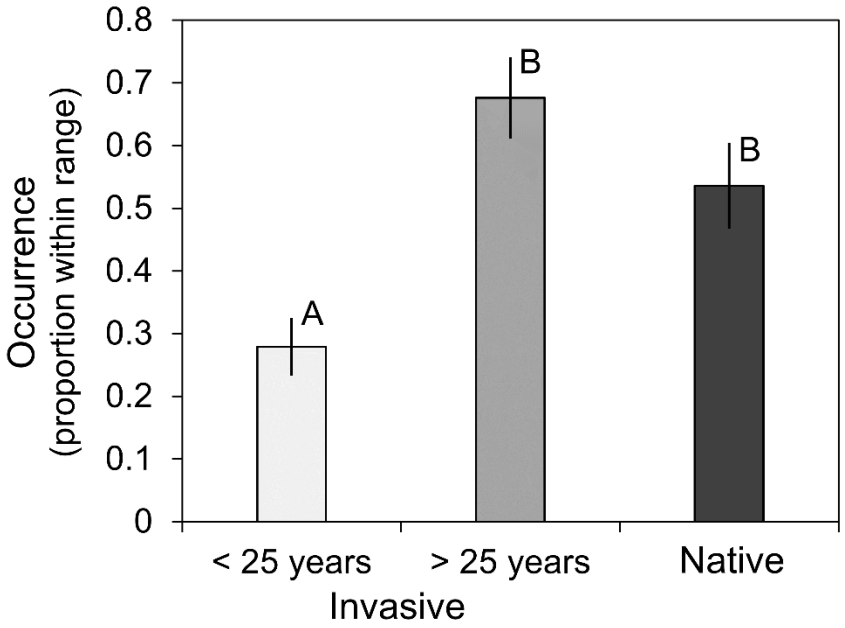


Figure 5.7 (see caption on p. 143)

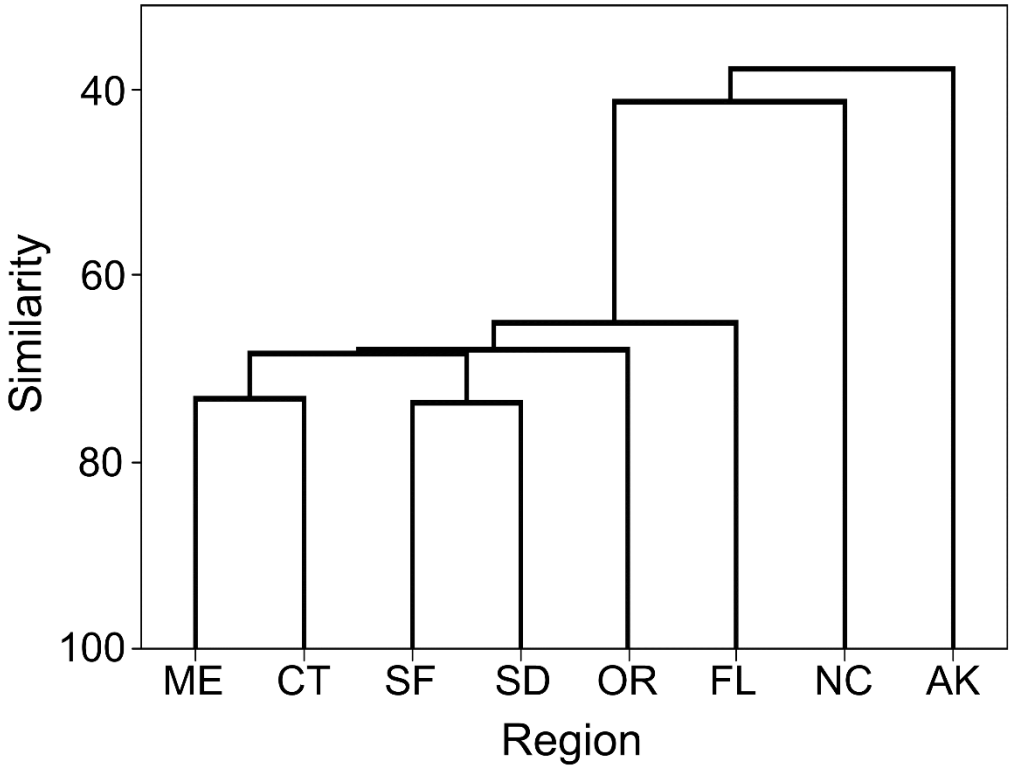
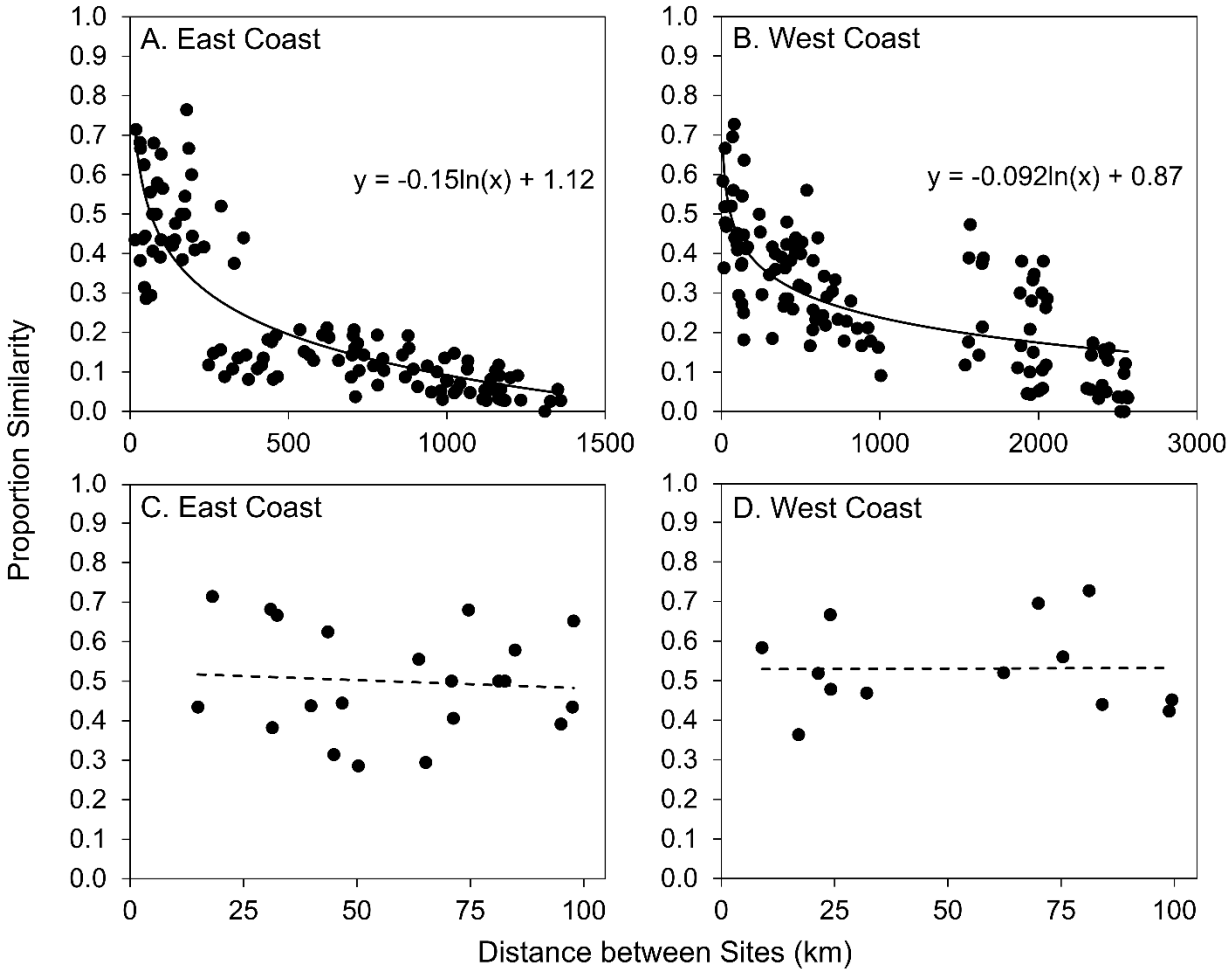


Figure 5.8 (see caption on p. 143)



General Conclusions

This dissertation examined the role that temperature plays in structuring communities and investigated the mechanisms behind shifts in predator-prey and competitive interactions. Previous climate research had established many species-specific growth responses to temperature (oysters Dame 1972; tunicates McCarthy et al. 2007; bryozoans Amui-Vedal et al. 2007, Saunders and Metaxas 2009), but many studies cited the need to assess how species interactions would be affected by global warming and climate change (Harley et al. 2006, Gilman et al. 2010, Somero 2012). In addition, studies that found changing community composition as a result of range shifts and increasing abundance of invasive species have been conducted in one geographic location, rendering them unable to differentiate causation between global warming and continued dispersal of invasive species within their present range (Sagarin 1999, Dijkstra et al. 2011, Sorte and Stachowicz 2011). The primary goals of this dissertation were: (1) quantify the impact of increased temperature on predator-prey and competitive interactions, and (2) compare thermal growth and metabolic responses to temperature over a wide geographic range to assess the context-dependency of responses to global warming.

One of the ways that eastern oysters are able to defend themselves from predation is through inducible defenses including producing thicker shells, which they have been shown to do in response to crushing predators like the blue crab *Callinectes sapidus* and mud crab *Rhithropanopeus harrissii* (Newell et al. 2007). The present study was the first to show that the eastern oyster *Crassostrea virginica* also produces thicker shells in the presence of drilling predators such as the predatory oyster drill *Urosalpinx cinerea* (Chapter 1). Oyster drill feeding had been quantified on a seasonal scale (Hanks 1957, Manzi 1970), but it is valuable to

understand how feeding rates respond to experimentally elevated temperatures along with seasonal variability. Chapter 2 quantified a 60% increase in drill feeding at temperatures elevated just 4°C above ambient seawater temperatures for 8 months, indicating that this species is likely to have more of an ecological impact with warmer temperatures in Long Island Sound. A necessary link was also established between drill feeding and oyster shell thickness, as drills preferentially (86%) consumed thinner-shelled oysters, highlighting the importance of the oyster inducible shell-thickening response (Chapter 1, 2). Temperature is known to control metabolic rate in oysters (Shumway and Koehn 1980, Cranford et al. 2011), and Chapter 3 highlighted this pattern and also showed that oyster responses to increased temperature varied with latitude, meaning that results of thermal metabolic experiments in one location should not be extrapolated to an entire species' range.

Increased temperatures also facilitated production of thicker shells in oysters in both laboratory experiments and in surveys conducted along the east coast of the United States (Chapter 3). This was likely due to the decreased solubility of calcium carbonate at higher temperatures, which allows oysters to more easily deposit calcite shells, potentially decreasing their susceptibility to predation (Chapter 2, 3) (Morse and Mackenzie 1990). This work suggests that oyster may be able to enhance their inducible response to predation in warmer temperature because of the increased capacity to deposit shell material, though further research on this subject is required. At warmer temperatures, *C. virginica* produced thicker shells (Chapter 3) and *U. cinerea* consumed far more oysters (Chapter 2), suggesting that oysters may reach a size or thickness refuge from oyster drill predation earlier in life. This would enhance survival of intermediate and large oysters, but even increased shell production will leave juvenile oysters (spat) vulnerable to the thermally-increased predation rate of *U. cinerea*. Therefore, it is expected

that with global warming, adult oysters will experience lower predation rates by drilling and crushing predators, but that juvenile oysters may be subject to increased predation pressure as a result of higher predator feeding rates.

As with oysters, growth rates of fouling organisms have been established, although usually in one location for each species (Amui-Vedal et al. 2007, McCarthy et al. 2007, Saunders and Metaxas 2009, Vance et al. 2009). Growth rates actually vary widely within species' ranges (Chapter 4), so measuring a single 'species' growth rate is not realistic. Temperature-dependent shifts in responses to experimentally elevated temperatures were observed across all fouling species studied, suggesting that responses to global warming may vary in a predictable manner depending on species' range position. Species in the northern portions of their latitudinal ranges displayed positive growth responses to increased temperature, while species near the southern end of their range responded negatively (Chapter 4). This not only allows for site-specific predictions of responses to global warming, but also enables prediction of shifts in competitive interactions, as changes in relative growth rates of species pairs were strongly correlated with competitive outcomes (Chapter 4). No previous research has focused on comparing growth rates of fouling species between different locations, and the thermal experiment component provided the capacity to identify the mechanism behind community-level responses to global warming.

Thermally-dependent growth rates and competitive outcomes provide information about community-level interactions after a species becomes established, but did not assess the impact of newly introduced or invasive species in these communities (Chapter 4). Research presented in Chapter 5 quantified the importance of commercial shipping traffic in introducing invasive species on a continental scale, while it was clear that whether or not a species becomes established in a new location is temperature-dependent. On large scales (100+ km), fouling

communities are controlled largely by both introductions of invasive species and by thermal regime, which controls which species can survive in each region (Chapter 5). On smaller scales (< 100 km) there is a large degree of stochasticity in species assemblages, with high levels of heterogeneity (low connectivity) and little importance of natural dispersal between sites.

This dissertation defined temperature-dependent growth rates for over 15 fouling species on a latitudinal scale, but the most important element was using these individual and species-level characteristics like growth, feeding, and metabolism to predict temperature-induced shifts in predator-prey and competitive interactions. Results from the predator-prey relationship between oysters and oyster drills highlighted the importance of testing thermal effects on more than just one species, and further experiments with inducible defenses and with other oyster predators would enhance the value of this. Fouling community surveys and experiments allowed for prediction of both the spread of invasive species within their ranges and the shift in competitive interactions with climate change. Species growth rates are important in defining competitive and predator-prey interactions, and the influence that temperature has on these relationships depends on physiological responses and latitudinal ranges of all species involved. Long-term predictions of shifts in community assemblages and abundance of individual species should take into account both interspecific interactions and latitudinal variability in species' thermal responses, as both can influence reactions to climate change at the individual, species, and community level.

References

Amui-Vedel A.-M., Hayward P.J., & Porter J.S. (2007) Zooid size and growth rate of the bryozoan *Cryptosula pallasiana* Moll in relation to temperature, in culture and in its natural environment. *Journal of Experimental Marine Biology and Ecology*, **353**, 1–12.

Cranford P., Ward J., & Shumway S. (2011) Bivalve filter feeding: variability and limits of the aquaculture biofilter. *Shellfish aquaculture and the environment* (ed. by S. Shumway), pp. 81–124.

Dame R. (1972) The ecological energies of growth, respiration and assimilation in the intertidal American oyster *Crassostrea virginica. Marine Biology*, **17**, 243–250.

Dijkstra J.A., Westerman E.L., & Harris L.G. (2011) The effects of climate change on species composition, succession and phenology: a case study. *Global Change Biology*, **17**, 2360–2369.

Gilman S.E., Urban M.C., Tewksbury J., Gilchrist G.W., & Holt R.D. (2010) A framework for community interactions under climate change. *Trends in ecology & evolution*, **25**, 325–31.

Hanks J. (1957) The rate of feeding of the common oyster drill, *Urosalpinx cinerea* (Say), at controlled water temperatures. *Biological Bulletin*, **112**, 330–335.

Harley C.D.G., Hughes R.A., Hultgren K.M., Miner B.G., Sorte C.J.B., Thornber C.S., Rodriguez L.F., Tomanek L., & Williams S.L. (2006) The impacts of climate change in coastal marine systems. *Ecology letters*, **9**, 228–41.

Manzi J. (1970) Combined effects of salinity and temperature on the feeding, reproductive, and survival rates of *Eupleura caudata* (Say) and Urosalpinx cinerea (Say)(Prosobranchia: *Biological Bulletin*, **138**, 35–46.

McCarthy A., Osman R.W., & Whitlatch R.B. (2007) Effects of temperature on growth rates of colonial ascidians: A comparison of *Didemnum* sp. to *Botryllus schlosseri* and *Botrylloides violaceus. Journal of Experimental Marine Biology and Ecology*, **342**, 172–174.

Morse J.W. & Mackenzie F.T. (1990) *Geochemistry of Sedimentary Carbonates.* Elsevier B.V., New York.

Newell R.I.E., Kennedy V.S., & Shaw K.S. (2007) Comparative vulnerability to predators, and induced defense responses, of eastern oysters *Crassostrea virginica* and non-native *Crassostrea ariakensis* oysters in Chesapeake Bay. *Marine Biology*, **152**, 449–460.

Sagarin R., Barry J., Gilman S., & Baxter C. (1999) Climate-related change in an intertidal community over short and long time scales. *Ecological Monographs*, **69**, 465–490.

Saunders M.I. & Metaxas A. (2009) Effects of temperature, size, and food on the growth of *Membranipora membranacea* in laboratory and field studies. *Marine Biology*, **156**, 2267–2276.

Shumway S. & Koehn R. (1982) Oxygen consumption in the American oyster *Crassostrea virginica. Marine Ecology Progress Series*, **9**, 59–68.

Somero G.N. (2012) The physiology of global change: linking patterns to mechanisms. *Annual Review of Marine Science*, **4**, 39–61.

Sorte C.J. & Stachowicz J. (2011) Patterns and processes of compositional change in a California epibenthic community. *Marine Ecology Progress Series*, **435**, 63–74.

Vance T., Lauterbach L., & Lenz M. (2009) Rapid invasion and ecological interactions of *Diplosoma listerianum* in the North Sea, UK. *Marine Biodiversity Records*, **2**, 1–5.