ABSTRACT

Title of Dissertation: THE ORGANISMAL AND POPULATION EFFECTS OF CLIMATE CHANGE ON JUVENILE BLUE CRAB (*CALLINECTES SAPIDUS*) IN THE PATUXENT RIVER, CHESAPEAKE BAY

Hillary Lane Glandon, Doctor of Philosophy, 2017

Dissertation directed by: Director and Professor Thomas J. Miller, Chesapeake Biological Laboratory, University of Maryland Center for Environmental Science

And

Associate Professor Kennedy T. Paynter, Chesapeake Biological Laboratory, University of Maryland Center for Environmental Science
and
Director, Marine, Estuarine, Environmental Sciences,
University System of Maryland

Future climate scenarios predict an increase in temperature and dissolved carbon dioxide (pCO_2) of the marine environment in the next century. Calcifying marine invertebrates are thought to be especially vulnerable to increases in pCO_2 and although the effect of increasing temperature in many of these taxa is understood, less is known about the interactive effects of these stressors on the physiology of calcifying

invertebrates. In the present study, juvenile blue crab (*Callinectes sapidus*) were exposed predicted future levels of temperature and pCO_2 in a 2x2 factorial design for two complete molts (approximately 30 days). Increased temperature caused a significant increase in crab growth rate and food consumption, but at a cost to carapace thickness and chemistry. The carapaces of individuals exposed to increased temperature were significantly thinner and had significantly lower percent high-magnesium calcite (HMC), the mineral from which the carapace derives its strength. Although there was a significant increase in percent HMC in response to increased pCO_2, this was paired with an increase in the concentration of magnesium, complicating the overall effect of increased pCO_2 on the carapace. The temperature range tested in this study was not large enough to elicit a significant difference in mean oxygen consumption rate. Crabs were resilient to exposure to extremely high levels of pCO_2; there was no significant effect of increased pCO_2 on crab growth rate, food consumption, or oxygen consumption rate.

Individual physiological response data were utilized in concert with historical and predicted water temperatures to determine effects of future predicted increases in water temperature on blue crab overwintering behavior in the Chesapeake Bay. Model data indicated a significant shortening of the overwintering period from approximately 3.5 months currently to between 1.5 and 3 months, depending on the climate model utilized for the predictions. Increases to growing season length, combined with predicted increases in crab growth rate and food consumption, indicate that in the future blue crab will mature faster and may possibly grow year-round, similar to individuals that live in the southern extent of the species' range.

THE ORGANISMAL AND POPULATION EFFECTS OF CLIMATE CHANGE ON JUVENILE BLUE CRAB (*CALLINECTES SAPIDUS*) IN THE PATUXENT RIVER, CHESAPEAKE BAY

by

Hillary Lane Glandon

Dissertation submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
2017

Advisory Committee:
Professor Thomas J. Miller, Chair
Associate Professor Kennedy T. Paynter
Assistant Professor James J. Pierson
Associate Professor Johan Schijf
Dr. A. Whitman Miller

Dedication

This document is dedicated to my Aunt Rita and my daughter Isabelle. I hope this accomplishment will make them both proud. L'Dor Vador.

Acknowledgements

I would not have been able to complete my dissertation research without the help of numerous people. My advisors, Dr. Thomas Miller and Dr. Ken Paynter have supported me in all ways possible throughout my time as a Ph.D. student. I am grateful to Dr. Paynter for introducing me to the Chesapeake Bay and the world of shellfish and to Dr. Miller for guiding me through coursework, lab experiments, and the publishing process, all while supporting me as a new parent. My dissertation committee, Dr. Jamie Pierson, Dr. Johan Schijf, and Dr. Whitman Miller have pushed me to my intellectual limits and helped me make this project as good as it could possibly be. Dr. Chris Rowe provided me with the instrumentation necessary to complete large portions of my research and also helped to fuel my interest in physiology throughout my time at CBL. Dr. Hali Kilbourne was instrumental in the success of many aspects of my project in addition to providing welcome support to me as a new mother. Additionally, Dr. Dong Liang and Dr. Viacheslav Lyubchich helped me work through experimental design and data analysis challenges throughout this project.

The Miller and Paynter lab members, especially David Loewensteiner, Danny Zaveta, Reed Brodnik, Adriane Michaelis, and Karen Kesler, have provided experimental and emotional help throughout my time as a Ph.D. student. The experimental component of my dissertation could not have been completed without the assistance of outstanding undergraduate and high school students Jeff Rice, Annie Nyffeler, Maddie Schwaab, Rose Miller, and Emmy Sheahan. I have been supported by the entire graduate student body and many faculty research assistants at CBL, but especially Carlos Lozano, Alex Atkinson, Christina Goethel, Suzan Shahrestani, Jessica Wingfield, Grey Redding, Brian

Gallagher, and Mike O'Brien. I have been fortunate to receive invaluable assistance from numerous CBL staff including Gail Canaday, Elissa Lee, Stacy Hutchinson, Brian Duke, Dale Garner, Kenny Rager, Thomas Darnall, Elaine Proctor, Samantha Mais, Jeannette Duran, and Dana Venneri. I would not have been able to begin construction of the experimental system without guidance from the staff and graduate students at the Smithsonian Environmental Research Center's Room of DOOM, particularly Becca Burrell and Andrew Keppel. I am grateful for Amanda Reynolds' patience with late nights and login failures on the titrator; she made it possible for my experimental system to run at its best.

The unwavering support of my family throughout my education has helped me to achieve the milestone of becoming a Ph.D. My husband, Avi, has been loving, patient, and kind through this project and I could not have done it without him. My parents and Charles and Megan have always believed in me and told me that I could do anything that I put my mind to; their encouragement has helped me every step of the way in achieving this final educational goal. The Glandons have been exceptionally supportive during my writing process and this document would not have been completed without their help.

Finally, this project was funded by the Chesapeake Biological Lab Graduate Education Committee, the University of Maryland Marine, Estuarine, and Environmental Science Reid E. Menzer Dissertation Research Fellowship, and the Maryland Sea Grant Coastal Resilience and Sustainability Fellowship.

Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction

Anthropogenic combustion of fossil fuels since the Industrial Revolution has caused an increase in the concentration of carbon dioxide (CO_2) in the atmosphere (Doney and Schimel, 2007). The Intergovernmental Panel on Climate Change (IPCC) predicts that atmospheric levels of CO_2 will continue to increase from 380 µatm currently to over 1,000 µatm by the end of the 21st century (Caldeira and Wickett, 2003; Orr et al., 2005; Stocker et al., 2013). Due to the greenhouse effect, this increase in CO_2 has caused an increase in atmospheric and, consequently, oceanic temperatures. Recent IPCC model predictions suggest that ocean temperatures will warm by 2.6-4.8°C by the year 2100 (Stocker et al., 2013). However, not all of the released CO_2 has remained in the atmosphere; the oceans have absorbed about 30% of this excess atmospheric CO_2 (Feely et al., 2009) resulting in an increase in dissolved CO_2 (pCO_2) in the ocean. The absorption of CO_2 by the ocean changes the equilibrium of the carbonate system, causing a decrease in oceanic pH due to an increased presence of acidic species (HCO_3^- and hydrogen ions) and a decreased presence of basic species (carbonate, CO_3^{2-}). As a result, oceanic surface pH has declined an average of 0.1 pH units (termed ocean acidification, OA) from preindustrial times and is predicted to decrease by another 0.3 pH units by 2100 (Caldeira and Wickett, 2003; Orr et al., 2005; Stocker et al., 2013).

Quantifying the effects of the changes in oceanic conditions described above on marine life is vital to understanding the future of the ocean and its function. Many processes critical to sustaining life in the ocean are dependent on specific ranges of temperature and pH (Pörtner, 2001; Whiteley, 2011) and the relatively rapid departure

from these ranges that is occurring in today's oceans will induce changes in the way many marine animals survive and reproduce. The impacts of changes in ocean temperature and pH on marine animals have been the focus of numerous studies, with special attention paid to species that utilize calcium carbonate in creating their carapace. My dissertation adds to this body of knowledge by quantifying the organismal and population level effects of warming and acidification on juvenile blue crab, *Callinectes sapidus,* in the Chesapeake Bay. I quantified the effect of changes in water temperature and pCO_2 on crab growth, oxygen consumption rates, and carapace properties and also used these data to predict the potential impacts of climate change on crab overwintering behavior.

The carbonate system and climate change

The carbonate system is defined by four analytical parameters: the total dissolved inorganic carbonate (C_T), total hydrogen ion concentration (pH), the total alkalinity (TA), and the concentration of CO_2 gas (pCO_2).

The C_T is the sum of all carbon species in a system and represented by the following equation:

$$C_T = [CO_2] + [HCO_3^-] + [CO_3^{2-}]$$

where [ ] indicates concentration.

The pH is representative of the level of hydrogen ions in a given system and is described by the following equation:

$$pH = -\log[H^+]$$

Since pH is described on a log scale, a 0.1 unit change in pH represents a 30% change in $[H^+]$. The pH of natural waters ranges from 6-9, with oceanic pH approximately 8.1. As more anthropogenic carbon dioxide is released into the atmosphere, $[H^+]$ in the ocean increases (see below), which causes the pH to decline, making the water more acidic.

The TA of a system is a representation of buffering capacity and is positively correlated with salinity in natural systems. TA is described by the following equation:

$$TA = [HCO_3^-] + 2*[CO_3^{2-}] + [OH^-] - [H^+]$$

Since TA represents buffering capacity, a complete definition includes all proton acceptors (bases formed from weak acids) and proton donors (weak acids). In natural systems, TA includes also borate, phosphate, silicate, and nitrate species in addition to the carbonate species listed above. TA can be measured in a variety of ways, most commonly (and simply) through titration of a seawater sample with a strong acid to prescribed inflection points (Dickson et al., 2007). Spectrophotometric determination of TA is a more accurate, while also more expensive and complex, method of quantifying the TA of a sample (Yao and Byrne, 1998).

The oceanic carbonate systems is an open system, and therefore the pCO_2 of the water is thought to closely track that of the atmosphere. While certain processes (e.g.: respiration and photosynthesis) may locally alter the pCO_2, most biological systems are at an average value of 380 µatm (Stocker et al., 2013) under current climate conditions. The concentration of CO_2 species in a given solution is represented by the following equilibria:

$$CO_{2(aq)} + H_2O_{(l)} \overset{\mathbf{1}}{\leftrightarrow} H_2CO_{3(aq)} \overset{\mathbf{2}}{\leftrightarrow} H^+_{(aq)} + HCO^-_{3(aq)} \overset{\mathbf{3}}{\leftrightarrow} 2H^+_{(aq)} + CO^{2-}_{3(aq)}$$

Since it is not possible to analytically distinguish $CO_{2(aq)}$ from $H_2CO_{3(aq)}$, the combination of the two is considered to represent pCO_2. At current (2017) ocean pH (about 8.1), approximately 85% of the carbon species present exist in the form of bicarbonate (HCO_3^-), with the remainder being mostly carbonate (CO_3^{2-}). Increases in atmospheric CO_2, and hence oceanic CO_2, push equilibria 1 and 2 in the equation above to the right, creating more free hydrogen ions in solution. The two free hydrogen ions on the right side of equilibrium 3 favor the movement of that equilibrium to the left, thereby creating even higher concentrations of bicarbonate and reducing the amount of carbonate available to form calcium carbonate.

These changes to the carbonate system equilibria have the potential to affect marine species in the variety of ways. Increasing atmospheric (and therefore surface ocean) pCO_2 will favor the formation of bicarbonate and will increase the amount of hydrogen released by the carbonate system. However, due to differences in the relative amounts of bicarbonate (high) and hydrogen (low) in the ocean, the relative increase in bicarbonate is very low compared to the relative decrease in pH that is resultant from an increase in atmospheric pCO_2. This large relative change in pH may impact ion regulation and acid-base balance in species as the environment acidifies. The decreased availability of carbonate may change the saturation state of calcium carbonate in acidified marine systems, increasing the corrosivity of the water and making the environment less favorable for the formation of calcium carbonate structures, such as marine invertebrate exoskeletons (Feely et al., 2004; Gazeau et al., 2007; Fabry, 2008). These changes, driven by the warming and acidification of the marine environment, have the potential to drastically change the ocean seascape.

Species response to climate change

The effects of a warming marine environment have been studied extensively, largely because the rates of many physiological processes in invertebrates and fishes are strongly temperature-dependent (Kinne, 1964; Pörtner, 2002). For example, temperature has been observed to directly influence the somatic growth rate of crustaceans by shortening the time between molts (Brylawski and Miller, 2006; Glandon and Miller, 2017). Importantly, research has shown that a failure to adapt to warming temperatures can have dramatic effects on the sustainability of valuable exploited species such as Atlantic cod, *Gadus morhua* (Pershing et al., 2015). Studies quantifying the impact of ocean acidification on marine animals are widespread. Work has ranged from exposure to highly acidic conditions for short periods of time (Bibby et al., 2007; Spicer et al., 2007; Checkley et al., 2009; Miller et al., 2009) to exposure to moderate, and potentially more realistic, pH levels for longer periods of time (Landes and Zimmer, 2012; Long et al., 2013b; Swiney et al., 2015; Long et al., 2016a). A theme throughout these studies is the inconsistency of the response of animals to declines in pH. A variety of factors may explain these patterns of variation: (1) the existence and type of calcium carbonate structures, (2) metabolic rate, and (3) phenotypic plasticity in response to acidification. Although acidification alone has been the focus of many studies, the interactive effects of temperature and acidification are less well studied. However, such interactive effects are important to quantify when considering the overall response of communities to climate change due to the potential for non-linear effects between environmental stressors (Pörtner et al., 2005; Kroeker et al., 2013; Todgham and Stillman, 2013).

The change in saturation state of calcium carbonate that occurs concurrently with ocean acidification (OA) has been observed to adversely affect shell deposition in marine animals. Shell dissolution under acidified conditions occurs due to the decline in calcium carbonate saturation state and the underlying mechanism for this dissolution is well understood (Feely et al., 2010; Findlay et al., 2011). However, increasing temperature causes the solubility of calcium carbonate to decrease, potentially counteracting the effect of acidification on the solubility of calcium carbonate species. The species-specific nature of the calcification process, combined with the effects of pCO_2 and temperature on the saturation state of calcium carbonate described above, induce considerable variability in the observed response of marine calcifying species to climate change stressors. Shell dissolution under acidic conditions has been observed in many taxa, from tropical corals (Kleypas et al., 1999; Hoegh-Guldberg et al., 2007; Anthony et al., 2008) to plankton (Riebesell et al., 2000; Iglesias-Rodriguez et al., 2008) and to bivalves including pteropods (Orr et al., 2005; Lischka et al., 2010) and oysters (Gazeau et al., 2007; Waldbusser et al., 2011b). However, increased calcification rates have been observed in certain species exposed to acidified conditions including crab (Ries et al., 2009; Long et al., 2013a), shrimp (Ries et al., 2009) and barnacles (McDonald et al., 2009). Currently the exact mechanism for the increase in calcification observed in these species is unknown but possible explanations might include the existence of a protective lipid layer on the carapace to prevent dissolution and tradeoffs between calcification rate and other physiological processes in certain species exposed to increased pCO_2. However, the lack of data on the effects of climate change stressors on the carapace structure and chemistry of species with complex carapaces, such as crustaceans, makes it difficult to identify the

effects of warmer, more acidic conditions on the mechanism of calcification in these species.

Another factor that climate change may affect is metabolic rate of poikilotherms. Metabolic rate is often tied to the thermal conditions of a species' habitat as temperature is a controlling factor in many organismal-level processes in cold-blooded marine animals (Pörtner, 2002). Levels of respiratory pigments, such as hemoglobin and hemocyanin, are thought to control thermal tolerance limits through oxygen limitation of cellular processes at temperature extremes (Pörtner, 2002). Species in warm water are able to maintain high metabolic rates while cold water species often have slower metabolisms. For example, polar crustaceans have been observed to have 2.5 to 7.5 fold lower respiratory pigment levels than mid-latitude crustaceans, and differences in respiratory pigment levels have been tied to metabolism in these species (Taylor et al., 1991; Pörtner et al., 2007). These cold water species may have reduced buffering capacity in comparison to their warm water counterparts and may therefore be more susceptible to the negative effects of climate change.

Phenotypic plasticity may provide animals resistance to potential climate stressors and has been observed in many marine invertebrates (Hadfield and Strathmann, 1996; Davis, 2000). In these cases, the expression of certain traits is dependent on the environmental context in which the animals develop. Species that live in naturally variable habitats may have a greater capacity for phenotypic plasticity than those that live in more stable environments due to the variable conditions under which they develop and to which they are adapted. In the context of climate change, species that live in habitats with a narrow range of temperature and pH may be more susceptible to environmental

changes than species living in more variable environments. For example, changes in thermal cues for gametogenesis, spawning, and the presence of larvae have been observed as a response to ocean warming in marine plankton (Hays et al., 2005) and gastropods (Moore et al., 2011). Similar phenotypic responses may exist in animals that live in variable pH environments, but the lack of data on this topic makes drawing overall conclusions regarding the phenotypic response to climate change difficult (Richardson and Poloczanska, 2008).

Climate change in estuaries

Much of the existing knowledge of the chemistry and biology of climate change presented above is based upon open ocean systems. For example, the IPCC predictions described above are based upon temperature and CO_2 data collected at the Earth System Research Laboratory located over 3,000 meters above sea level at Mauna Loa, Hawaii (Doney and Schimel, 2007). Acidification in the open ocean is primarily driven by increasing atmospheric concentrations of CO_2, a concept known as "Ocean Acidification v 1.0" (MdOA, 2015), where small changes in pH occur relatively slowly (but more rapidly than have occurred in several million years in the ocean) and have a large impact on the system. However, the open ocean system is not characteristic of all oceanic habitats. In "Ocean Acidification v 2.0", also known as coastal acidification, the influence of physical oceanographic factors, such as upwelling of CO_2-rich deep ocean water, are incorporated into prediction scenarios (MdOA, 2015). While the overall change in pH is slow in this scenario, abrupt but temporary changes in pH can occur due to movement of individual water masses. Unlike the impacts of "Ocean Acidification v

1.0", certain effects of "Ocean Acidification v 2.0" can be mitigated if natural or commercial processes can be halted during periods when the location is affected by upwelling.

However, neither of these scenarios applies to near-coastal or estuarine systems such as the Chesapeake Bay. In these systems, a combination of low natural alkalinity, eutrophication from wastewater, storm water, and agricultural runoff, and high natural productivity and respiration, causes a combination of hypoxia and acidification. Najjar et al. (2009; 2010) examined potential impacts of climate change on mid-Atlantic estuaries, including the Chesapeake Bay. Likely changes in the future include increased flooding and submergence of wetlands, increased variability in salinity, harmful algae, and hypoxia, and a reduction in the amount of submerged aquatic vegetation. The magnitude of the effects of climate change on estuaries is related to the CO_2 emission trajectory and therefore actions taken now to reduce emissions may reduce impacts on estuarine systems. This scenario, termed "Ocean Acidification v 3.0" (also known as estuarine acidification), is characterized by substantial, rapid, and cyclic changes in pH (MdOA, 2015).

In contrast to the relatively stable water chemistry of the oceanic carbonate system, the estuarine carbonate system is quite variable (Cai and Wang, 1998; Waldbusser and Salisbury, 2014). Future climate scenarios may make the environment even more variable (Miller et al., 2009) through an increasing atmospheric CO_2 baseline. The absorption of CO_2 gas into estuarine waters is only one of multiple processes that impact pH in estuaries (Blackford and Gilbert, 2007; Soetaert et al., 2007; Miller et al., 2009). Freshwater influx can alter alkalinity and therefore carbonate species

concentration (Howland et al., 2000; Swarzenski et al., 2008) while plankton photosynthesis/respiration cycles can cause strong diel cycling of pH (Sampou and Kemp, 1994). Increased rain events caused by climate change could impact freshwater influx into estuaries (Najjar et al., 2010) and increased temperatures can increase phytoplankton respiration and photosynthesis (Richardson and Schoeman, 2004; Hays et al., 2005). Considering the important differences between the drivers of climate in the estuary and the open ocean, estuarine-specific studies are necessary to understand the impact of climate change on this critical environment.

Estuaries form a connection between the terrestrial and marine environment and provide critical habitat for many species. The estuarine habitat is influenced by stressors from both the marine (tides, waves, salt) and riverine (freshwater and nutrient inputs) environment. Estuaries are often very productive environments and are consequently nursery habitat for the early life stages of many species. The Chesapeake Bay is the largest estuary on the east coast of North America and is home to many ecologically and commercially important species. Effects of climate change have already been observed in both water temperature and pH in the Chesapeake Bay (Najjar et al., 2010). For example, the climate regime of the Chesapeake Bay is projected to represent present day South Carolina by the year 2100 (Boesch et al., 2008). The effect of increased atmospheric pCO_2 has also been observed in the Chesapeake Bay (Miller et al., 2009; Waldbusser et al., 2013), although the time series on these data is much shorter than the available temperature data. As compared to open ocean systems, the Chesapeake Bay may be especially vulnerable to OA due to lower buffering capacity associated with low salinity (Cai and Wang, 1998; Miller et al., 2009) and high riverine nutrient input (Howland et

al., 2000; Swarzenski et al., 2008). The complexity of the estuarine system combined with the lack of studies quantifying climate change impacts in estuaries was motivation for my study on the effects of climate change on juvenile blue crab in the Chesapeake Bay.

Blue crab population demography

The blue crab, *Callinectes sapidus*, is a decapod crustacean found along the coast of the western Atlantic, from Massachusetts to Uruguay (Williams, 1984). The *Callinectes* genus originated in the shallow coastal waters of the neotropical Atlantic but has radiated to a wide geographical range over time (Williams, 2007). Despite the large range of environmental conditions which contain populations of adult crabs, larvae of blue crab are stenohaline and stenothermal, following the characteristics of their ancestors (Costlow and Bookhout, 1965; Kalber, 1970). After 7-8 molts in the coastal ocean, blue crab larvae ingress into the closest estuary, which is determined by wind and circulation conditions during the marine larval development period (Epifanio, 2007). The timing of this ingress is dependent upon the breeding population of interest; females in low latitudes spawn year-round, mid-latitude females spawn in both the fall and spring, and females from northern populations may only spawn once per year in the late spring/early summer (Hines, 2007). Larvae develop into megalopae, then juveniles, and finally reach adulthood in the estuarine environment, where mating occurs at the terminal female molt (Hines, 2007; Jivoff et al., 2007; Lipcius et al., 2007). Stage duration and growth rates are temperature (and therefore latitude) dependent in this species such that individuals in the southern extent of the range may reach sexual maturity in one year

while individuals from the northern extent can take up to three years to reach maturity (Williams, 1984; Fogarty and Lipcius, 2007).

The blue crab is a commercially and ecologically important species in the Chesapeake Bay (Hines et al., 1990; Hines, 2007; Miller et al., 2011). Blue crab diets contain many taxa including other crustaceans, bivalves, and fish and varies with crab size (Hines et al., 1990; Mansour and Lipcius, 1991). Smaller crabs eat small invertebrates and thin-shelled bivalves while larger individuals eat thick-shelled bivalves and are known to be cannibalistic (Dittel et al., 1995; Hines and Ruiz, 1995). Blue crab are opportunistic, aggressive, benthic predators and their broad diet makes them an integral part of the benthic food web in the Chesapeake Bay (Eggleston et al., 1992; Hines, 2007). Blue crab have been fished commercially in the Chesapeake Bay since the turn of the 19th century (Kennedy et al., 2007) and currently support important commercial and recreational fisheries in the region (Kennedy et al., 2007; Miller et al., 2011).

Blue crab growth and molting

Blue crab display a discontinuous growth pattern (Smith and Chang, 2007) achieved through the shedding of the external carapace, a process termed molting. The protective external carapace of the blue crab is composed of four layers which lay on top of a permanent hypodermis layer (Figure 1.1). The outermost epicuticle is composed of spherical "islands" of calcium carbonate (in the form of calcite) surrounded by a lipid-protein matrix (Roer and Dillaman, 1984). The second layer inward is the exocuticle, composed of calcite crystals interspersed within untanned chitin-protein fibers that are

organized into layers called lamellae (Roer and Dillaman, 1984). The third layer is the endocuticle, also composed of calcite crystals but interspersed within tanned chitin-protein fibers organized into lamellae (Roer and Dillaman, 1984). The innermost layer is the membranous layer, composed of chitin and protein but no calcite (Roer and Dillaman, 1984). These four layers are connected to the hypodermis by numerous pore canals, which are cytoplasmic extensions of the outer epithelial cells and allow for the movement of the organic and mineral components of the carapace to be absorbed and deposited as the molt cycle progresses (Roer and Dillaman, 1984).

The molt cycle in blue crab consists of four stages: pre-molt (D_0 – D_4), ecdysis (E), post-molt (A, B, C_1 – C_3), and inter-molt (C_4). The onset of pre-molt (stage D_0) occurs when the pore canals are severed and the connection between the hypodermis and the old cuticle is interrupted (Green and Neff, 1972). Pre-molt stages D_1 to D_4 are characterized by the dissolution of the old cuticle and the deposition of the new epi- and exocuticles. The dissolution of the mineral part of the cuticle is achieved by lowering the pH of the molting space through the addition of H^+, thereby driving the dissolution of calcite into calcium and bicarbonate ions. After this dissolution, calcium and bicarbonate ions are actively pumped out of the molting space by the Ca/ATPase pump and carbonic anhydrase (CA), respectively (Roer, 1980). The end of the pre-molt stage is characterized by an increase in ventilation to increase haemolymph [O_2], which leads to an increase in haemolymph pH which buffers the acidic byproducts of the post-molt process (Mangum, 1992). In addition, increased Na/K/ATPase activity in the gills creates an osmotic water influx, thereby increasing hydrostatic pressure in the body cavity leading to ecdysis (Neufeld and Cameron, 1994).

Exuviation of the old exoskeleton defines molt stage E (ecdysis). Exuviation is achieved by the rapid pumping of water that began during late post-molt, increasing the hydrostatic pressure in the body cavity to levels that split the old carapace and allow the animal to emerge (Neufeld and Cameron, 1994). The process of exuviation is rapid, and in many individuals it occurs in only a few minutes time (Cameron, 1985). After ecdysis the crab is considered a "soft shell", indicating that the carapace has not yet hardened and movement is limited due to lack of firm attachment sites for muscle and gill structures (Smith and Chang, 2007). Early post-molt (stages A and B) is characterized by rapid uptake of water; two-thirds achieved by drinking and the remaining one-third by osmotic uptake in the gills (Neufeld and Cameron, 1994). This uptake of water results in an increase in blood pressure to five times inter-molt levels, forcing the new cuticle to become taut and conform to the crab's new, larger dimensions that will exist until the next molt event (Mangum, 1992). Active ventilation through the gills is at 50% capacity during early post-molt, but oxygen can diffuse directly through the soft cuticle at this time, compensating for some of the reduced ventilation capacity (Smith and Chang, 2007). Haemolymph pH declines during early post-molt due to anaerobic respiration and the formation of calcium carbonate for the new exoskeleton, both of which have acidic byproducts. The increase in haemolymph alkalinity during late pre-molt counteracts this acidosis and allows the crab to survive ecdysis and early post-molt (Smith and Chang, 2007).

Late post-molt is characterized by deposition of the new endocuticle and mineralization of the new cuticle. Mineralization of the new cuticle occurs after water uptake and exoskeleton expansion are complete, likely because elongation of the cuticle

is more rapid without calcium carbonate structures (Roer and Dillaman, 1993). Mineralization occurs from the outermost layer (exocuticle) inwards and involves the transport of both calcium and bicarbonate into the crab from the external environment. Calcium enters the crab passively across the gills and actively from the hypodermis to the cuticle through calcium/ATPase pumps (Roer and Dillaman, 1993). Bicarbonate transport is mediated by the enzyme carbonic anhydrase (CA), with peak gill CA activity observed during mid to late post-molt. This is contrasted with peak cuticular CA activity which is observed during the pre-molt stage (Henry and Kormanik, 1985). Secretion of the membranous layer of the new cuticle during molt stage C_3 marks the end of the post-molt stage (Roer and Dillaman, 1984).

The molting process is costly for blue crab both energetically and in terms of predation vulnerability. Many of the processes described above involve active pumping of ions, requiring energy in the form of ATP and others rely on the presence of an ionic gradient to facilitate diffusion. Another energetic cost of molting is the lack of feeding that occurs during late pre-molt through early post-molt, due to lack of calcification in newly molted mouthparts (Smith and Chang, 2007). Blue crab are vulnerable to predators during early post-molt due to the absence of a fully calcified cuticle. This limits crab movement (and feeding) because the musculature necessary for movement does not have a hard anchor on which to pull until the cuticle is fully calcified (Smith and Chang, 2007). The warmer, more acidic conditions of the future may make molting more energetically costly and less efficient for crabs. Understanding how crab molting and growth will be affected by climate change is a central question in my dissertation research.

Blue crab regulatory capacity

Environmental variability plays a major role in determining species diversity and spatial distribution (Sanders, 1968; Heerebout, 1970; De Jonge, 1974); highly variable environments often contain low numbers of species in a small area due to the relationship between environmental variability and osmotic and thermal stress (Kinne, 1964; Parry, 1966). The range of environmental conditions where blue crab thrive underscore the impressive regulatory capacity of this species. Although the molting process (summarized above) puts the individual under enormous physical stress, it represents an ephemeral increase in the cost of living for blue crab. The permanent existence of blue crab in a wide range of environmental conditions is a testament to the regulatory capacity of this species, which is primarily achieved through the gills and carapace (Smith and Chang, 2007; Towle and Burnett, 2007). The outermost layer of the blue crab carapace is coated with a lipid-protein matrix, forming an impermeable layer which protects the individual from the external environment (Roer and Dillaman, 1984). The protective ability of the carapace is therefore twofold in blue crab; to provide the animal with protection against predation through a hard shell and to create a barrier to fluctuations in external conditions.

The complex structure of the blue crab gill aids in the ability of ion transport systems to regulate the conditions inside of the crab. Blue crab have eight pairs of gills with approximately eight lamella per mm of gill length (Aldridge and Cameron, 1982; Taylor and Taylor, 1992). The lamella contain a single layer of epithelial cells which create a simple interface between the external environment and the inside of the crab and

aid in gas exchange, ion transport, acid-base regulation, and nitrogen excretion (Towle and Burnett, 2007). Na/K/ATPase exchanger is primarily responsible for gill ion transport in blue crab through increased activity when the environmental conditions (specifically salinity) change (Towle et al., 1976; Neufeld et al., 1980). The increase in Na/K/ATPase activity has been linked to an increase in oxygen consumption in crabs acclimated to low salinity (Towle et al., 1976). Transfer to low salinity also increases hemolymph ammonia, which is excreted through the substitution of K^+ for NH_4^+ in the Na/K/ATPase enzyme (Towle and Holleland, 1987).

Gas uptake and excretion in the blue crab also occur in the lamella of the gills. Deoxygenated hemolymph accumulates in the body of the individual and the hemolymph hemocyanin (the oxygen-carrying protein in blue crab) becomes fully saturated with oxygen as it passes through the gills (Mangum et al., 1985; Towle and Burnett, 2007). Carbon dioxide is excreted through the gills with the assistance of the enzyme carbonic anhydrase, which accelerates the interconversion of CO_2 and carbonic acid (Henry, 1996). Carbonic anhydrase has also been linked to acid-base balance in blue crab, such that metabolic alkalosis is mediated through mechanisms in the gills for translocating ions (Henry and Cameron, 1983). The ability of blue crab to regulate internal conditions in a wide range of environments may allow this species to acclimate, and even adapt, to changing environmental conditions. My dissertation research questions focus on whole-animal responses to climate change, but the mechanisms described above form the basis of the physiological response to climate change that my research aims to quantify.

Blue crab population response to climate change

Although understanding the response of individuals to environmental stressors such as climate change is a critical step in predicting the fate of species in the future, modeling population level responses is another important aspect of climate change research. Global climate models (GCM) are complex simulations of weather patterns and physical factors that allow researchers to predict future climate conditions. GCMs predict many aspects of climate including temperature, precipitation, and severe storm frequency. Despite the power of GCMs, they are most useful on a global scale, which is too broad for many research questions. Downscaling of GCMs to local scales is a way to understand how GCM predictions may play out in specific environments (Taylor et al., 2012; Maloney et al., 2014). Downscaled models use predictions from a single GCM to inform a model that has more accurate physical forcing and weather data at a smaller geographic scale than the GCMs. It is important to note that the downscaled information can be no more reliable than the climate model simulation that underlies it and therefore downscaled GCM results should be taken with caution. However, these downscaled model predictions are the most accurate local scale predictions currently available. Utilizing local environmental dynamics in concert with downscaled GCM projections may improve the reliability of predictions from downscaled models (Cho and Lee, 2011). Long-term, high resolution environmental data can aid in understanding the climate dynamics of local systems and can improve downscaled GCM predictions.

The Chesapeake Biological Laboratory (CBL) has been recording daily air and water temperature since 1938. This high-resolution, long term dataset is ideal to characterize the local climate in the Chesapeake Bay to aid GCM future temperature

predictions. Accurately characterizing the relationship between air and water temperature in the local climate can allow researchers to use GCM air temperature projections to predict future water temperature in their region. Future water temperature can then be used to predict population level effects of expected changes in temperature on marine species. For example, blue crab in the Chesapeake Bay overwinter when temperatures fall below 9°C (Smith and Chang, 2007). Understanding the temperature dynamics of the Chesapeake Bay in the future can help to predict changes in blue crab behavior, specifically the length of the overwintering period. Changes to this period may have widespread effects including an increase in the length of the growing season, increased predation by blue crab, and the potential for an increase in the length of the crab fishing season. The critical role of blue crab in the Chesapeake Bay food web underscores the importance of determining population level effects of climate change on this species.

Dissertation research summary

My dissertation evaluated the effect of increased temperature and pCO_2 on juvenile blue crab, *Callinectes sapidus*, in the Patuxent River, Chesapeake Bay. I conducted four studies to quantify the response of juvenile blue crab to climate change.

In Chapter 2, I determined the effect of increased temperature and pCO_2 on the growth and food consumption of juvenile blue crab. I designed a flow-through system in the Cronin Laboratory at the Chesapeake Biological Laboratory (CBL) that allowed for the control of the temperature and pH of experimental water. Temperature was controlled through the addition of heated seawater provided in the Cronin Laboratory. pH was controlled through a "pH-stat" system, similar to a thermostat. pH was lowered by the

controlled addition of CO_2 gas, moderated by a controller to pre-determined set points. Once gas flow stopped, the continual flow of water into the experimental tanks increased the pH thereby initiating the flow of CO_2 gas once again. 160 individual crabs were held for two complete molts (approximately 30 days) in one of four temperature/pCO_2 treatment combinations. Crabs were fed daily and growth and food consumption were determined for each individual. I tested the hypotheses that increased temperature would have a positive effect on crab growth and food consumption while increased pCO_2 would have a negative effect on those responses. I also hypothesized that there would be a significant antagonistic interaction of temperature and pCO_2 on crab growth and food consumption, such that individuals exposed to high temperature/high pCO_2 would display the lowest growth and food consumption of all treatment combinations. The data from this study indicated that increased temperature significantly increased the growth rate and food consumption of crabs while increased pCO_2 had no significant effect on either response. This work is published in the ICES Journal of Marine Science (Glandon and Miller, 2017).

In Chapter 3, I quantified changes in the thickness and chemistry of the carapaces of juvenile blue crab exposed to increased temperature and pCO_2. In this study, the carapaces of individuals from the previous study were analyzed for thickness and chemistry. Carapace thickness was determined by light microscopy of thin sections. The concentration of calcium and magnesium in individual carapaces was determined through Inductively Coupled Plasma – Atomic Emission Spectrometry (ICP-AES), and these concentrations were used to quantify the amount of high-magnesium calcite (HMC) present in the carapace. I tested the hypothesis that increased temperature would cause

carapaces to be thinner and lower the content of HMC while increased pCO_2 would not affect carapace thickness but would increase the HMC content of carapaces. The data from this study indicated a significant effect of temperature on the carapaces of crabs; carapaces of crabs exposed to high temperature were significantly thinner and contained significantly less weight percent HMC than the carapaces of crabs exposed to ambient temperature. Increased pCO_2 significantly increased the weight percent HMC and the concentration of magnesium in the carapace. This work has been accepted for publication to the Journal of Experimental Marine Biology and Ecology with Drs. Hali Kilbourne, Johan Schijf, and Thomas Miller as co-authors.

In Chapter 4, I determined how future predicted increases in temperature and pCO_2 may affect the oxygen consumption rate of juvenile blue crab. In this study, individuals were exposed to one of four treatments of combinations similar to the treatment levels in Chapter 2. After the crabs had completed two molts in the acclimation system, the rate of oxygen consumption was determined after a 24 hour starvation period. Individuals were placed in a flow-through respiration chamber overnight for at least 10 hours and the concentration of oxygen in the inflow and outflow water as well as the flow rate of water was determined every second. The water in the chamber during each trial was the same temperature and pCO_2 as the acclimation water for each crab. Based on the known impacts of temperature and pCO_2 on other marine species combined with the expected outcomes of the growth study, I hypothesized that increased temperature would increase the oxygen consumption rate of crabs and increased pCO_2 would also increase the oxygen consumption rate of crabs due to increased rates of ion pumping in a more acidic environment. I expected there would be an additive interactive effect of

temperature and pCO_2 on oxygen consumption rate, such that individuals exposed to increased temperature and pCO_2 would have the highest oxygen consumption rate of any treatment combination. Although the temperatures tested in this study did not elicit a significant difference in mean oxygen consumption rate, Q_{10} values indicated that oxygen consumption rates did predictably rise with temperature. High individual variability and a relatively small difference in temperature treatments are likely the cause for the lack of a statistically significant difference between mean oxygen consumption rates by temperature. Additionally, there was no significant effect of increased pCO_2 on the oxygen consumption rate of crabs in this study. This work is currently being prepared for submission to the Journal of Shellfish Research with Drs. Chris Rowe, Kennedy Paynter and Thomas Miller as co-authors.

In Chapter 5, I characterized the air-water temperature relationship in the observational temperature data and used this relationship to predict future water temperature in the Chesapeake Bay using projected air temperature from downscaled global climate models (GCM). These predictions were then used to determine changes in the length of the overwintering period of blue crab in the Chesapeake Bay, using 9°C as the temperature below which overwintering would occur (Smith, 1997). Specifically, I tested the hypothesis that downscaled GCM trends would predict a more significant decline in the overwintering period than the decline in the overwintering period predicted by the extension of the current trend in the observed water temperature. The length of the overwintering period predicted from the extension of the current temperature trend as well as downscaled GCM data were both significantly shorter than the length of the current blue crab overwintering period in the Chesapeake Bay. Additionally, the length of

the overwintering period predicted by the downscaled GCM data was significantly shorter than the length of the overwintering period predicted by the extension of the current temperature trend. These data were then used to discuss possible population level impacts from a decline in the length of the overwintering period (and therefore an increase in the length of the growing season) in the Chesapeake Bay in the future. This work is currently being compiled as a manuscript for future publication.

My dissertation research indicates that juvenile blue crab are resilient to the potential negative effects of climate change. Crab growth, food consumption, and carapace properties responded predictably to increases in temperature. The growth, food consumption, carapace thickness, and metabolism of crabs were not significantly affected by exposure to extremely high levels of pCO_2. A source of this resilience is likely the highly variable estuarine environment, where large swings in both temperature and pH are the norm. My research also determined that blue crab overwintering behavior will be inconsistent in the future, warmer climate of the Chesapeake Bay will process. However, blue crab are just one component of the complex food web of the Chesapeake Bay. Although not the focus of my dissertation, the impact of other species' responses to climate change on blue crab survival, growth, and reproduction will ultimately determine the fate of this species in the future.

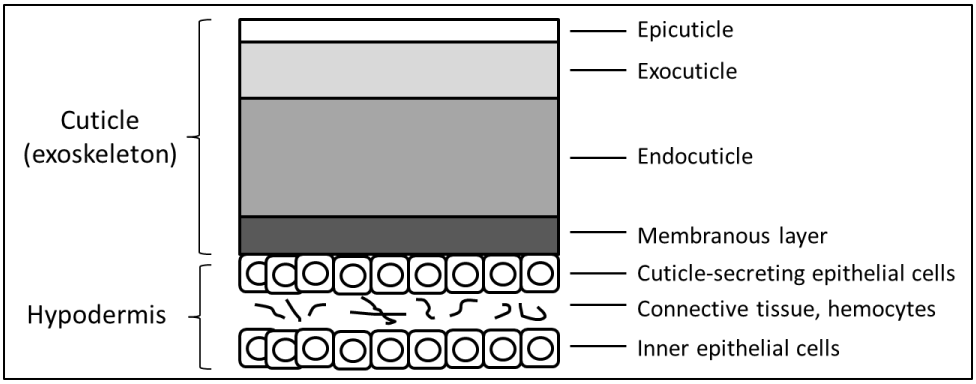

Figure 1.1. Diagram of the blue crab carapace during C4 (inter-molt) phase. From Smith and Chang (2007).

Chapter 2: No effect of high pCO_2 on juvenile blue crab, *Callinectes sapidus*, growth and consumption despite positive responses to concurrent warming.

Abstract

Future climate scenarios predict increases in both ocean temperature and dissolved carbon dioxide (pCO_2) over the next century. Calcifying invertebrates, which depend on specific conditions of temperature and carbonate chemistry for many processes, may be especially affected by these changes. In our study, juvenile blue crab, *Callinectes sapidus*, were exposed to one of four temperature/pCO_2 treatments (ambient/low, ambient/high, high/low, high/high) for two complete molts. Our study is the first to examine the effect of multiple climate stressors on blue crab and therefore basic responses, including the growth per molt (GPM), inter-molt period (IMP), and food consumption, were quantified. GPM was not affected by either increased temperature or pCO_2. Although increased pCO_2 did not significantly influence the duration of crab IMP, crabs in warm water had significantly shorter IMP (10.6±3.1days, (±SD)) than crabs in ambient water (12.5±2.8days). Increased pCO_2 did not significantly affect the amount of food consumed, but crabs in warm water ate significantly more food than those in ambient water. These data suggest that the impact of warming outweighs the impact of acidification in juvenile blue crab. The effects of these changes on more complex physiological parameters such as metabolism and shell chemistry remain to be examined. Additionally, quantifying the changes to the Chesapeake Bay food web that may occur due to the observed increase

in crab growth and consumption is important to ensure sustainability of this resource in the face of future climatic changes.

Introduction

Recent projections from the Intergovernmental Panel on Climate Change (IPCC) indicate that by the year 2100 mean global ocean temperature is likely to warm by 2.6-4.8°C and that mean global surface ocean pH is likely to decrease by up to 0.3 units (Stocker et al., 2013). The effects of a warming marine environment have been studied extensively, largely because the rates of many physiological processes in invertebrates and fishes are strongly temperature dependent (Kinne, 1964; Pörtner, 2002) and this work has become even more relevant in the face of a warming ocean. For example, temperature has been observed to directly influence the somatic growth rate of crustaceans by shortening the time between molts (Brylawski and Miller, 2006). Importantly, research has shown that a failure to adapt to warming temperatures can have dramatic effects on the sustainability of exploited species (Pershing et al., 2015). Ocean acidification (OA) has the potential to have drastic system-wide impacts on marine life (Hendriks et al., 2010; Doney et al., 2012; Kroeker et al., 2013; Stocker, 2015) and a range of responses to acidified conditions have been observed in many marine species, including decapod crustaceans (Pane and Barry, 2007; Ries et al., 2009; Long et al., 2013a; Dodd et al., 2015)

The documented response of crustaceans to declines in pH/increases in pCO_2 have been variable, likely related to life stage and the variety of processes, from ion regulation to calcification, which can be impacted by changes in water chemistry.

Negative responses to acidification have been observed in the development time of larval stages of the spider crab, *Hyas araneus,* (Walther et al., 2010) and the development and growth of juvenile and adult Tanner crab, *Chionoecetes bairdi*, and red king crab, *Paralithodes camtschaticus* (Long et al., 2013a; Swiney et al., 2015). However Carter et al. (2013) observed no effect of acidification on juvenile porcelain crab metabolism and energetics and in an examination of the response of 18 benthic species to acidification, Ries et al. (2009) observed an increase in the calcification rate of adult blue crab, *Callinectes sapidus,* as measured by changes in buoyant weight before and after treatment. The effect of acidification on blue crab found by Ries et al. (2009) suggests that the crab in our study may also be effected by acidification, although calcification rates were not measured in this study and we focused on the juvenile life stage of blue crab, rather than adults.

While acidification may impact crustaceans in a variety of ways, a primary factor in the response of these animals to acidification is likely related to the complex processes involved in molting and growth in these animals. Crustacean exoskeletons are physiologically active (Roer and Dillaman, 1993; Dillaman et al., 2005) and have a structure that provides optimal mechanical strength while still allowing for movement flexibility (Roer and Dillaman, 1984; Dillaman et al., 2005; Raabe et al., 2005; Boßelmann et al., 2007; Chen et al., 2008). Crustaceans display discontinuous growth through molting, a process that involves dramatic increases in active ion pumping in order to generate osmotic water influx prior to molting (Mangum, 1992) as well as the active pumping of calcium and bicarbonate ions after molting in order to form the calcite necessary for crustacean shell strength (Roer, 1980; Roer and

Dillaman, 1993; Dillaman et al., 2005). Therefore, changes in the balance of the carbonate system that accompanies ocean acidification may alter the energetic requirements for crustacean molting and calcification. This energetic cost could be manifested in a variety of ways, including changes in growth and/or consumption.

Understanding the impacts of increased temperature and pCO_2 in estuarine ecosystems is important because of the role of estuaries as productive nursery areas and because estuarine systems do not respond to changes in these parameters in the same manner as the open ocean. In estuarine systems, a combination of tidal influx and efflux, low natural salinity and therefore lowered alkalinity, eutrophication from wastewater, storm water and agricultural runoff, and high natural productivity and respiration cause strong, rapid cycling of pH, leading to episodic acidification of estuarine waters (Cai and Wang, 1998; Miller et al., 2009; Doney et al., 2012; Duarte et al., 2013; Waldbusser and Salisbury, 2014). The interaction between temperature and pH in estuarine ecosystems underscores the importance of studying the combined effects of warming and acidification on these ecosystems, especially because multiple stressors may interact to produce either antagonistic or synergistic effects (Boyd and Hutchins, 2012; Kroeker et al., 2013; Todgham and Stillman, 2013). The Chesapeake Bay is the largest estuary in the continental United States and provides nursery and other habitats for many species (Wagner and Austin, 1999; Harding and Mann, 2001; Orth et al., 2006; Coen et al., 2007; Wingate and Secor, 2008). Although not a focus of the current study, the increasing relevance of the Chesapeake Bay hypoxic zone has motivated multiple-stressor studies in this region (Aumann et al., 2006; Tanner et al., 2006; Keppel et al., 2015) and is another important factor in understanding the

susceptibility of estuarine species to future climate scenarios. The integral role that the blue crab plays in the Chesapeake Bay food web (Baird and Ulanowicz, 1989; Eggleston et al., 1992; Hines, 2007; Kennedy et al., 2007; Miller et al., 2011), combined with the dearth of existing knowledge on the impact of climate change on both estuarine and crustacean species, make it an ideal species for examining the effects of climate change in this system.

Effects of climate change have already been observed for both water temperature and pH in the Chesapeake Bay. For example, the water temperature in the Patuxent River near Solomons, Maryland has increased approximately 1.5°C since 1940 (Najjar et al., 2010) and the climate regime of the Chesapeake Bay could be similar to that in present day South Carolina by the year 2100 (Boesch et al., 2008). The effect of increased atmospheric pCO_2 has also been observed in the Chesapeake Bay (Miller et al., 2009; Waldbusser et al., 2011b; Waldbusser et al., 2013), although the time span of these data is much shorter than that available for temperature. Our work is relevant to projected future conditions in the Chesapeake Bay ecosystem specifically, and to understanding the impact of climate change on estuaries generally.

The primary goal of our study was to determine the independent and interactive effects of acidification and warming on the growth and consumption of juvenile *Callinectes sapidus* from the Patuxent River, Chesapeake Bay. We chose to examine juveniles because previous work has indicated that this life stage is important in regulating overall crab population dynamics (Miller, 2001) and juveniles are the first complete life stage of blue crab to live entirely in the estuary. Crab were

exposed to projected future levels of warming and acidification in the estuarine environment in order to quantify impacts of climate change in a more realistic, multi-stressor environment, using temperature and pCO_2 targets determined specifically for the estuarine system. Since our study is the first to examine the combined effects of warming and acidification on blue crab, we aimed to quantify the effects of these stressors on two basic parameters of crab physiology, growth and consumption. The two-fold quantification of growth coupled with consumption allowed us to understand the impact of both acidification and warming on blue crab physiology, creating a foundation for future work on the effect of multiple climate stressors on estuarine species.

Methods

Carbonate system parameters

In order to determine pCO_2 treatment targets for this study, data from the Chesapeake Bay Program Water Quality Database (CBPWQD) were used to determine the maximum pCO_2 values experienced in the Patuxent River from 2008-2012 (MdDNR, 2017). pCO_2 was calculated using pH, salinity, and temperature measured at three sites in the Patuxent River represented in the CBPWQD and the total alkalinity (TA) measured in the experimental system during pilot experiments. The mean pCO_2 was 3,266.7 ± 818.9 µatm (mean ± standard deviation) during this time period. There was a five-fold difference in the observed range of pCO_2 values; the average minimum pCO_2 was 1,237.9 ± 775.0 µatm and the average maximum pCO_2 was 6,363.8 ± 1,610.5 µatm among all years and months sampled. Using these

data, 800 µatm was identified as the low treatment level and 8,000 µatm was identified as the high pCO_2 treatment level for our experiment.

All pH electrodes were calibrated weekly to a pH of 3 in a 0.25M NaCl solution (approximately equal to the molarity of the experimental system) assuming Nernstian behavior of the electrodes, which had previously been verified. Since the salinity was stable during the course of the experiment (12.42±.75), no correction for changes in salinity was employed during this study. Total alkalinity (TA; µmol/kg-sw) was determined weekly in each experimental tank and three randomly selected sample tanks per header tank through two-point Gran titration using 0.1 M hydrochloric acid (Edmond, 1970). Accuracy comparisons were performed using certified reference materials (Dickson et al., 2003). Water samples collected for alkalinity were filtered through 0.45 µm filters and stored in sterilized Pyrex glass bottles (Dickson et al., 2007). pCO_2 levels were calculated in each experimental and sample tank using the measured pH and total alkalinity data, in addition to recorded temperature and salinity, in the software CO2SYS (Lewis and Wallace, 1998). The CO_2 constants used (K_1, K_2) were from Mojica Prieto and Millero (2002), the protonation constant of bisulfate (HSO_4^-) was taken from Dickson (1990), and the total borate concentration was calculated from salinity (chlorinity) as per Uppstrom (1974).

Experimental setup

A controlled laboratory experiment was conducted at the Chesapeake Biological Laboratory (CBL) using flowing Patuxent River water in order to evaluate

the effect of increased temperature and pCO_2 on juvenile blue crab growth and consumption. A blocked, 2x2 factorial design was employed with pCO_2 and water temperature as the factors. Figure 2.1 outlines the experimental system employed for this study, which was conducted during the summer of 2015 over the course of two experimental blocks. Filtered Patuxent River water (sand filtered to approximately 5µm) flowed into two 100 gallon pre-treatment tanks. Water temperature in each pre-treatment tank was maintained at either 26°C or 32°C using a combination of heated and chilled water. Water in the pre-treatment tanks was vigorously bubbled with compressed air in order to reduce the influence of microbial respiration and photosynthesis on the CO_2 concentration in the water and maintain the pCO_2 at 800 µatm in the pre-treatment tanks. Water from the pre-treatment tanks then flowed continuously into eight 35-gallon experimental tanks (labeled A-D in Figure 2.1) at a rate adjusted to maintain the desired temperature in each experimental tank. Manipulation of pCO_2 also took place in each experimental tank. The assignment of experimental tanks to both temperature and pCO_2 treatment levels was random. Experimental tanks were covered with plastic lids to limit the exchange of gases between the water and the surrounding air. Each experimental tank contained a glass combination pH electrode that monitored and recorded the pH of the water once per second. pH in the experimental system was controlled through a "pH-stat" system, whereby pH was lowered by a controller to pre-determined set points through the controlled addition of CO_2 gas. Once gas flow stopped, the continual flow of water into the experimental tanks increased the pH thereby reinitiating the flow of CO_2 again. Manipulation of pCO_2 occurred in two experimental tanks per pre-treatment

tank (four experimental tanks total) to achieve the acidified treatment pCO_2 target of 8,000 µatm (Tanks B and D in Figure 2.1) while the low treatment pCO_2 remained at 800 µatm (Tanks A and C in Figure 2.1). This resulted in a full factorial design with two levels of replication within each experimental block.

Treated water from each experimental tank flowed continuously and gravimetrically to eleven 2.5-gallon sample tanks. Ten of these sample tanks contained an individual crab; the last tank (Food Consumption Tanks in Figure 2.1, outlined in textured grey) was used to estimate the food recovery efficiency for our consumption measurements (see below for methods). Water from individual sample tanks never interacted with water from other sample tanks; sample tank water flowed into the drain directly from the tank it entered. Crab were kept in individual tanks in order to minimalize cannibalism and accurately determine inter-molt period. Sample tanks were also covered with plastic lids in order to limit the exchange of gases between the water in the tanks and the surrounding air. pH was determined once daily in each sample tank using an Orion Ross double junction refillable glass electrode. Temperature (°C) and salinity were recorded once daily in the pre-treatment and experimental tanks using a YSI Pro-plus meter.

Crab collection and measurements

Juvenile blue crab (30-35 mm carapace width) were collected at night from the CBL pier, located at the mouth of the Patuxent River, Maryland, by catching tidally migrating animals. Crabs were transferred to holding tanks containing filtered Patuxent River water in order to acclimate to laboratory conditions and food (frozen

bay scallops) prior to experimentation. After the acclimation period, initial carapace width (mm) was determined using vernier calipers and the mass (mg) determined for each crab. Individual crabs were then randomly assigned to sample tanks and monitored until they had completed two molts.

Crab growth per molt (GPM) was determined after each molt event as the increase in crab carapace width (mm) and was later calculated as percent change in growth. The carapace width of newly molted individuals was determined after three days, to allow the crab to fully harden. Inter-molt period (IMP) was determined as the number of days between observed molting events.

Crab were provided with pre-weighed food (frozen bay scallop) each morning and food was left in the sample tank for a period of 24 hours. Crab were provided with new food immediately after the old food was removed, to ensure food was always available to each crab. Food consumption was determined for each crab after feeding events by collecting the food remaining in the sample tanks once daily. Consumption was determined as the difference in wet mass (mg) between the initial weight of the food and the weight of the food after it had been in the tank for 24 hours. The amount of food consumed was normalized for crab mass by determining the percent of crab mass consumed by each crab each day. Food recovery efficiency was accounted for each day by determining the difference in wet mass (mg) between the initial weight of food and the weight of food after it had been in a tank without a crab for 24 hours. The correction for food recovery efficiency was incorporated into the consumption data for each temperature/pCO_2 replicate, using the appropriate daily correction factor for those sample tanks.

Statistical analyses

Data are presented as mean ±SD by temperature/pCO_2 treatment. All analyses were conducted in R (version 3.2.2 -- R Core Team, 2015) using R-Studio (version 0.98.1103). An α level of $P < 0.05$ was used for all analyses. Experimental data were analyzed as a full factorial design, with two levels of both temperature and pCO_2. Analysis of Variance (ANOVA) using type II sum of squares was used to test for the effects of temperature, pCO_2, and their interaction on each response variable. To assure the data met the assumptions of ANOVA, data were visually assessed for normality and then analyzed using the Shapiro-Wilk normality test. Equality of variance was assessed by examining plots of residuals by predicted values for any patterns. In order to accurately represent the experimental design, temperature and pCO_2 were treated as fixed effects, and block and experimental tank were treated as random effects. Response variables included growth per molt (GPM) after molt 1 (mm), GPM after molt 2 (mm), inter-molt period (IMP; days), and consumption (% of crab mass). Significance of fixed effects and their interactions was assessed using the *Anova* function on linear, linear-mixed effects models using the *car* (Fox and Weisberg, 2010) and *nlme* packages (Pinheiro et al., 2015). When the analysis indicated no significant interaction between the effects of temperature and pCO_2, the model was rerun without the interaction term in order to improve the power of tests of main effect.

Results

Carbonate system parameters

Table 2.1 presents the principal parameters of the carbonate system for each temperature/pCO_2 treatment combination. Much of the variability present in the pCO_2 concentrations was due to the high cycling present in estuarine ecosystems and in our experimental control system. The experimental control system maintained clear differences among all temperature and pCO_2 treatments throughout the experiment, while maintaining consistent TA among all treatments (Figure 2.2; Table 2.1). Total alkalinity titrations were accurate to 0.20% as compared to certified reference materials (Dickson et al., 2003).

Crab growth

The crabs experienced no mortality during the course of the experiment; all 160 crabs molted at least once and survived for the duration of the experiment. The average growth per molt (GPM) for crabs in our experiment was 7.3 ± 2.0mm (22.4 ± 6.1%) after the first molt and 8.9 ± 2.2mm (22.6 ± 5.4%) after the second molt. A two-way ANOVA indicated no significant temperature/pCO_2 interaction on growth per molt for either the first ($F=0.855$, $P=0.375$) or second ($F= 0.081$, $P=0.782$) molt, and therefore main effects were considered. There was no significant effect of temperature or pCO_2 on GPM after the first (Temperature: $F=0.336$, $P=0.574$; pCO_2: $F=0.294$, $P=0.599$) or second (Temperature: $F=0.828$, $P=0.382$; pCO_2: $F=0.442$, $P=0.520$) molt.

The duration of crab inter-molt period (IMP) was affected by increased temperature, but increased pCO_2 had no significant effect on the length of crab IMP (Figure 2.3). A two-way ANOVA indicated no significant temperature/pCO_2 interaction (F=2.379, *P*=0.151), and therefore main effects were considered. Although increased pCO_2 did not significantly affect the duration of crab IMP (F=0.547, *P*=0.475), temperature did have a significant effect on the duration of crab IMP (F=8.200, *P*=0.002). The IMP of crab in the warmer treatment was almost 2 days (8.5%) shorter than the IMP of crab in ambient treatment (IMP=10.6±3.1 and 12.5±2.8 days for warm and ambient temperatures, respectively).

Crab consumption

Similar to the IMP data, the amount of food crab consumed was affected by increased temperature, but increased pCO_2 had no significant effect (Figure 2.4). A two-way ANOVA indicated no significant temperature/pCO_2 interaction (F=2.838, *P*=0.120), and therefore main effects were considered. Although increased pCO_2 did not significantly affect the amount of food crab consumed (F=0.251, *P*=0.626), temperature was found to be a significant driver of the amount of food eaten (F=9.342, *P*=0.007). Crabs in warm water ate almost 4% more food (as a percentage of crab body mass) than crabs in ambient water (Consumption=24.3±8.0% and 20.4±5.5% for crab in warm and ambient temperatures, respectively).

Discussion

Our study shows that juvenile blue crab may be able to mitigate some of the potential negative effects of increased temperature and pCO_2. Our data indicate that when juvenile blue crab are exposed to increases in both temperature and pCO_2, the effect of temperature is present in the absence of any effect of pCO_2. The effect of temperature we observed was characteristic of ectothermic animals, in as much as increased temperature led to a significant increase in crab growth rate, brought about by a decrease in the inter-molt period. Furthermore, our results show that the observed increase in growth rate caused a significant increase in the consumption of food. Additionally, the crabs experienced no mortality throughout the course of the experiment, further underscoring their robustness in the face of increased temperature and pCO_2. Together, these results highlight the tolerance of juvenile blue crab to acidification and indicate that crab growth and consumption will respond predictably to projected future increases in temperature, regardless of projected changes in pCO_2.

Although the response of blue crab to increases in temperature is well characterized (Brylawski and Miller, 2006), the effect of the combined stress of increased temperature and pCO_2 had not previously been examined in this species. Therefore, our study was designed to push individuals to their limits to determine if a response to acidification existed, while still exposing the animals to realistic, future levels of both acidification and warming. Our data analysis methods were specifically designed to account for pseudoreplication as identified by Hurlbert (1984) and more recently by Cornwall and Hurd (2015). In order to accommodate the large range of pCO_2 levels currently present in the Chesapeake Bay ecosystem, our high pCO_2

treatment level (8,000 µatm) was ten times higher than our low treatment level (800 µatm). Considering the severity of the high pCO_2 treatment level combined with the large difference between pCO_2 treatments, we believe that our experiment was adequate to measure the effect of increased pCO_2, if such an effect existed on juvenile crab growth and/or consumption. In this light, the absence of a significant effect of pCO_2 treatment on juvenile crab growth or consumption in our results indicates a lack of response of blue crab to this stressor, rather than an inadequate experimental design.

The absence of a response to increased pCO_2 of juvenile blue crab in this study may be related to the ability of estuarine species to tolerate a high level of environmental variability. Much of the existing research on OA effects on decapod crustaceans has been conducted in the marine environment (Walther et al., 2009; Carter et al., 2013; Long et al., 2013a; 2013b; Swiney et al., 2015; Long et al., 2016a), which maintains a pCO_2 that is much more stable than levels found in the estuarine environment. However, little work has been done to quantify the response of estuarine crustaceans to multiple climate stressors (but see Walther et al., 2010), despite the ecological and economic importance of these species as well as mounting evidence of non-linear effects in multiple stressor studies (Boyd and Hutchins, 2012; Kroeker et al., 2013; Todgham and Stillman, 2013). The estuarine environment of the juvenile blue crab is highly variable for many environmental parameters (temperature, salinity, dissolved oxygen, and pH at scales of hours, days and months) and animals that live in this environment must be capable of survival and growth under such variability. Although the highly variable responses observed in this study

make it difficult to determine the significance of effects, the plasticity of response of blue crab in our study suggests that individuals have the capacity to thrive in a variety of conditions. This plasticity may be advantageous to this population in the face of future climate changes and further studies on the impact of climate change on estuarine crustaceans may help to clarify the role of environmental variability in apparent resistance to climate change in this phylum.

Crustaceans initially form their new carapace inside of the old one, utilizing their impressive ability to precisely control internal ion concentrations to create an environment well-suited to shell formation under a variety of external conditions (Wheatly, 1985; Smith and Chang, 2007). Due to the increase in hydrogen ion concentration concurrent with the acidification of seawater, animals in acidic environments must counteract a less favorable proton gradient when converting bicarbonate to carbonate in order to form their shells, increasing the energetic cost of shell creation and maintenance in acidic environments. However, blue crab molt and thrive in a variety of external conditions and experience large variability in many environmental parameters, including temperature, salinity, pH, and dissolved oxygen. Estuarine crustaceans, such as the blue crab, have the favorable combination of highly efficient ion regulation to counteract large swings in external conditions in addition to the formation of the new carapace inside of the protection of the old one. These mechanisms may have contributed to the ability of the crabs in our study to maintain growth rates in the face of increased pCO_2, but do not address the large impact of temperature on the growth of crabs, which was significant despite crabs being exposed to high variability in temperature in their natural environment.

Although the data from our study indicate that juvenile blue crab in highly acidified conditions can maintain levels of growth and consumption equal to that of crab in control conditions, there may be other negative effects of acidification that were not detected in this study. For example, the effect of acidification may be detectable through changes in metabolic rate and/or influences on internal pH of crab hemolymph, as crabs in acidic water may need to shift metabolic energy in order to compensate for the changes in water chemistry concurrent with acidification. This has been observed in allocation of metabolic energy in larval sea urchin, *Strongylocentrotus purpuratus*, exposed to acidification, paired with the absence of effects on more global parameters such as growth (Pan et al., 2015). Additionally, Meseck et al. (2016) observed a tradeoff between maintaining defense mechanisms and energy towards reproduction in Tanner crab, *Chionecetes tanneri*, exposed to acidified conditions for two years, despite the absence of an impact on overall hemolymph pH. Similarly, Pane and Barry (2007) observed a negative effect of short term hypercapnia-induced acidosis on acid-base regulatory capacity. Quantification of these physiological parameters, such as metabolic rate, will provide a more complete picture of the impact of acidification on juvenile blue crab.

The impact of environmental stressors may also depend on when during the molt cycle response variables are quantified. Crab are in intimate contact with their external environment during the molt event. In addition to increased interaction with external conditions due to the lack of a fully calcified cuticle during molting, crabs also vigorously pump water into their new, larger body cavity during the molt event (Roer, 1980; Henry and Kormanik, 1985). Therefore, changes in the ion

concentration of seawater associated with acidification may affect the ability of crabs to maintain acid-base balance disproportionately during the molt event, rather than during the inter-molt period once pumping rates have slowed. Quantification of the metabolic rate of molting crab as well as crab that are in inter-molt may lead to a greater understanding of the influence of molt stage on the apparent resistance of juvenile blue crab to acidification. We suggest experiments to quantify the metabolic response throughout the molt cycle of blue crab to OA are of a high priority.

We observed a high level of individual variability with regards to both growth rate (inter-molt period) and food consumption in this study and the magnitude of this variation likely has important biological consequences. For example, an especially large range was observed in the inter-molt period (IMP) data, where the minimum and maximum duration of IMP for individual crab within a single treatment differed by 11-12 days. A change in inter-molt period this large would have profound effects on individual crabs, considering that the average inter-molt period of crabs in this study was 11.6 days. Although the identification of the source of this variation was outside the scope of this study, a combination of the genotypic and phenotypic variation inherent in the traits of many estuarine species may be at play here. Differences in growth rate of this magnitude, scaled to the crab life cycle, could have a significant influence on the growth and maturation of blue crab in the Chesapeake Bay, and are ideal for examination through individual-based modeling techniques.

Blue crab plays an important role in the food web of the Chesapeake Bay, serving as prey for an array of species in addition to aggressively consuming many small benthic species (Eggleston et al., 1992; Hines, 2007). Various prey items of

blue crab, including benthic bivalve species, have displayed slowed growth and/or calcification rates in the face of acidification (Michaelidis et al., 2005; Berge et al., 2006; Waldbusser et al., 2011a; 2011b; Parker et al., 2012), making these prey more vulnerable to predation. Our results indicate that crabs will be larger as water temperatures increase and these larger crabs will need to consume more food in order to sustain higher growth rates. Therefore, in the warmer, more acidic conditions of the future, larger crabs with increased energetic demands may be targeting more vulnerable prey. However, the extension of our laboratory results to crabs in the field should be applied with caution, as the results from studies on the impact of acidification on predator-prey interactions in crustaceans are mixed. Additionally, this potential increase in food consumption may come at a cost to crab survival as crabs venture out of protected areas more frequently to feed (Lipcius et al., 2003; Lipcius et al., 2007). Another tradeoff of increased growth in warmer environments could be that while crabs will reach a size refuge from predation faster in warmer water, the increased molting frequency at which this would be achieved will place crabs in the more vulnerable state of preparing for, completing, and recovering from the molt event more frequently than if the crabs grew at a slower rate. An approach to understanding the influence of these factors on an ecosystem level are large scale modelling efforts that can capture the dynamic nature of species interactions in a changing environment. We believe that incorporating the data from this study into a larger ecosystem model would be a useful extension of this work.

Our data show no effect of acidification on the growth and consumption in juvenile blue crab, *Callinectes sapidus*, and a predictable response to warming

conditions. These results suggest that juvenile blue crab may not be as susceptible to the potential negative consequences of climate change observed in other species. A recent theme issue in the ICES Journal of Marine Science titled "Towards a Broader Perspective on Ocean Acidification Research" acknowledged the bias in the published literature towards studies showing statistically significant effects of climate change as well as the paucity of multi-stressor climate studies (Browman, 2016), although many studies in that volume addressed those issues (e.g., Campanati et al., 2015; Schram et al., 2015; Vicente et al., 2015; Zhang et al., 2015). As the first to examine the effect of multiple climate stressors on blue crab, our study focused on large-scale, whole-animal response variables in order to gain information on the impact of climate change on blue crab. Although no large-scale effects of acidification were observed in our study, this does not mean that crabs are not influenced in some way by the dramatic changes in water chemistry that will occur in projected future climate scenarios. Understanding the role that climate-induced changes in the blue crab population may have in the complex ecosystem dynamics of the Chesapeake Bay ecosystem is vital to our ability to protect and manage this ecologically and economically important environment in the future.

Table 2.1. Mean and standard deviation of carbonate system parameters for the duration of the experiment, by block and temperature/pCO_2 treatment. Temperature and salinity were measured daily, pH was measured once per second, and TA (total alkalinity) was measured weekly.

			Temperature (°C)		Salinity (ppt)		pH (Total Scale)		TA (µmol/kg-sw)		*p*CO2 (µatm)	
Block	**Temp**	***p*CO2**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
1	Amb	Amb	26.0	1.0	12.8	0.3	7.69	0.11	1,420.3	41.5	855.1	22.7
		High	26.0	1.0	12.8	0.3	6.70	0.10	1,431.7	42.1	8,749.3	172.7
	High	Amb	31.7	0.9	12.7	0.3	7.69	0.11	1,417.7	42.2	843.4	22.8
		High	31.6	0.9	12.7	0.3	6.72	0.05	1,430.8	42.7	8,172.5	98.3
2	Amb	Amb	26.4	0.4	11.8	0.7	7.71	0.12	1,407.6	28.8	807.6	24.4
		High	26.4	0.5	11.8	0.7	6.70	0.08	1,407.4	20.5	8,605.7	142.6
	High	Amb	31.7	0.3	11.8	0.7	7.69	0.14	1,411.4	30.6	871.1	63.6
		High	31.6	0.2	11.8	0.7	6.70	0.06	1,411.2	17.5	8,566.5	150.4

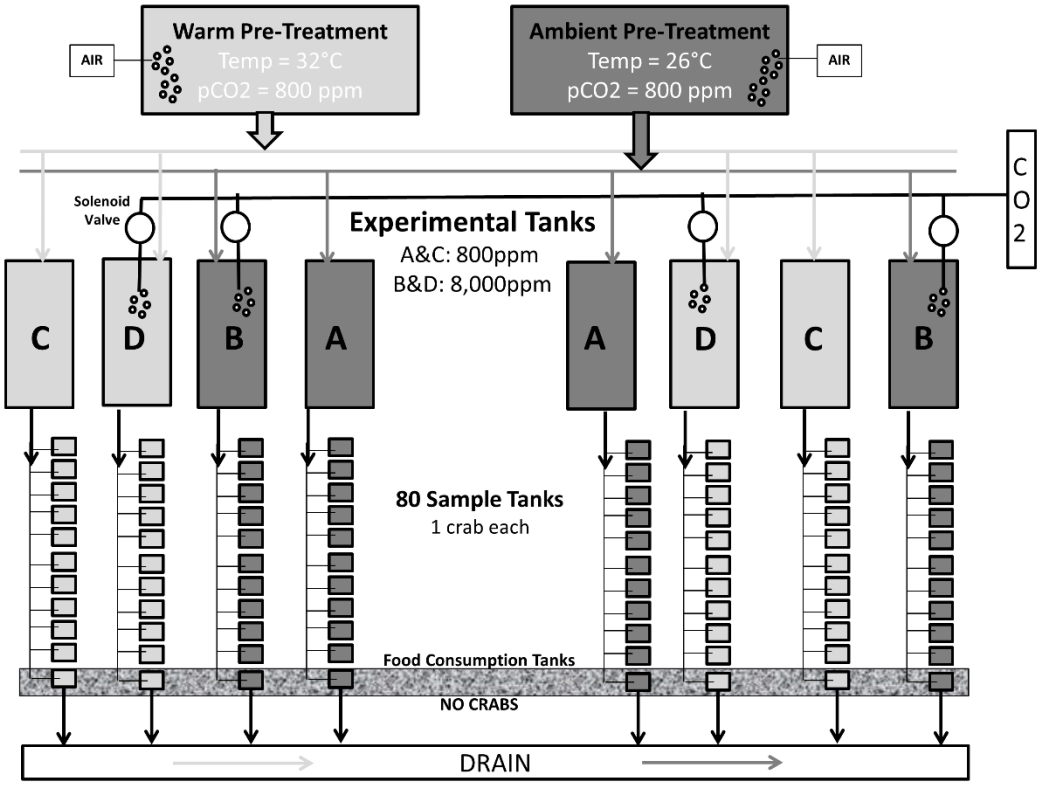

Figure 2.1. Schematic of experimental system used in this study, which was run over two blocks ensuring 4 independent replicates of each temperature/pCO_2 treatment combination. Pre-treatment tanks (100 gallons) were filled with Patuxent River water and maintained at either 26°C or 32°C using a combination of heated and chilled water and vigorously bubbled with compressed air. Water from the pre-treatment tanks then flowed continuously into eight 35-gallon experimental tanks (tanks A-D) where manipulation of pCO_2 took place. Each experimental tank contained a glass combination pH electrode that monitored and recorded the pH of the water once per second. Manipulation of pCO_2 occurred in tanks B and D, acidifying the water in those tanks to 8,000 µatm while the pCO_2 in tanks A and C remained at 800 µatm. Treated water flowed continuously from each experimental tank gravimetrically to

eleven 2.5-gallon sample tanks. Ten of these sample tanks contained an individual crab and the last tank in each line (outlined in textured grey) was used to estimate the food recovery efficiency for our consumption measurements. Water from individual sample tanks never interacted with water from other sample tanks; sample tank water flowed into the drain directly from the tank it entered.

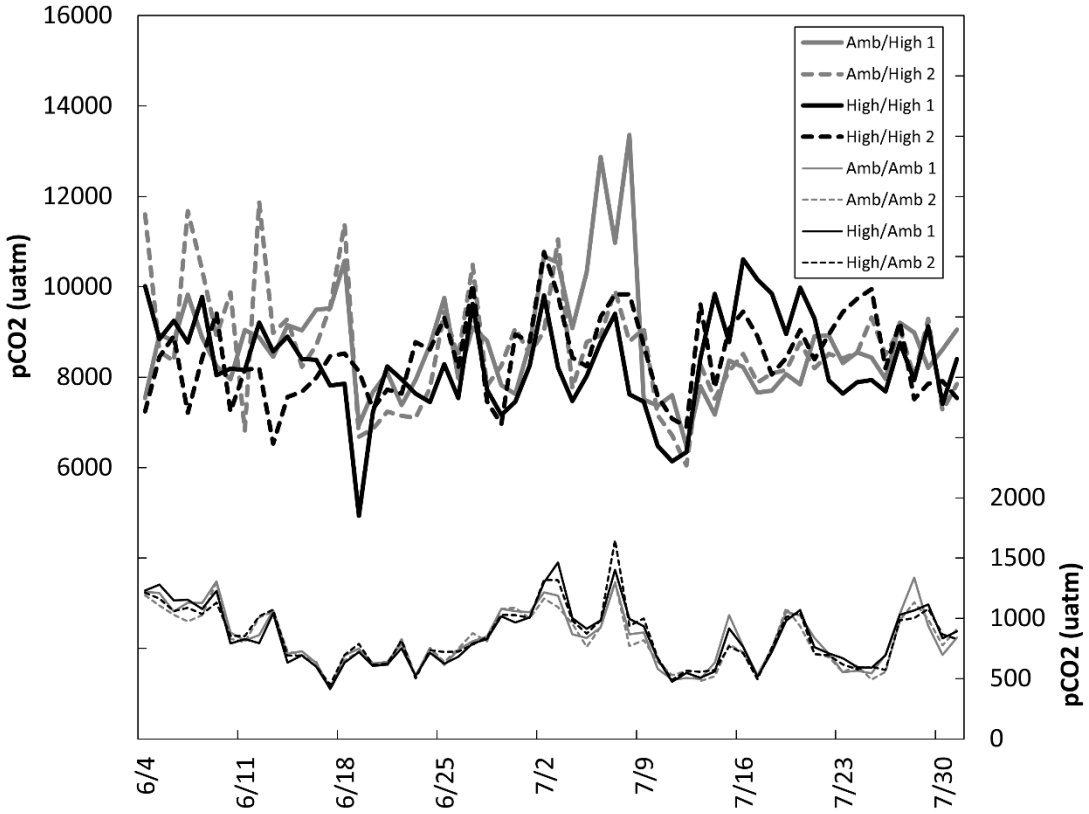

Figure 2.2. Mean pCO_2 in experimental tanks by temperature/pCO_2/replicate for the duration of the experiment. Line thickness distinguishes between pCO_2 treatments (thick=high, thin=ambient). Dashed lines are ambient temperature and solid lines are high temperature.

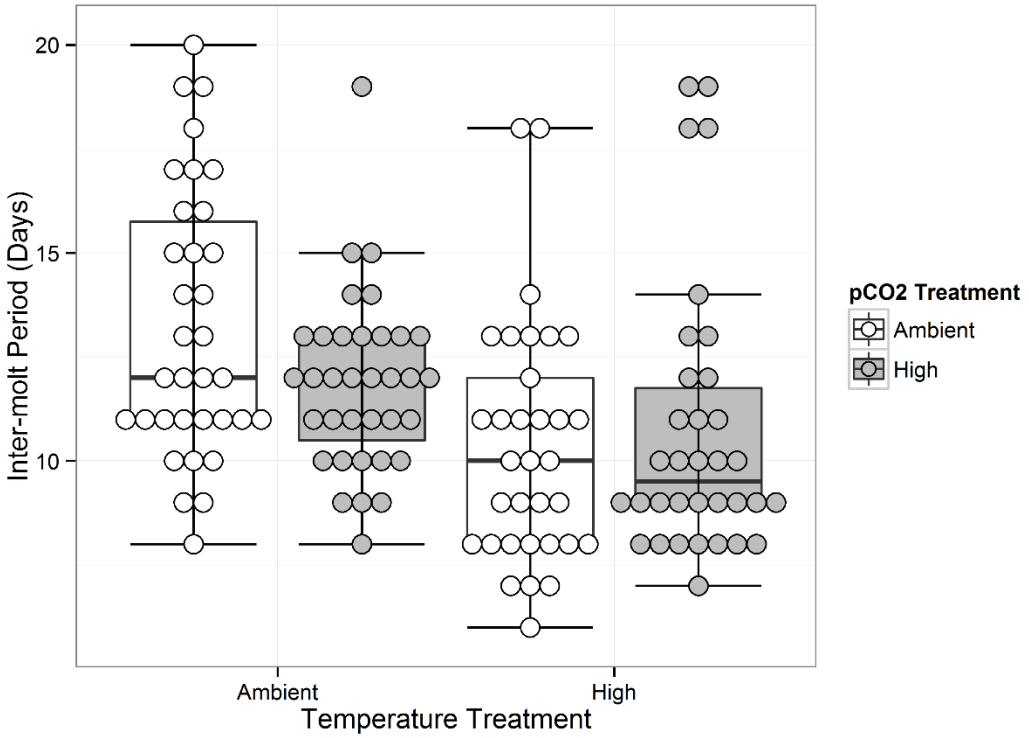

Figure 2.3. Inter-molt period (days) of blue crab by temperature/pCO_2 treatment. Dots represent individual observations of inter-molt period. Solid dark line represents the median of each treatment combination, boxes represent inter-quartile range, whiskers represent the sum of 1st (lower whisker) or 3rd(upper whisker) plus 1.5 times the inter-quartile range. Crabs in high temperature water had significantly shorter inter-molt periods (P=0.002) than crabs in ambient temperature water, regardless of pCO_2 treatment.

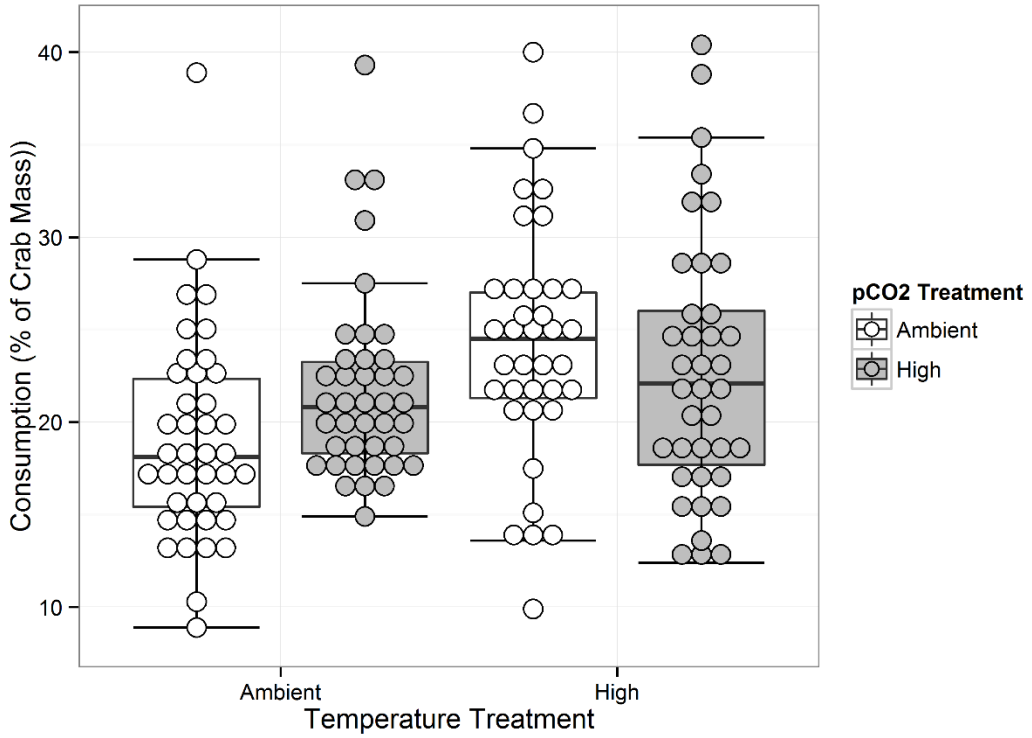

Figure 2.4. Consumption (% of crab mass) of blue crab by temperature/pCO_2 treatment. Dots represent the mean consumption of individual crabs over the course of the experiment. Solid dark line represents the median of each treatment combination, boxes represent inter-quartile range, whiskers represent the sum of 1st (lower whisker) or 3rd (upper whisker) plus 1.5 times the inter-quartile range. Crabs in high temperature water ate significantly more food (P=0.007) than crabs in ambient temperature water, regardless of pCO_2 treatment.

Chapter 3: Counteractive effects of increased temperature and pCO_2 on the thickness and chemistry of the carapace of juvenile blue crab, *Callinectes sapidus,* from the Patuxent River, Chesapeake Bay.

Abstract

Exoskeletons are central to the physiology and survival of marine invertebrates, but future acidification and warming of the marine environment may affect invertebrate's exoskeletons through changes in the carbonate system and the processes of shell formation. Thus it is important to consider the impacts of a changing climate on the functionality of the exoskeleton in these species. In this study, juvenile blue crab, *Callinectes sapidus,* from the Chesapeake Bay were exposed to increased temperature and pCO_2 in a 2x2 factorial design for a period of two molts (approximately 30 days). Treatment levels were chosen to represent current (26°C and 800 µatm CO_2) and predicted future conditions in the year 2100 (32°C and 8,000 µatm CO_2) in the Chesapeake Bay. Thickness was determined by light microscopy and carapace calcium (Ca) magnesium (Mg) content were determined by Inductively Coupled Plasma – Atomic Emission Spectrometry. All Ca and Mg in the carapace were assumed to be present in the form of high-magnesium calcite (HMC, presented at weight percent). The carapaces of juvenile blue crab exposed to increased temperature were significantly thinner and had significantly less weight percent HMC than the carapaces of crabs exposed to ambient temperatures. Increased pCO_2 significantly increased the weight percent HMC but a significant increase in magnesium content was also observed. The observed counteractive effects of

temperature and pCO_2 on weight percent HMC underscores the importance of determining the effects of multiple stressors in studies that quantify the impacts of environmental stressors. Combined with the knowledge of the influence of temperature and pCO_2 on crab growth, the results from this study indicate tradeoffs between carapace thickness and chemistry with growth in juvenile crab exposed to future warming, and that juvenile crab carapaces may be stronger under more acidic conditions.

Introduction

Understanding the response of species to a changing climate is critical to determining the dynamics of the marine environment in the future. Recent IPCC scenarios predict that ocean pH will decrease by 0.3 units and ocean surface temperatures will increase by 2.6-4.8°C by the year 2100 (Caldeira and Wickett, 2003; Orr et al., 2005; Stocker et al., 2013). These changes in the marine environment may especially impact marine invertebrates with exoskeletons that contain calcium carbonate. The exoskeleton is critical for protection from predators (Boßelmann et al., 2007), control of the chemistry of the internal environment (Roer and Dillaman, 1984), resistance to mechanical loads (Fabritius et al., 2012), attachment for musculature to facilitate swimming and gill movement in mobile marine invertebrates, and attachment to substrate for sedentary invertebrates (Vincent, 2002; Smith and Chang, 2007). Both acidification and warming of the marine environment may affect the invertebrate exoskeleton through changes in the availability of specific carbonate species for incorporation into the exoskeleton and

impacts on the strength of ion gradients necessary for shell formation. Considering the role that the exoskeleton plays in the survival of marine invertebrates, it is important to consider the impacts of a changing climate on the functionality of the exoskeleton in these species.

Changes in the chemistry of the marine environment due to acidification and warming may be manifested in a variety of ways. One of the consequences of acidification is a decline in carbonate ion concentration. As the water acidifies, the saturation state of calcium carbonate will decline, moving towards a state in which dissolution of calcium carbonate is favored, creating a more adverse environment for shell formation (Gazeau et al., 2007). Additionally, acidification of water (the increase in hydrogen ions) may impact the strength of ion gradients utilized during the shell formation process (Roer and Dillaman, 1984; Orr et al., 2005). However, the solubility of calcium carbonate is also affected by temperature. Increasing temperature causes the solubility of calcium carbonate to decrease (i.e., make dissolution less likely), although the effect of temperature on the solubility of calcium carbonate species is small compared to the effect of acidification (Brezonik and Arnold, 2011). Temperature may also impact the thickness of the exoskeleton, since the kinetics of carapace formation are largely governed by external temperature, as is the case with many physiological processes in marine invertebrates (Kinne, 1964). The possible interaction between the effects of increased temperature and pCO_2 on the exoskeleton of marine invertebrates underscores the importance of quantifying both of these factors in multi-stressor studies.

Much of the existing work on the effect of climate change on the exoskeletons of invertebrates has been conducted under acidic conditions on species where calcium carbonate is found in the form of calcite or aragonite. Shell dissolution under acidic conditions has been observed in many taxa, from tropical corals (Kleypas et al., 1999; Hoegh-Guldberg et al., 2007; Anthony et al., 2008) to coccolithophores (Riebesell et al., 2000; Iglesias-Rodriguez et al., 2008), pteropods (Orr et al., 2005; Lischka et al., 2010), and oysters (Gazeau et al., 2007; Miller et al., 2009; Waldbusser et al., 2011b). However, there is less information on the response to increases in pCO_2 in species where calcium carbonate is found in the form of high-magnesium calcite, such as crustaceans. Unlike exoskeletons composed mainly of calcite or aragonite, the exoskeleton of crustaceans are complex biological composite materials composed of chitin and protein fibers, and high-magnesium calcite crystals with small amounts of amorphous calcium phosphate (Roer and Dillaman, 1984; Boßelmann et al., 2007; Ries, 2011). These crystals are incorporated into chitin-protein fibers that are arranged in a multi-layered structure (Raabe et al., 2005; Chen et al., 2008). This complex structure provides the strength necessary to protect the animal from both predators and the external environment while also allowing for the flexibility to periodically shed the exoskeleton to grow through molting. In contrast to the dissolution observed in species with calcite or aragonite exoskeletons, increased calcification rates have been observed in some crustacean species exposed to acidified conditions, including crab (Ries et al., 2009; Long et al., 2013a), shrimp (Ries et al., 2009) and barnacles (McDonald et al., 2009). The complexity of the

crustacean exoskeleton underscores the necessity to examine the impact of environmental changes on the exoskeleton of these species in detail.

The mineralogy of the crustacean carapace has been examined using a variety of techniques to understand how the carapace changes in response to various stressors (Dillaman et al., 2005; Boßelmann et al., 2007; Ries, 2011; Long et al., 2013b). Ries (2011) determined that over 97% of the carapace of adult blue crab is high-magnesium calcite at a variety of acidities using X-ray diffraction (XRD). XRD is effective for distinguishing relative abundances of calcium carbonate minerals and polymorphs (e.g., calcite, aragonite, high-magnesium calcite), but the absolute concentration of the individual calcium carbonate polymorphs (including amorphous phases) is difficult to quantify. Inductively coupled plasma atomic emission spectrometry (ICP-AES) has been used to examine the elemental content of a variety of calcifying species with very high precision, from Ca, Mg, and Sr in coral (Deng et al., 2010) to Cd, Ni, Hg, and Pb in crab (Hamilton et al., 2008; Mutlu et al., 2011). Since the crustacean cuticle gains much of its strength from calcium carbonate (Jordaens et al., 2006; Amato et al., 2008; Fabritius et al., 2012), it is important to quantify the variability in the calcium carbonate content of the carapace in response to climate change. Recently, Coffey et al. (2017) used electron dispersive spectroscopy to examine the microstructure of the carapace of juvenile red and blue king crab and found no connection between elemental composition and hardness in the carapaces of crabs exposed to increased temperature and pCO_2.

Although the protection provided by a thick, heavily calcified carapace may contribute to the overall fitness of an individual crab, the creation of such a carapace

is also energetically costly. The molting process in blue crab involves the active transport of various ions out of and into the cuticular space in order to allow for the formation of the new cuticle inside the animal while still maintaining optimal internal hemolymph chemistry (Roer and Dillaman, 1984; Roer and Dillaman, 1993). Therefore, tradeoffs may exist between the many energy-intensive processes that must occur after molting and the creation of an optimal exoskeleton. For example, in juvenile blue crab, rapid growth is prioritized for the crab to achieve a size refuge from predation as quickly as possible (Hines, 2007), possibly at the expense of shell thickness or strength. Long et al. (2013b) observed a tradeoff between condition index and calcium content in red king crab (*Paralithodes camtschaticus*) and Tanner crab (*Chionoecetes bairdi*) exposed to acidified conditions. Acidification did not impact the calcium content of red king crab carapaces but the condition index did decrease. Conversely, Tanner crab condition index remained unchanged while calcium content decreased when crab were exposed to acidified conditions.

The objective of this study was to examine the impact of increased water temperature and pCO_2 on the carapace thickness and chemistry of juvenile blue crab in the context of possible tradeoffs between crab growth dynamics and exoskeleton functionality in a changing climate. We chose to examine the impact of climate change on the exoskeleton of juvenile blue crab, *Callinectes sapidus*, because of the lack of existing knowledge on the impacts of climate change on estuarine crustaceans and their economic and ecological importance (Baird and Ulanowicz, 1989; Kennedy et al., 2007). Glandon and Miller (2017) exposed juvenile blue crab to realistic future levels of warming and acidification for the Chesapeake Bay and found no effect of

increased pCO_2 on the growth and food consumption, despite positive responses to concurrent warming. Here we examine the consequence of these exposures to the chemistry and thickness of juvenile blue crab exposed to changes in temperature and pCO_2

Methods

Experimental setup and specimen selection

Specimens for carapace structure and mineralogy were obtained from animals exposed to increased water temperature and pCO_2, as described in Glandon and Miller (2017). Briefly, a flow-through experimental tank system was constructed using Patuxent River water at the Chesapeake Biological Laboratory. Juvenile blue crab (30-40 mm carapace width) were exposed to a 2x2 temperature x pCO_2 factorial experimental design for a period of two molts (approximately 30 days). Each treatment was replicated twice in each of two experiment blocks. Treatment levels were chosen to represent current summer conditions in the Chesapeake Bay (26°C and 800 µatm) and predicted future conditions in the year 2100 (32°C and 8,000 µatm). Temperature was manipulated through a combination of heated and chilled seawater. pCO_2 was manipulated with the addition of CO_2 gas into aquaria mediated by a "pH-stat" system, whereby pH was lowered by a controller to pre-determined set points. At least 10 crab were maintained individually at each treatment combination. Once each individual crab reached the inter-molt phase of the molt cycle (stage C4; Freeman et al., 1987) after its second molt, it was immediately frozen at –20°C for analysis. Carapaces from four crabs, selected at random per temperature x pCO_2

treatment per experimental block (total $n = 64$), were analyzed for both carapace thickness and elemental concentrations (g per g dry carapace) of calcium (Ca) and magnesium (Mg).

Carapace thickness

Selected specimens were thawed and the dorsal carapace was fully removed from the remaining carcass. Dorsal carapace pieces were manually cleaned of any residual tissue and dried to constant weight at 60°C. To minimize the effects of differences in thickness due to location on the carapace (Waugh et al., 2009), samples were taken from a standardized location on each crab. One millimeter square sections of the central portion of the dorsal carapace (location A in Figure 3.1) were removed for thickness analysis.

Following the methods of Waugh et al. (2009) and Secor et al. (1992), carapace sections were prepared for examination by light microscopy. Carapace sections were embedded in EpoFix cold-setting epoxy resin (Struers Inc., Cleveland, Ohio) blocks and allowed to cure for at least 24 hours at room temperature. Epoxy blocks were then thin-sectioned using an Isomet low-speed diamond saw (Buehler, Lake Bluff, Illinois) and thin sections mounted onto a slide for further polishing. Thin section slides were polished using alumina powder through a series of three polishing papers: 320 grit, 1200 grit, and finally a microcloth fine polishing pad in order to obtain maximum clarity of the carapace section. Thin section slides were photographed under light microscopy at 100x magnification and the thickness of the entire cuticle section (mm) as well as the endocutile layer was determined using

ImageJ software (Rasband, 2012). Exocuticle thickness was determined as the difference between whole cuticle thickness and endocuticle thickness. Figure 3.2 shows a section of carapace mounted and viewed under light microscopy, with the endocuticle and exocuticle layers clearly visible.

Carapace calcium (Ca) and magnesium (Mg) analysis

A 200 mg portion was taken from location B on the dried carapace (Figure 3.1) for ICP-AES analysis. Each ~200 mg carapace sample was dissolved in 8 mL of 16 N nitric acid in a Milestone Ethos EZ microwave digester, in Teflon vessels with quartz inserts. A mixture of 30% H_2O_2 and Milli-Q water was placed in the Teflon vessels outside the inserts. Each vessel was linearly ramped to 200°C in 10 min, then held at 200°C for 10 min, and finally cooled under forced air for about 80 min until a safe temperature of <50°C was attained. Upon cooling, the contents of the quartz inserts were transferred to 30-mL Teflon vials and diluted 250-fold with Milli-Q water in 15-mL centrifuge tubes before analysis. Cation determinations were made on the dilute solutions using a Perkin-Elmer Optima 8300 ICP-AES. Calibration standards were made gravimetrically in trace metal grade 2% nitric acid by combining certified standards of known Mg, Ca, and Sr concentrations in proportions to match those of a typical crab, as determined by a trial run using standard methods for measuring these elements in coral skeletal material (Schrag, 1999). Accuracy and precision were determined by measuring, alongside the crab samples, an in-house gravimetric standard solution and in-house coral solution with known Mg, Ca, and Sr concentrations that have been cross-calibrated by multiple laboratories using ICP-

AES and Thermal Ionization Mass Spectrometry methods. Analytical precision is estimated to be ±1.6% (1 sigma) relative standard deviation (RSD) for Ca and 4.6% (1 sigma) RSD for Mg, based on 30 determinations of the coral standard solution, with similar values obtained for the gravimetric standard. Accuracy was within 1% for both elements based on the same standards. The relatively high uncertainty for Mg is due to its low concentrations relative to Ca and instrument drift over the course of each run.

Elemental concentrations were expressed as mg/g carapace dry weight. It is assumed that all Ca and Mg found in the carapace are present in the form of-high magnesium calcite (HMC), based upon the findings of Ries (2011) and the molar ratio of Ca:Mg in the samples being typical of HMC (~3-4% MgCO3; Reeder, 1983; Morse et al., 2006), indicating no significant organic source of Ca or Mg in the carapace. Our calculations do not quantify the type of crystal structure the Ca and Mg form within the carapace. HMC was determined from elemental concentrations of Ca and Mg. The total number of moles of Ca and Mg were determined from elemental concentrations and then summed to determine the number of moles of carbonate. The elemental concentration of carbonate was determined from the moles of carbonate. Elemental concentration of HMC was determined as the sum of the elemental concentrations of Ca, Mg, and carbonate. HMC is presented as percentage by weight of the crab carapace throughout the results.

Statistical analyses

Data are presented as mean ± standard deviation by temperature/pCO_2 treatment. However, experimental data were analyzed as a full factorial design, with two levels of both temperature and pCO_2. All analyses were conducted in R (version 3.2.2 -- R Core Team, 2015) using R-Studio (version 0.98.1103). An alpha level of $P < 0.05$ was used for all analyses. Analysis of Variance (ANOVA) using type II sum of squares was used to test for the effects of temperature, pCO_2, and their interaction on each response variable. In order to accurately represent the experimental design, temperature and pCO_2 were treated as fixed effects, and block and experimental tank were treated as random effects. Response variables included total carapace thickness (mm), endocuticle layer thickness (mm), exocuticle layer thickness (mm), carapace calcium content (mg/g dried shell), carapace magnesium content (mg/g dried shell), weight percent HMC in the carapace, and the molar ratio of calcium to magnesium in the carapace. Significance of fixed effects and their interactions was assessed using the *Anova* function on linear, linear-mixed effects models using the *car* (Fox and Weisberg, 2010) and *nlme* packages (Pinheiro et al., 2015). When the analysis indicated no significant interaction between the effects of temperature and pCO_2, the model was rerun without the interaction term in order to improve the power of tests of main effect.

Results

Carapace thickness

The carapace thickness of 64 individual blue crab was determined for this study. Table 3.1 shows the sample size, mean, and standard deviation of the whole cuticle, endocuticle, and exocuticle thickness of the crabs by temperature x pCO_2 treatment. A two-way ANOVA indicated no significant temperature x pCO_2 interaction ($P>0.05$) on whole cuticle, endocuticle, and exocuticle thickness, and therefore main effects of temperature and pCO_2 were considered. The ANOVA indicated an effect of temperature on whole cuticle thickness ($P=0.052$), but no significant effect of pCO_2 on whole cuticle thickness ($P=0.118$). The carapaces of crabs in high temperature water (0.283±0.058 mm) were thinner than the carapaces of crabs in ambient temperature water (0.310±0.051 mm), regardless of pCO_2 treatment (Figure 3.3). The ANOVA indicated that neither temperature nor pCO_2 had a significant effect on the thickness of the endocuticle or the exocuticle ($P>0.05$ for all tests), despite the observed effect of temperature on whole cuticle thickness.

Carapace calcium (Ca) and magnesium (Mg) content

The calcium and magnesium content (mg/g dried carapace) of 64 individual crabs were determined for this study. Table 3.2 shows the number, mean, and standard deviation of the concentration of each element found in the carapace of blue crab by temperature x pCO_2 treatment. Percent high-magnesium calcite (HMC), calculated from the concentrations of calcium and magnesium observed, and the molar ratio of magnesium to calcium, are also reported in Table 3.2.

A two-way ANOVA indicated no significant temperature x pCO_2 interaction (P>0.05) on carapace calcium content, and therefore main effects of temperature and pCO_2 were considered. The ANOVA indicated a significant effect of temperature on carapace calcium content; the carapaces of crabs in high temperature water contained significantly less calcium than the carapaces of crabs in ambient temperature water (P=0.00016; high temperature mean = 253.2±8.8 mg/g, ambient temperature mean = 261.1±8.3 mg/g). Figure 3.4 shows the mean carapace calcium content by temperature treatment. Although a trend of decreasing carapace calcium content was observed in carapaces of crabs exposed to high pCO_2 compared to the carapaces of crab exposed to ambient pCO_2, the ANOVA indicated that it was not significant (P=0.064).

A two-way ANOVA indicated a significant temperature x pCO_2 interaction on carapace magnesium content (P=0.025), therefore pCO_2 effects were considered at each temperature level separately. The effect of increased pCO_2 was significant at both ambient and high temperature (P=0.003 and 1.05×10^{-6} for ambient and high temperatures, respectively); the carapaces of crabs at high pCO_2 contained more magnesium than the carapaces of crabs at ambient pCO_2 at both ambient and high temperature. However, the pCO_2 effect was greater at high temperature than at ambient temperature. The molar ratio of magnesium to calcium (Mg:Ca) followed similar trends to the carapace magnesium content data. A two-way ANOVA indicated a significant temperature x pCO_2 interaction on the ratio of magnesium to calcium in the carapace (P=0.033), therefore pCO_2 effects were considered at each temperature level separately. The effect of increased pCO_2 was significant at both ambient and

high temperature (P= 0.02 and 0.00019 for ambient and high temperatures, respectively); the carapaces of crabs at high pCO_2 had higher ratios of magnesium to calcium than the carapaces of crabs at ambient pCO_2 at both ambient and high temperature. However, the pCO_2 effect on the ratio of magnesium to calcium was greater at high temperature than at ambient temperature, which is clear from the interaction plot in Figure 3.5.

A two-way ANOVA indicated no significant temperature/pCO_2 interaction (P>0.05) on carapace weight percent HMC, and therefore main effects of temperature and pCO_2 were considered. The ANOVA indicated a significant effect temperature on carapace weight percent HMC; the carapaces of crabs in high temperature water had a significantly lower weight percent HMC than the carapaces of crabs in ambient temperature water (P=0.007; high temperature mean = 67.5±2.3%, ambient temperature mean = 69.0±2.2%). Additionally, the ANOVA indicated a significant effect of pCO_2 on the weight percent HMC of juvenile crab carapaces; the carapaces of crabs in high pCO_2 water had significantly more weight percent HMC than the carapaces of crabs in ambient pCO_2 water (P=0.012; high pCO_2 mean = 68.9±2.1%, ambient pCO_2 mean = 67.6±2.4%). Figure 3.6 shows the mean carapace weight percent HMC of crabs by temperature x pCO_2 treatment.

Discussion

The mineral component of blue crab shells is almost exclusively composed of high-magnesium calcite (HMC; Ries, 2011) and therefore the changes in HMC are the most relevant in quantifying the effects of increased temperature and pCO_2 on

juvenile blue crab carapaces. The data from this study reveal a counteractive effect of increased temperature and pCO_2 on the carapace chemistry of juvenile blue crab. Increased temperature significantly reduced the weight percent HMC in crab carapaces, which was largely caused by a decrease in the concentration of calcium with temperature (Table 3.2). Increased pCO_2 significantly increased the percent HMC by weight in crab carapaces, which was caused by an increase in the concentration of both calcium and magnesium with increased pCO_2 (Table 3.2).

However, the results of the molar ratio of magnesium to calcium complicates the interpretation of the HMC data. The observed changes in the percent HMC by weight were accompanied by changes in the elemental composition of HMC by temperature and pCO_2 treatment, since both temperature and pCO_2 caused a significant increase in the molar ratio of magnesium to calcium in this study. The effect of increased magnesium content on the protective ability of the carapace is unclear. Increased proportion of magnesium in the carapace may cause the carapace to be less stable due to the positive relationship between solubility and magnesium content in magnesium-calcite compounds (Berner, 1975). Additionally, increases in the ratio of magnesium to calcium in the carapace may be an indicator of stress and could be used to gauge the condition of animals exposed to external stressors, as has been suggested for other calcifying organisms such as bivalves and foraminifera (Lorens and Bender, 1980; Toler et al., 2001). However, increased magnesium content has been positively correlated with increased hardness in biogenic calcites (Kunitake et al., 2012).

There was a clear negative effect of temperature on the thickness and chemistry of juvenile crab carapaces in this study. The carapaces of crabs exposed to high temperature were approximately 8% thinner than the carapaces of crabs exposed to ambient temperature. Not only were these carapaces thinner, but they contained approximately 1.5% less HMC than the carapaces of crabs exposed to ambient temperature. A significant decline in calcium ion concentration was responsible for this decline in HMC, which was accompanied by an increase in magnesium. This decline in calcium concentration concurrent with an increase in magnesium concentration represents a double negative effect of increased temperature on blue crab carapace chemistry, since carapace calcium content has been correlated to shell strength in a variety of invertebrate species (Jordaens et al., 2006; Amato et al., 2008; Fabritius et al., 2012) and increased carapace magnesium content could be an indicator of stress (Lorens and Bender, 1980; Toler et al., 2001). Yet, there must be a balance between carapace thickness, calcium, and magnesium content in order to provide protection while still allowing for growth through molting (Roer and Dillaman, 1984; Boßelmann et al., 2007). The data from this study indicate that increased temperature may cause a change in this balance through declines in carapace thickness and percent HMC, as well as changes to the elemental composition of HMC in blue crab carapaces exposed to increased temperature.

The effect of increased pCO_2 on carapaces of juvenile blue crab was markedly different than the effect of increased temperature. Although there was no effect of increased pCO_2 on carapace thickness or calcium content, these carapaces contained approximately 1.5% more HMC, suggesting a potential benefit of being in high pCO_2

conditions on the blue crab carapace. However, the molar ratio of Mg:Ca increased in response to increasing pCO_2, indicating that although more HMC was present, the elemental composition of that HMC was more soluble in the carapaces of crabs exposed to increased pCO_2. However, Kunitake et al. (2012) showed that increased magnesium content of biogenic calcites increased the hardness of the material, pointing to a possible increase in carapace strength under high pCO_2 conditions in this study. Determining the relative importance of the amount of HMC and the elemental composition of that HMC in juvenile blue crab carapaces warrants future attention.

The biological effects of the observed changes in blue crab carapace thickness and chemistry may be profound. Declines in carapace thickness and percent HMC have the potential to not only effect the ability of the carapace to protect the crab again predation, but also could affect the process of carapace formation in this species. Increased pCO_2 will cause an increase in the bicarbonate concentration in seawater and may affect the amount of free calcium and magnesium in seawater (Brezonik and Arnold, 2011). Since blue crab mobilize free ions to create HMC in the carapace (Roer and Dillaman, 1984; Roer and Dillaman, 1993), acidification-related changes to the carbonate system may affect the carapace formation process in this species. Additionally, the excellent ability of blue crab to regulate their internal chemistry (Towle et al., 1976; Henry and Kormanik, 1985; Mangum et al., 1985) may reduce the impact of changes in environmental chemistry on crab carapace thickness and chemistry since blue crab form the new carapace inside of the old one in a controlled environment (Roer and Dillaman, 1984). Hemolymph pH was not

quantified in this study, but understanding the effects of increased environmental pCO_2 on blue crab hemolymph chemistry is an important next step in determining the effects of environmental change on crab carapace structure and chemistry.

The counteractive effects of increased temperature and pCO_2 on the percent of HMC by weight are evident in Table 3.2; the mean percent HMC by weight of the crabs exposed to ambient temperature and ambient pCO_2 is almost identical to the mean percent HMC by weight of crabs exposed to high temperature and high pCO_2. As stated previously, there was a synergistic effect of temperature and pCO_2 on the molar ratio of Mg:Ca in the carapaces of juvenile blue crab in this study. The carapaces of crabs exposed to increased pCO_2 contained higher ratios of Mg:Ca regardless of temperature treatment, but the impact of increased pCO_2 was greater at high temperature than at ambient temperature (Figure 3.5) in these data. Non-linear responses have been observed in other species exposed to multiple stressors (e.g.: Crain et al., 2008; Dissanayake and Ishimatsu, 2011; McBryan et al., 2013), underscoring the importance of conducting multiple stressors studies when attempting to quantify species response to climate change. The presence of non-linear responses in the data from this study are another example of the importance of conducting multi-stressor studies when quantifying the effects of environmental change.

Integrating the results of the thickness and mineral content of crab carapaces with the effects of increased temperature and pCO_2 on crab growth rate observed by Glandon and Miller (2017), indicate tradeoffs between crab growth and protective ability of the carapace in the face of a changing climate. These effects are summarized in Table 3.3. Glandon and Miller (2017) determined that juvenile blue

crab exposed to increased temperature grew significantly faster than crabs exposed to ambient temperature conditions. The data reported here indicate that blue crab carapace thickness and chemistry decline in order to sustain rapid growth when exposed to increased temperature. These data also suggest that while crabs can maintain growth rates similar to ambient conditions when exposed to increased pCO_2, the maintenance of growth is accompanied by an increase in the amount of HMC the carapace. However, the increase in the ratio of Mg:Ca in the carapaces of crabs exposed to increased pCO_2 indicates a more soluble HMC, which could also be generating a harder material. The effect of an increase in the Mg:Ca ratio on the hardness or protective ability of the carapaces of crab exposed to increased pCO_2 remains to be seen.

The results of this study underscore the importance of examining secondary effects when quantifying the impact of environmental changes on organism fitness. Although Glandon and Miller (2017) observed no effect of increased pCO_2 on juvenile blue crab growth, the results of the current study suggest changes in the thickness and chemistry of the carapaces of crabs exposed to increased pCO_2. Similar patterns have been observed in other species, highlighting the importance of secondary effects. McDonald et al. (2009) observed no impact of low pH on overall growth in the barnacle, *Amphibalanus amphitrite*, however a weakening of shell plates was observed, indicating dissolution of the barnacle shells as they grew. Shell growth in rocky intertidal snails, *Nucella lamellose*, was positively correlated to increasing CO_2 levels but significant shell dissolution was also observed, indicating different impacts of ocean acidification on shell deposition and dissolution in this

species (Nienhuis et al., 2010). The data from our study indicate a secondary effect of acidification on juvenile blue crab; significant changes in carapace composition in addition to the maintenance of growth.

Impacts of environmental change and stress may be variable according to life history stage. The data from this study represent the juvenile life stage of blue crab, a time when rapid growth is prioritized in order to achieve a size refuge from predation (Hines, 2007). The importance of growth at the juvenile life stage is clear when considering the results of this study paired with the data from Glandon and Miller (2017). However, the data from this study do not speak to the impact of increased temperature and pCO_2 on the carapaces of adult blue crab; whether crabs held in conditions for many years would have more severe impacts, or acclimation to those conditions would occur. Exposure studies over entire life histories are difficult and expensive to maintain, creating a dearth of data on acclimation response over a realistic period of time. Swiney et al. (2015) examined survival and development of Tanner crab, *Chionoectes bairdi*, embryos from female crab held in acidified conditions for two years. Oocyte and embryonic development, hatching success, and carapace calcium content were all negatively impacted as exposure to acidified conditions lengthened. Additionally, Long et al. (2016a) observed a greater impact of acidified conditions on Tanner crab larvae raised from adults held in acidified conditions than from larvae only exposed during the larval period, indicating significant carryover effects in this species. Recently, Coffey et al. (2017) observed no effect of pH or temperature on cuticle thickness in juvenile red and blue king crab exposed to conditions for one year, despite elevated calcium content. Although

Tanner, king, and blue crab are all from the infraorder Brachyura, few additional similarities exist between the species. Tanner and king crab are found in the Bering Sea and Gulf of Alaska while blue crab are found in the coastal and estuarine waters of the western Atlantic Ocean. Considering the large differences in habitat between the species, cross-species conclusions are difficult to make. Long-term studies of the effects of environmental change on estuarine crustaceans would greatly aid in understanding the success of these species in future climate conditions.

Understanding the effects of environmental variability on physiology is critical to predicting species response to future climate scenarios (Lefevre, 2016). Increased temperature and pCO_2 have counteractive effects on the weight percent HMC in the carapaces of juvenile blue crab; increased temperature causes a thinning of the carapaces and a decline in weight percent HMC, while increased pCO_2 causes no change in carapace thickness and an increase in weight percent HMC. However, a significant positive effect of increased temperature and pCO_2 was observed in the molar ratio of Mg:Ca in the crabs in this study, indicating that changes in the amount of HMC were accompanied by changes in the elemental composition of that HMC. Understanding the relative importance of the amount of HMC and the molar ratio of Mg:Ca in the carapaces of juvenile blue crab would help to determine the biological significance of the observed changes in carapace composition in this study. Additionally, studies to quantify hardening time after the molt may shed light on how increased temperature and pCO_2 would impact recovery from molting. Any lengthening of the time to hardness could be detrimental to individuals, both as an increased energetic cost of time to recover and an increased risk of predation.

Understanding how these effects may manifest through crab life history would be valuable to quantify the impact of changes in carapace thickness and chemistry at the population level. Finally, continuing to explore the secondary effects (e.g., metabolism, energy content) of increased temperature and pCO_2 on juvenile blue crab would help to paint a complete picture of the possible impact of climate change on this economically and ecologically valuable species.

Table 3.1. Sample size, mean, and standard deviation of the whole cuticle, endocuticle, and exocuticle thickness (mm) of blue crab by temperature x pCO_2 treatment. Whole cuticle and endocuticle thickness were measured using image analysis software ImageJ. Exocuticle thickness was determined as the difference between whole cuticle and endocuticle thickness. SD: standard deviation.

			Whole cuticle (mm)		**Endocuticle (mm)**		**Exocuticle (mm)**	
Temperature	***p*CO₂**	***n***	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Ambient	Ambient	16	0.324	0.051	0.267	0.045	0.056	0.014
	High	16	0.296	0.048	0.234	0.050	0.062	0.009
High	Ambient	16	0.290	0.063	0.238	0.058	0.053	0.008
	High	16	0.276	0.052	0.223	0.050	0.053	0.010

Table 3.2. Sample size, mean (mg/g dried carapace) and standard deviation of calcium and magnesium content, weight percent high-magnesium calcite (HMC), and the molar ratio of magnesium to calcium found in the carapaces of juvenile blue crab by temperature x pCO_2 treatment. n:n represents molar ratio and SD represents one standard deviation.

			Calcium (mg/g)		**Magnesium (mg/g)**		**% HMC**		**Mg:Ca (n:n)**	
Temperature	***p*CO₂**	**N**	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Ambient	Ambient	16	259.1	10.7	10.5	0.6	68.4	2.8	0.067	0.003
	High	16	263.2	4.6	11.2	0.6	69.6	1.2	0.070	0.004
High	Ambient	16	251.4	6.4	11.6	0.7	66.8	1.7	0.076	0.004
	High	16	255.1	10.6	13.2	1.1	68.3	2.6	0.085	0.009

Table 3.3. Summary of results of two-way ANOVA to test the impact of increased temperature and pCO_2 on juvenile blue crab growth and consumption (shown in italics; from Glandon and Miller 2017) and carapace thickness and mineral content. Alpha level for all tests was 0.05. # = significant interaction between temperature and pCO_2 was observed.

Response	**Temperature**	***p*CO$_2$**
Growth Rate	*Increase*	*No effect*
Carapace Thickness	Decrease	No effect
Carapace [Ca]	Decrease	No effect
Carapace [Mg]$^{\#}$	Increase	Increase
Carapace % HMC	Decrease	Increase
Carapace Mg:Ca$^{\#}$	Increase	Increase

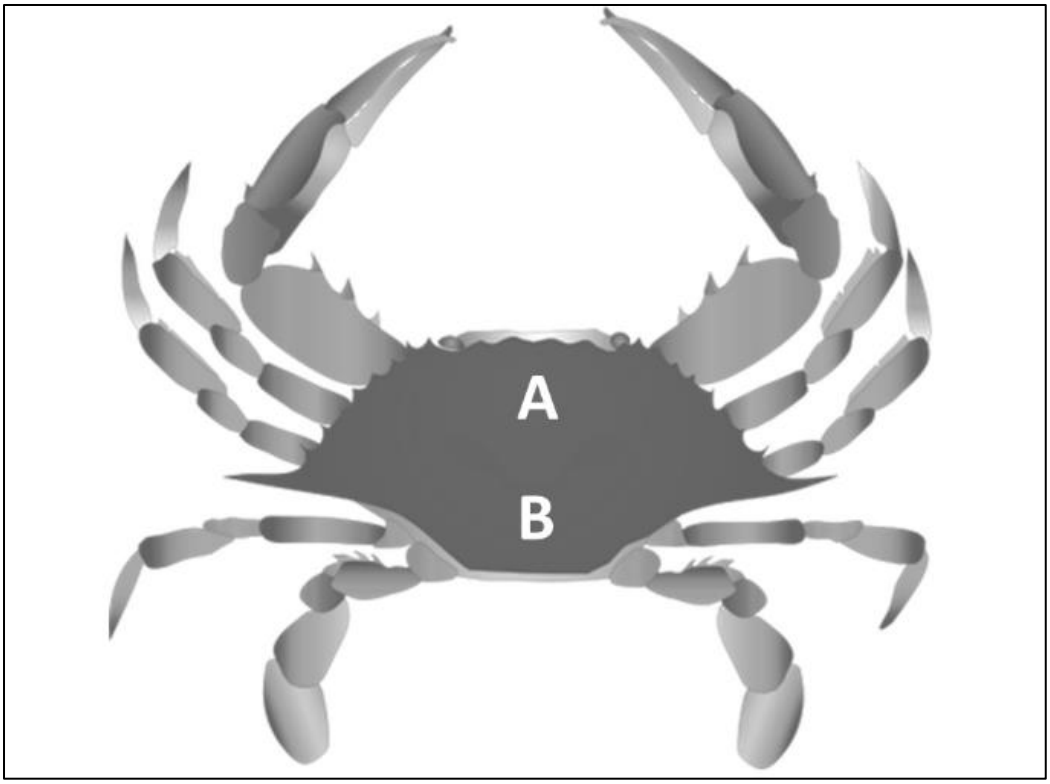

Figure 3.1. Sampling location for specimens to be used for carapace thickness analysis (letter A) and carapace elemental content (letter B)). Image from: http://ian.umces.edu/imagelibrary/.

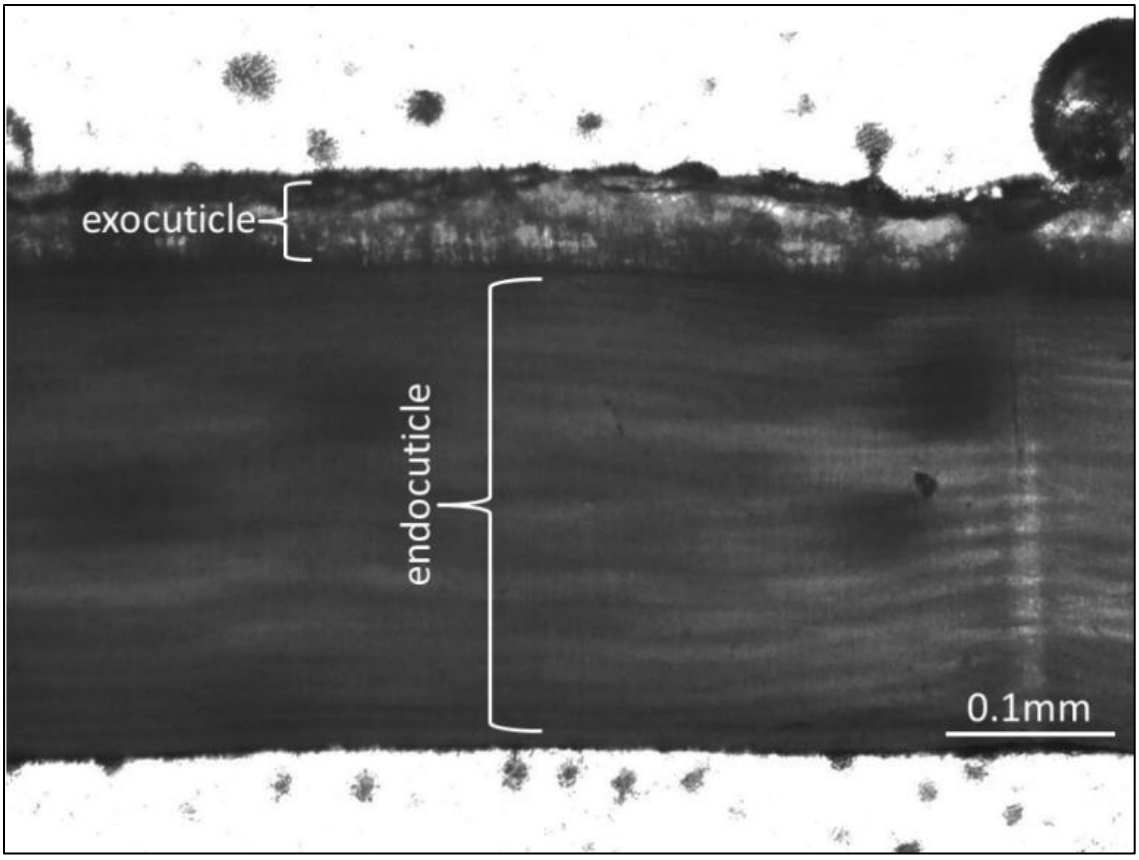

Figure 3.2. Thin section of blue crab carapace, prepared using the methods described and viewed under light microscopy.

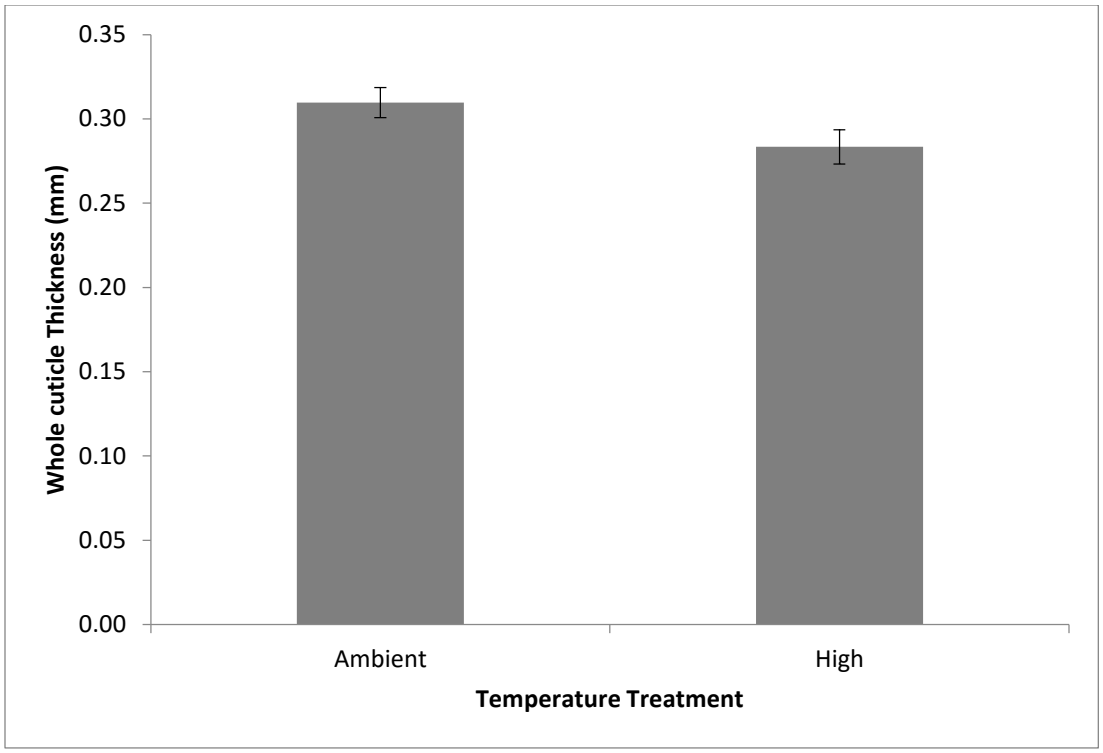

Figure 3.3. Juvnile blue crab whole cuticle thickness (mm) by temperature treatment. The ANOVA indicates a significant effect of temperature on whole cuticle thickness (P=0.052). The carapaces of crabs in high temperature water (0.283±0.058 mm) were significantly thinner than the carapaces of crabs in ambient temperature water (0.310±0.051 mm), regardless of pCO_2 treatment. Error bars represent the standard error of the mean.

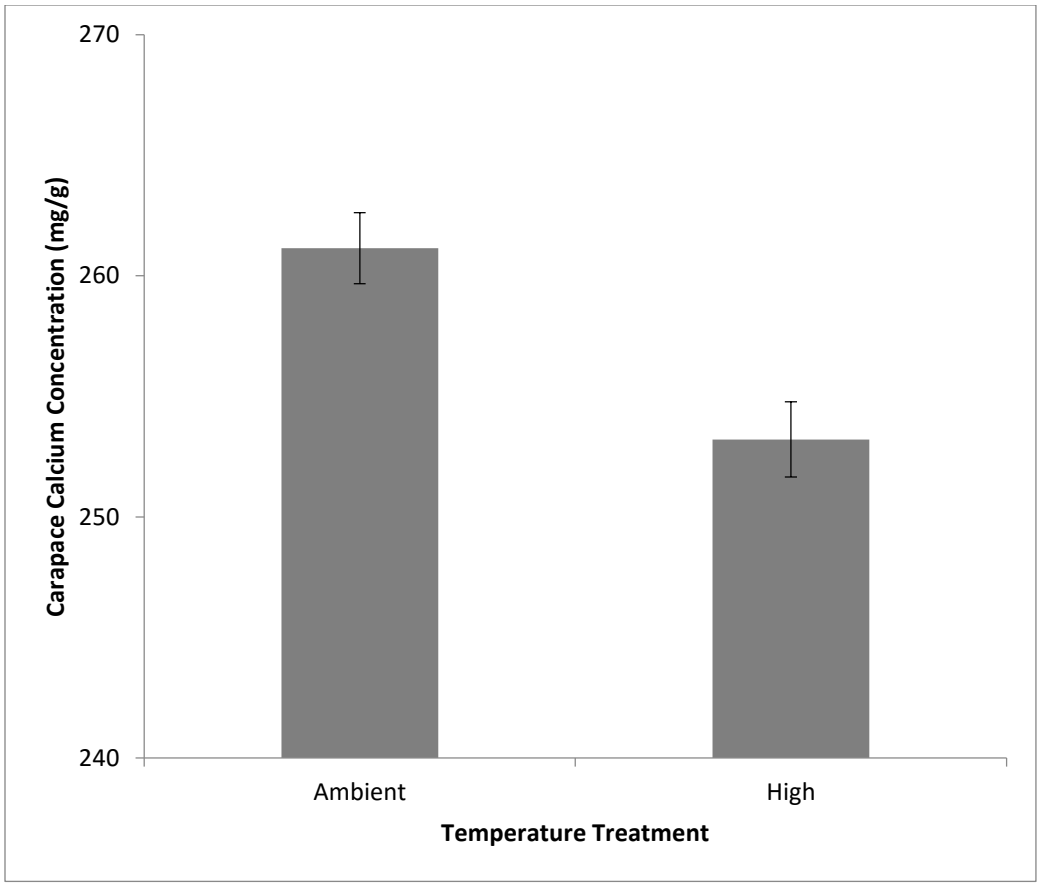

Figure 3.4. Juvenile blue crab carapace calcium content (mg/g) by temperature treatment. The carapaces of crabs exposed to high temperature water contained significantly less calcium than the carapaces of crabs exposed to ambient temperature water (P=0.00018). There was no significant effect of increased pCO_2 on the calcium content of the carapaces. Error bars represent the standard error of the mean.

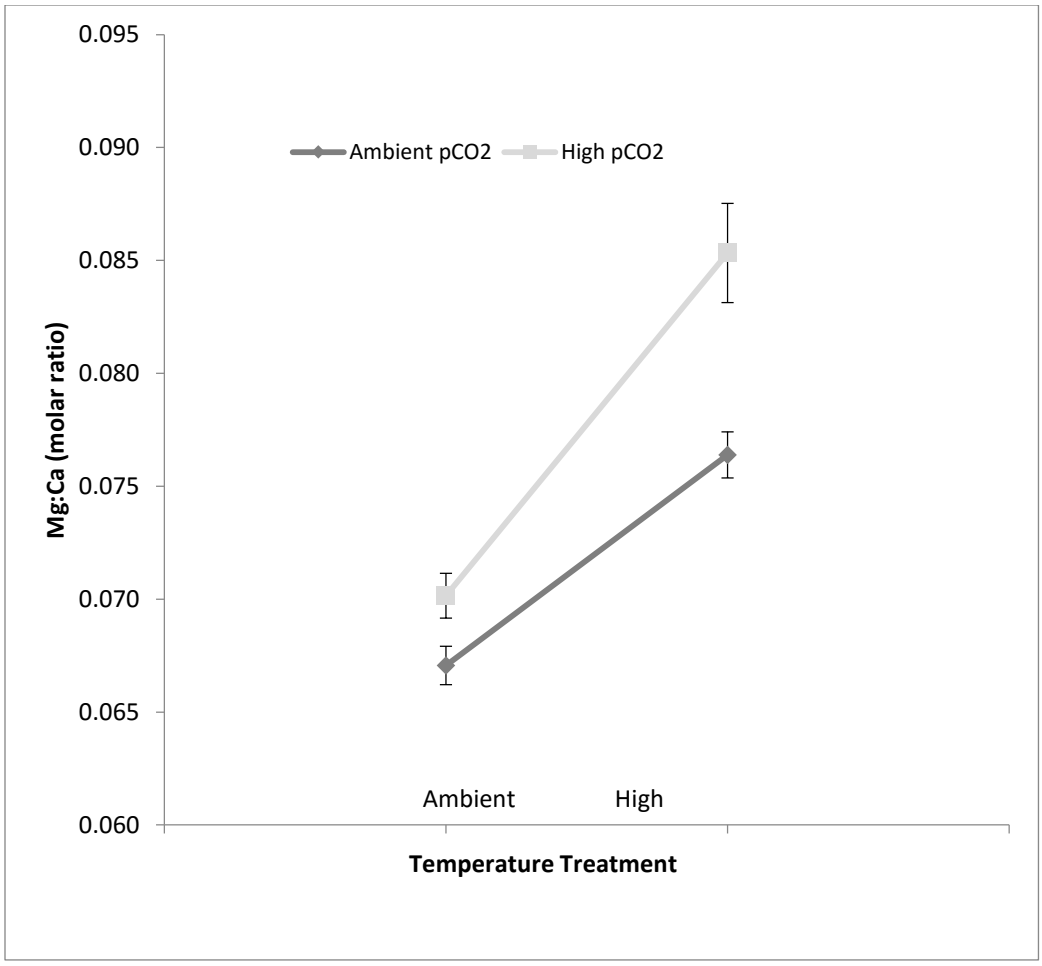

Figure 3.5. Interaction plot of the ratio of magnesium to calcium (molar ratio) in juvenile blue crab carapaces by temperature and pCO_2 treatment, showing a greater effect of pCO_2 treatment at high temperature than ambient temperature (significant two-way ANOVA interaction; P=0.033). At both temperature levels, the carapaces of crabs exposed to high pCO_2 had significantly higher ratios of magnesium to calcium than the carapaces of crabs exposed to ambient pCO_2 (P=0.02 and 0.00019 for ambient and high temperatures, respectively). Error bars represent the standard error of the mean.

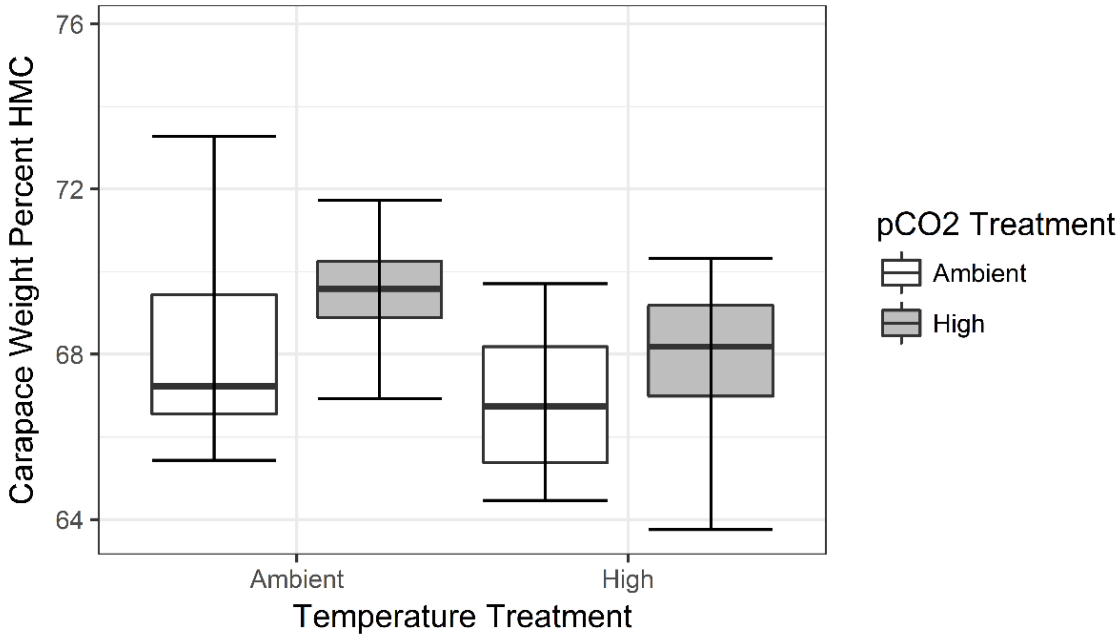

Figure 3.6. Juvenile blue crab carapace weight percent high-magnesium calcite (HMC) by temperature and pCO_2 treatment. Solid dark line represents the median of each treatment combination, boxes represent inter-quartile range, and whiskers represent the sum of 1st (lower whisker) or 3rd (upper whisker) plus 1.5 times the inter-quartile range. There was a significant negative effect of increased temperature (P=0.007) and significant positive effect of increased pCO_2 (P=0.012) on carapace weight percent HMC.

Chapter 4: Oxygen consumption of juvenile blue crab, *Callinectes sapidus*, from the mesohaline Chesapeake Bay in response to future predicted increases in environmental temperature and pCO_2.

Abstract

Quantifying the physiological impact of environmental stressors is critical to the ability to predict the response of species to future climate scenarios. We quantified oxygen consumption rate (µmol O_2/g/min) to examine the response of juvenile blue crab, *Callinectes sapidus*, from the Chesapeake Bay (Patuxent River, Maryland) to elevated temperature and pCO_2 reflective of projected future climate scenarios. Treatment levels were selected to represent current conditions in the Chesapeake Bay (26°C and 800 µatm) and conditions predicted to occur by the year 2100 (31°C and 8,000 µatm). Crabs were exposed to these conditions throughout two successive molts (approximately 30 days). At the end of the exposure, the oxygen consumption rate of individual crabs was determined over at least a 10 hour period using a flow-through respiration chamber equipped with optical oxygen electrodes. Metabolic measurements were also made on crabs that experienced a non-acclimated, rapid increase in temperature. Although the range of temperatures used in this experiment did not cause a significant difference in the mean oxygen consumption of crabs in this study, Q_{10} values indicate that oxygen consumption rates did predictably rise with temperature. High individual variability and a relatively small difference in temperature treatments are likely the cause for the lack of a statistically significant difference in mean oxygen consumption rates by temperature. Additionally, there was no significant effect of increased pCO_2 on crab oxygen consumption, suggesting an absence of a bioenergetic impact of increased pCO_2 on

juvenile crab in general. The results of this study have implications for the resilience of blue crab to future climate stressors and underscore the need for multispecies studies to quantify the ecosystem-level impacts of climate change on the Chesapeake Bay ecosystem.

Introduction

Highly variable environments, both naturally occurring and human-induced, favor species with high reproductive output and hence increased genetic variability and/or a high degree of phenotypic plasticity. This variability can be expressed ontogenetially at individual- and population-levels. Individuals with the optimal combination of traits for a given set of environmental conditions will have a higher likelihood of survival and these "natural athletes" are more likely to pass their traits on to the next generation (Rice et al., 1993; Fuiman and Cowan, 2003). Dramatic ontogenetic shifts in morphology and habitat often cause the same individual to experience substantially different conditions throughout life, thereby favoring genetic and phenotypic variability. This variability can be seen particularly in traits such as growth and metabolism which regulate the length of exposure to life-stage specific sources of mortality, as has been observed in a variety of processes from larval fish growth rates and stage duration (Houde, 1989) to amphibian size at metamorphosis (Wilbur and Collins, 1973; Werner, 1986). Thus, quantifying the response of key physiological parameters to a changing marine environment is critical to understanding how populations may respond to climate stressors into the future.

Anthropogenic release of fossil fuels has caused substantial increases in the temperature and pCO_2 of the marine environment, with further increases predicted by the end of the century (Stocker et al., 2013). Indeed, increasing anthropogenic influences are

creating a warmer, more acidic marine environment, unlike any that has been observed to date (Feely et al., 2009). Marine invertebrates are an ideal taxa in which to examine the physiological effects of increased temperature and pCO_2 because they are prolific in a wide range of environmental conditions. Metabolic rate is an important metric of an individual's ability to grow and survive, and has been the focus of many studies quantifying the physiological impact of climate change (see below for examples). In studies of the effects of environmental stressors on metabolic rate, both the variability of measured respiration rates and the Q_{10} value can be useful indices of species resilience. The Q_{10} quantifies the change in metabolic rate for a 10°C increase in temperature and provides a standardized index of thermal response. Changes to the variability of metabolic rates as well as Q_{10} can have large impacts on individual survival and fitness, and therefore they are important to quantify along with average metabolic rate.

The relationship between temperature and metabolic rate in marine invertebrates is well established (Newell and Branch, 1980; Gillooly et al., 2001) and has been linked to oxygen limitation of thermal capacity in these species (Pörtner, 2001; Pörtner, 2010). In contrast, studies examining the effects of increased pCO_2 on metabolic rate have reported a range of responses (Kroeker et al., 2010). Metabolic depression as a result of exposure to decreased pH/ increased pCO_2 is well documented (Pörtner et al., 2005) and has been observed in a variety of species including infaunal brittlestar, *Amphiura filiformis* (Hu et al., 2014), late stage larval lobster, *Homarus gammarus* (Small et al., 2015) and mussel, *Mytilus chilensis* (Navarro et al., 2016). Conversely, increases in metabolic rate in response to decreased pH/ increased pCO_2 have been reported in juvenile eastern oyster, *Crassostrea virginica* (Beniash et al., 2010) and early stage larval

lobster (Small et al., 2015), while decreased pH/ increased pCO_2 had no effect on metabolic rate in other species such as abalone, *Haliotis iris* (Cunningham et al., 2015), cuttlefish *Sepia officinalis* (Sigwart et al., 2015) and larval Pacific oyster, *Crassostrea gigas* (Frieder et al., 2016). Importantly, synergistic effects of increased temperature and pCO_2 on metabolic rate have been observed in a variety of species including the nektonic crustacean, *Metapenaeus joyneri* (Dissanayake and Ishimatsu, 2011), Pacific oyster, *Crassostrea gigas* (Lannig et al., 2010), and the Arctic pteropod, *Limacina helicina* (Comeau et al., 2010), underscoring the importance of multi-stressor studies. The duration and type of exposure (e.g.; acute, long-term, cycling) to increased pCO_2 may contribute to the variability of these responses (Todgham and Stillman, 2013). Additionally, individual and life-stage specific capacity for acid-base regulation, which can be impacted by ventilatory capacity, type and efficiency of ion exchangers, and overall activity levels, is known to affect species response to increased pCO_2 (Pörtner et al., 2004).

A positive relationship between temperature and metabolism has been well characterized in decapod crustaceans from the larval stage through the adult life stage (Leffler, 1972; Breteler, 1975; Eriksson and Edlund, 1977; Gutermuth and Armstrong, 1989; Booth and McMahon, 1992) and has been linked to strong ventilatory capacity of this taxa (Frederich and Pörtner, 2000). Coefficients of variation among individuals in these taxa range from 14% to 60%, depending on the temperatures tested. Similarly, Q_{10} values ranged from approximately 1 to 12, again depending on the temperatures tested. Similar to the trend in marine invertebrates in general, the effect of decreased pH/ increased pCO_2 on the metabolism of decapod crustaceans appears to be species and life-

stage specific. A synergistic effect of temperature and pCO_2 were observed in adult edible crab, *Cancer pagurus*, such that the thermal tolerance window significantly narrowed in response to increased pCO_2 (Metzger et al., 2007). Similarly, in adult velvet swimming crab, *Necora puber*, metabolism significantly declined under acidified conditions (Small et al., 2010). However, only the embryonic stage of the porcelain crab, *Petrolisthes cinctipes*, experienced a significant decline in metabolic rate when exposed to increased pCO_2; no effect was observed in the larval or juvenile stage (Carter et al., 2013). The variability in response to increased temperature and pCO_2 experienced by decapod crustaceans underscores the need for life stage and species-specific studies examining the effects of climate stressors on key physiological parameters, such as metabolic rate.

Much of the knowledge regarding the effects of climate change presented above is based upon marine species. In contrast to the relative consistency of the marine environment, estuarine systems are characterized by large fluctuations in environmental parameters including temperature and pCO_2 (Cai and Wang, 1998; Waldbusser and Salisbury, 2014). Estuarine and coastal systems are some of the most productive regions on earth, thereby amplifying the importance of quantifying the effects of climate change on estuarine species (Cooley and Doney, 2009). The blue crab (*Callinectes sapidus*) is an ecologically and economically important species in the Chesapeake Bay estuary (Baird and Ulanowicz, 1989; Kennedy et al., 2007), and understanding the impact of potential future climate stressors on this species is therefore of great regional concern. The wide distribution of the blue crab, along the eastern seaboard of the US and throughout the

Gulf of Mexico (Williams, 1984), makes it an excellent candidate for examining the impact of climate change on estuarine crustaceans in general.

Here, we quantified the oxygen consumption rate of juvenile blue crab from the Chesapeake Bay (Patuxent River, Maryland) in response to two key climate stressors (increased temperature and pCO_2). Treatment levels of temperature and pCO_2 were chosen to reflect current and future predicted climate conditions for the Chesapeake Bay by the year 2100 (see Glandon and Miller, 2017). We chose to examine the juvenile life stage because of its role in regulating overall crab population dynamics (Miller, 2001) and because juveniles are the first life stage to live entirely in the estuary. This study adds to the growing body of knowledge on the impact of climate stressors in estuarine crustaceans; other work in our lab has quantified the effects of increased temperature and pCO_2 on the growth and food consumption (Glandon and Miller, 2017) and shell properties (Glandon et al., *submitted*) of juvenile blue crab.

Methods

Crab collection and maintenance

Juvenile blue crab, approximately 30mm carapace width, were collected from two principal tributaries of the Chesapeake Bay. Trials were conducted in a blocked experimental design. Block 1 crabs were collected in May 2016 from the Patuxent River, Maryland and block 2 crabs were collected in September 2016 from the York River, Virginia. Crabs were kept in a flow-through acclimation system at the Chesapeake Biological Laboratory at one of four temperature/pCO_2 treatments. Treatment levels were selected to represent current summer temperature and pCO_2 in the Patuxent River (26°C

and 800 µatm) and conditions forecast for the year 2100 (31°C and 8,000µatm; Boesch et al., 2008). Details on the flow-through system for acclimation of individuals to treatment conditions are given in Glandon and Miller (2017). Crabs were kept in the flow-through acclimation system for a period of two molts (approximately 30 days) and fed frozen bay scallops ad libitum in order to alleviate known effects of food limitation on metabolic rate (O'Connor et al., 2009).

Respiration chamber design

Oxygen consumption of individual juvenile crabs were quantified in a flow-through respiration chamber (see Figure 4.1). Flow-through respirometers were selected so that extended trials could be conducted in order to minimize handling effects on respiration rates. Treatment water was mixed in a header tank, specific for each temperature/pCO_2 combination, and then flowed, gravimetrically but separately, into respiration chambers and the water bath. Since chambers and the water bath were filled from a single header tank, only one treatment combination was run during a given assay. A maximum of four individual chambers were run per assay (one night), with at least one empty chamber serving as a blank to take into account microbial respiration and ambient conditions during each assay. Crab and blank treatments were assigned randomly to specific chambers. Each respirometry chamber was constructed of 1.3 cm thick plexiglass with a 0.6 cm 70 durometer silicone gasket. Holes (0.6 cm OD) were drilled in two opposite sides of the chamber to allow for inflow and outflow of water and barbed brass fittings were added to each hole. A digital flow meter was constructed using a Hall-effect sensor (0.6 cm OD plastic; Uxcell, Hong Kong) connected to an Arduino Uno R3 board

(Arduino AG, Italy). The Arduino was used to measure and record the flow into the chamber throughout the trial. The flow meter and a Pyro Science FireSting flow-through oxygen electrode (model OXFTC; Pyro Science GmbH, Aachen Germany) were placed before the inflow hole and an additional flow-through oxygen electrode was placed on the after the outflow hole. Water flow and oxygen concentration of the inflow and outflow water were recorded once per second in each chamber throughout the duration of each assay.

Oxygen consumption calculations

Oxygen consumption (R; µmol/g/min) was determined for each respirometry chamber individually. First, the residence time (T_R; min) of the water in the chamber was determined as

Equation 1: T_R (min) = (V/F_A);

where V was the volume of the chamber (L) and F_A was the average flow (L/min) for each chamber in each trial. The residence time was used to determine a shift that was applied to the outflow data, such that the oxygen concentration of the water used in calculations was shifted by T_R, to take into account the residence time of the water in the chamber. The shifted outflow oxygen concentration was then represented as O_{oTR} (µmol/L). The difference between the concentration of oxygen in the inflow and outflow water was determined as

Equation 2: $D_O = O_i - Oo_{TR}$;

where O_i was the concentration of oxygen (µmol/L) in the water flowing into the chamber and O_{OTR} was the time-shifted concentration of oxygen (µmol/L) in the water

flowing out of the chamber. The uncorrected oxygen consumption rate, R_U, in µmol/min was determined by

Equation 3: $R_U = D_O * F$;

where D_O was as defined in Equation 2, and F was the flow rate (L/min) at that same time point. To account for microbial respiration in the respirometer, we further corrected the estimate of oxygen consumption as:

Equation 4: $R_C = R_U$ (crab) – R_U (blank);

where R_U (crab) was the average oxygen consumption rate (µmol/min) for an individual crab (Equation 3) and R_U (blank) was the average oxygen consumption rate of all blanks conducted during an individual overnight trial. Finally, the mass-specific oxygen consumption rate was determined as

Equation 5: $R = R_C / m$;

where R_C was the corrected oxygen consumption rate of an individual crab (µmol/L; Equation 4) and m was the wet mass of that crab. The Q_{10} value was determined for each pCO_2 treatment separately as

Equation 6: $Q_{10} = (R_{T2}-R_{T1})^{[10/(T2-T1)]}$;

where R_{T2} was the average oxygen consumption of high temperature treatment (31°C) and R_{T1} was the average oxygen consumption of the ambient temperature treatment (26°C). The temperature (°C) range used to calculate the Q_{10} values are indicated in the subscript as $Q_{10\ T1-T2}$ in the following text, where T1 is the lower temperature and T2 is the upper temperature.

Temperature/pCO_2 experiment

Oxygen consumption of crabs were determined after an individual had completed two molts in the acclimation system. All trials were conducted for at least 10 hours between 18:00 and 9:00, the time of day that blue crab are the least active. Crabs were fed daily while in the acclimation system and were starved for 24 hours prior to measurement. Oxygen electrodes were calibrated in freshwater fully saturated with air before each overnight trial and electrodes were configured to compensate for the salinity of water in the flow-through system (salinity: 14-17). Measurements of oxygen concentration and flow were taken once per second (60 times per minute). Oxygen consumption was measured 4-10 days after the second molt to control for molt stage, which was approximately 15 days in the crabs in this study. Oxygen electrodes and flow meters were cleaned in a solution of soapy water and then flushed for at least 1 hour with freshwater after each overnight trial. Respirometry chambers were cleaned with bleach and freshwater after each overnight trial.

The oxygen consumption rate of most individual crabs were measured at the same temperature and pCO_2 conditions at which they were held. However, to determine the sensitivity of the flow-through respirometry chamber design to the variability in oxygen consumption rates by juvenile blue crab, a preliminary experiment was conducted to determine the oxygen consumption of crabs that were temperature shocked. Crabs were acclimated to ambient temperature and ambient pCO_2 conditions for a period of two molts, as described above and the oxygen consumption of these crabs was determined following an immediate transfer to high temperature (31°C) water. It was expected that the oxygen consumption of these individuals would be significantly higher than the

individuals that were tested at their acclimation temperature, confirming that the system design could accurately measure the response of crab to environmental stressors, such as increased temperature and pCO_2.

Data analysis

Mean, mass-specific oxygen consumption rate (R in µmol/g/min; ± standard deviation (SD)) was determined for each crab for each overnight trial, excluding the first hour of measurement in order to avoid influences of acute handling stress. In order to account for possible deviations from an isometric relationship between crab mass and oxygen consumption rate (Beaupre and Dunham, 1995; Poehlman and Toth, 1995), differences in mean oxygen consumption rate (R in µmol/min) by treatment were determined using analysis of covariance (ANCOVA) with crab mass as the covariate. For ease of comparison with other studies, mass-specific oxygen consumption rates are reported in the results.

To test for the effect of a rapid increase in temperature (temperature shock experiment) on the oxygen consumption rate of juvenile blue crab, a 1-way ANCOVA was conducted with oxygen consumption as the response, treatment as the fixed effect, and crab mass as the covariate. Treatments compared for this analysis were crabs acclimated and tested at ambient temperature, crabs acclimated and tested at high temperature, and crabs acclimated at ambient temperature and tested at high temperature. A 3-way ANCOVA was conducted with oxygen consumption as the response, block, temperature, and pCO_2 as fixed effects, and crab mass as the covariate. To test for the effect of temperature and pCO_2 on oxygen consumption within each block, a 2-way

ANCOVA was conducted with oxygen consumption as the response, temperature and pCO_2 as fixed effects, and crab mass at the covariate. A tukey-post hoc test was conducted if significant main effects were observed. All analyses were conducted in R (version 3.2.2 - R Core Team, 2015) using R-Studio (version 1.0.136). Significance of fixed effects and their interactions was assessed using the *Anova* function on linear-mixed effects models using the *car* (Fox and Weisberg, 2010)and *nlme* packages (Pinheiro et al., 2015).

Results

The oxygen consumption of 46 individual crabs were quantified during this study. An example of data produced from a single overnight trial is shown in Figure 4.2, highlighting the variability in observations. Data from a representative blank chamber are shown in Figure 4.2A, with the difference between the inflow and outflow between zero and 5 µmol/min for the entire trial. Figure 4.2B shows data from an individual crab, with the difference between the inflow and outflow being closer to 10 µmol/min for the trial. The sample size, mean, standard deviation, and $Q_{10\ 26\text{-}31}$ of the oxygen consumption of crabs by block and temperature/pCO_2 treatment are shown in Table 4.1.

Temperature shock preliminary experiment

The 1-way ANCOVA of the indicated a significant effect of temperature treatment on the oxygen consumption of crabs ($F_{2,24}$=4.62, P = 0.020). Tukey post-hoc tests revealed that the oxygen consumption of crabs acclimated and tested at ambient

temperature (R = 0.17±0.07 µmol/g/min) was significantly lower than the oxygen consumption of crabs acclimated at ambient temperature and tested at high temperature (R = 0.65±0.33 µmol/g/min; $P = 0.018$). Additionally, the oxygen consumption of crabs acclimated and tested at high temperature (R = 0.26±0.18 µmol/g/min) was lower than the oxygen consumption of crabs acclimated at ambient temperature and tested at high temperature (R = 0.65±0.33 µmol/g/min; $P = 0.090$). However, there was no significant difference between the oxygen consumption of crabs acclimated and tested at ambient temperature and crabs acclimated and tested at high temperature ($P = 0.34$).

Temperature/pCO_2 experiment

The 3-way ANCOVA indicated a significant effect of block on the oxygen consumption rate of crabs ($F_{1,37} = 6.11$, $P = 0.018$). Due to the significant block effect, data for block 1 and 2 were analyzed separately using 2-way ANCOVA. Under the levels of temperature and pCO_2 tested in this experiment, the 2-way ANCOVA of the block 1 data indicated no significant effect of temperature or pCO_2 on the oxygen consumption rate of juvenile crabs ($F_{1,19} = 1.453$, $P = 0.234$ and $F_{1,19} = 0.582$, $P = 0.455$ for temperature and pCO_2, respectively). Similarly, under the levels of temperature and pCO_2 tested in this experiment, the 2-way ANCOVA of the block 2 data indicated no significant effect of temperature or pCO_2 on the oxygen consumption rate of juvenile crabs ($F_{1,19} = 0.567$, $P = 0.462$ and $F_{1,19} = 0.480$, $P = 0.498$ for temperature and pCO_2, respectively).

Discussion

The data from this study indicate that juvenile blue crab are metabolically resilient to predicted future levels of temperature and pCO_2 in the Chesapeake Bay. The temperature shock experiment showed that the oxygen consumption rate of crabs does increase significantly when exposed to a sudden increase in temperature; the oxygen consumption rate of crabs that were acclimated to and measured at high temperature was lower than the oxygen consumption rate of crabs acclimated to ambient temperature but measured at high temperature in the temperature shock experiment.

The goal of this study was to examine potential impacts of future climate conditions on blue crab oxygen consumption rate, when individuals will gradually acclimate to temperature rather than experience an acute temperature shock. Thus, we exposed individuals to increased temperature and pCO_2 for a period of two molts (approximately 30 days) which provided a period of acclimation to conditions prior to the measurement of oxygen consumption. Our analysis revealed a significant difference in the oxygen consumption rate of crabs by block. This was likely due to differences in the time of year (spring versus fall) and collection location (Maryland versus Virginia) between the two blocks. However, patterns in oxygen consumption rate were similar between the two blocks of data. Following exposure to future climate conditions for a period of two molts, neither temperature nor pCO_2 significantly influenced oxygen consumption rate of crabs in either block.

The relationship between temperature and metabolic rate in blue crab is well established (Leffler, 1972; Booth and McMahon, 1992). The lack of a significant temperature effect in the oxygen consumption rate data reported by this study is likely

due to the relatively small difference between treatment temperatures and high individual variability in oxygen consumption rates. Both temperature treatments (26°C and 31°C) are near the maximum of the thermal window for blue crab in the Chesapeake Bay. In other studies on the metabolic rate of juvenile decapod crustaceans, temperatures that showed a metabolic response were farther apart and near the lower end of the thermal window (Leffler, 1972; Gutermuth and Armstrong, 1989; Brown and Terwilliger, 1999). Another explanation for the lack of a significant effect of temperature in this study could be that the crabs acclimated to conditions during the 30 day period prior to measurement of oxygen consumption rate, as has been observed in other species such including fish (Fangue et al., 2014), zooplankton (Zeis et al., 2004), and salamanders (Brown and Fitzpatrick, 1981). However, oxygen consumption rates throughout the acclimation period would be necessary to conclusively determine if the individuals in this study had truly acclimated to conditions.

The high individual variability in oxygen consumption rate of crabs in our study limited the finding of statistical significance, despite the well documented response of the oxygen consumption rate of poikilotherms to increased temperature (Kinne, 1964; Pörtner, 2002). For example, the coefficients of variation by treatment in our study ranged from 28% to 73%, underscoring the variability both within and among treatments. Since the estuarine environment is variable on spatial and temporal scales (both seasonally and daily), variability in physiological responses is advantageous for species, such as blue crab, that live in this environment. Juvenile blue crab migrate from their ingress location at the mouth of the Chesapeake Bay throughout the estuary over the course of a few months and are thus exposed to a wide range of environmental conditions

during this time (Etherington and Eggleston, 2003). Phenotypic plasticity in physiological parameters such as oxygen consumption rate may help crabs survive this migration, and our data suggest that this plasticity may allow for individuals to acclimate to a changing climate.

The data from this study fit well with previous work conducted on the effect of temperature on the metabolic rate of crustaceans. Table 4.2 summarizes results from several previous studies quantifying the effects of temperature on the metabolic rate of *C. sapidus* and another crab species, *Cancer magister*. Although there is variability in the mean oxygen consumption values observed at a given temperature, the coefficient of variation (CV) for the data are remarkably similar and highlight large variability in metabolic rates throughout this taxonomic group. Additionally, apart from the extreme values observed by Leffler (1972), the Q_{10} values are similar among studies. However, it is worth noting that variability in estimates in Table 4.2 is likely due to the considerable differences in species, ontogeny, and measurement methods. For example, the geographic range of the two species in Table 4.2 is markedly different; *Cancer magister*, is found in the deep waters of the north Pacific while *Callinectes sapidus* is largely found in the estuarine waters of the north Atlantic.

More recent studies have generally observed a negative effect of increased pCO_2 on crustaceans, but the magnitude and variability of this effect is species- and life-stage dependent. In a meta-analysis of the respiratory effects of elevated temperature, high pCO_2, and their interaction, Lefevre (2016) found a small, negative and variable effect of increased pCO_2 on the metabolic rate of crustaceans. However this effect was species- and life-stage dependent, and it was not observed in juveniles. Decline in metabolic rate

in response to increased pCO_2 has been observed in embryonic porcelain crab, *Petrolisthes cinctipes* and adult velvet swimming crab, *Necora puber* (Small et al., 2010; Carter et al., 2013). However, increased pCO_2 had no effect on the metabolic rate of larval and juvenile porcelain crab (Carter et al., 2013) and early stage larval lobster, *Homarus gammarus* (Small et al., 2015). The data from the current study indicate no effect of increased pCO_2 on the oxygen consumption rate of juvenile blue crab, contributing to the variety of responses previously reported on the effects of increased pCO_2 on metabolic rate in crustaceans.

Although there was no effect of increased pCO_2 on the oxygen consumption rate of juvenile blue crab, reductions in Q_{10} of individuals at high pCO_2 indicate a reduction in the plasticity of the metabolic response to increased pCO_2 as compared to the plasticity of response to increased temperature. The $Q_{10\ 26\text{-}31}$ of crabs at ambient pCO_2 was at least 2 in both blocks, which is a predictable metabolic response to increased temperature. Conversely, the $Q_{10\ 26\text{-}31}$ of crabs at high pCO_2 was just one-fourth of the $Q_{10\ 26\text{-}31}$ of crabs at ambient pCO_2 in both blocks. The low Q_{10} value of crabs at high pCO_2 may indicate a reduction in metabolic scope when individuals were exposed to acidified conditions. Additionally, the estimate of the oxygen consumption rate at ambient conditions was almost identical to the estimate of the oxygen consumption rate at high temperature/high pCO_2 in both blocks of data examined. Although no significant main effects were observed, it is worth noting the similarity in the observed oxygen consumption rates in the "least stressful" and the "most stressful" conditions. This could be evidence for a narrowing of the thermal window (the threshold identified by the onset of anaerobic metabolism) of juvenile blue crab when exposed to both increased temperature and pCO_2,

which has also been observed in adult edible crab, *Cancer pagurus*, (Metzger et al., 2007) and adult spider crab, *Maja squinado* (Frederich and Pörtner, 2000). Although not commonly observed in the juvenile life stage, antagonistic effects of increased temperature and pCO_2 have been observed in a variety of marine invertebrates (Byrne and Przeslawski, 2013), and could be at play in this system as well, underscoring the importance of measuring multiple stressors when quantifying climate effects on species.

The results from this study highlight the resilience of juvenile blue crab to projected future levels of temperature and pCO_2 and expand the existing knowledge of the effect of increased temperature and pCO_2 on blue crab physiology. The lack of a significant effect of increased pCO_2 on crab growth (Glandon and Miller, 2017) and metabolism (this study) indicate that blue crab are metabolically resilient to future predicted levels of pCO_2 in the Chesapeake Bay. Brylawski and Miller (2006) and Glandon and Miller (2017) have quantified the temperature dependence of growth in juvenile blue crab. Despite the lack of a significant difference in mean oxygen consumption at the temperatures tested in this study, the Q_{10} values from crab at ambient pCO_2 in this study are similar to those determined from the growth rate observations in both studies mentioned above. This highlights the strong relationship between temperature, metabolism, and growth in this species.

The remarkable ability of blue crab to regulate their internal chemistry (Towle et al., 1976; Henry and Kormanik, 1985; Mangum et al., 1985) may reduce the impact of changes in environmental chemistry on crab oxygen consumption and physiology in general. Hemolymph pH was not quantified in this study, but understanding the effects of increased environmental pCO_2 on blue crab hemolymph chemistry is an important next

step in determining the effects of environmental change on blue crab. Regardless of changes to internal conditions, the increase in Q_{10} paired with the growth response to increased temperature may have ecological tradeoffs for blue crab in the future warmer, more acidic Chesapeake Bay. The increased growth rates associated with increased temperature may come at a cost to carapace thickness and chemistry (Glandon et al., *submitted*) and might affect crab protection and mating success as adults. Another tradeoff may be between food consumption and prey quality/availability. Increased temperature will cause individuals to consume more food (Glandon and Miller, 2017). However, bivalve prey of blue crab are expected to experience decreased calcification and growth as water acidifies in the future (Waldbusser et al., 2011b) which may cause crabs to more heavily target weaker prey items and could impact the ability of crabs to maintain high growth rates when exposed to increased temperature. Future studies quantifying the predator/prey interactions of blue crab would help to elucidate the magnitude of future environmental conditions on the Chesapeake Bay food web. Similarly, understanding the combined effects of food availability and climate stressors on the metabolism of blue crab would place the data from this study in a more realistic ecological framework.

Table 4.1. Sample size, mean, and standard deviation (SD) of the oxygen consumption rate (µmol/g/min) of juvenile blue crab by block and temperature/pCO_2 treatment. The 3-way ANCOVA indicated a significant block effect but no significant effect of temperature or *p*CO2 on the oxygen consumption of juvenile blue crab in this study. Q_{10} [26-31] for ambient and high pCO_2 treatments are reported separately.

			Oxygen Consumption Rate (µmol/g/min)			
Block	***p*CO₂**	**Temperature**	***n***	**Mean**	**SD**	**Q_{10}**
1	Ambient	Ambient	8	0.185	0.076	2.308
		High	7	0.281	0.121	
	High	Ambient	5	0.218	0.073	0.504
		High	4	0.155	0.040	
2	Ambient	Ambient	5	0.155	0.074	4.761
		High	8	0.338	0.308	
	High	Ambient	5	0.166	0.055	1.167
		High	8	0.179	0.050	

Table 4.2. Summary of the effects of temperature on the metabolic rate of decapod crustaceans. Coefficient of variation (CV) was calculated as the ratio of standard deviation (SD) to the mean metabolic rate. Q_{10} temperature coefficient was calculated as $(R_2/R_1)^{[10/(T_2-T_1)]}$ for temperature pairs as listed in the table.

Study	Year	Species	Method	Temperature °C	*n*	Mass (g)	Mean O_2 (µmol/g/min)	SD	CV	Q_{10}
This Study	2017	*Callinectes sapidus*	Flow-through chamber	26	13	5.4	0.173	0.074	0.427	3.53
				31	15	5.3	0.259	0.178	0.689	
Booth and McMahon	1992	*Callinectes sapidus*	Respiratory mask	13	6	162	0.010	0.004	0.373	3.10
				20	6	170	0.022	0.005	0.224	2.00
				28	6	154	0.040	0.015	0.368	
Leffler	1972	*Callinectes sapidus*	Closed chamber	13	4	4	0.010	0.004	0.398	12.29
				20	4	4	0.055	0.011	0.208	0.74
				27	4	4	0.045	0.006	0.140	3.01
				34	4	4	0.097	0.019	0.196	
Brown and Terwilliger	1999	*Cancer magister*	Closed chamber	10	4	4	0.021	0.006	0.286	2.14
				20	4	4	0.045	0.015	0.333	
Guteruth and Armstrong	1989	*Cancer magister*	Differential respirometer	6	20	4.8	0.223	0.067	0.298	1.00
				10	23	4.8	0.186	0.071	0.384	4.50
				14	15	4.8	0.335	0.173	0.516	5.50
				18	29	4.8	0.670	0.401	0.598	

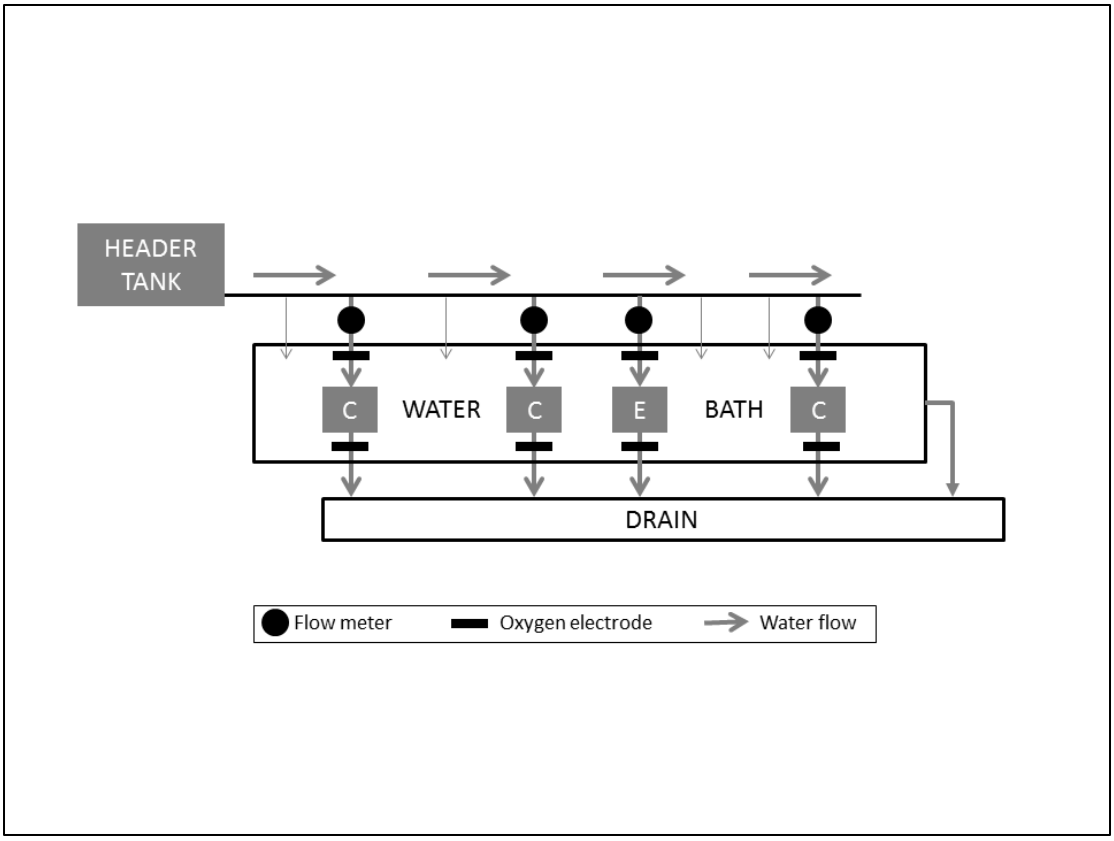

Figure 4.1. Schematic of respirometry chambers used to quantify oxygen consumption of juvenile blue crab in this study. Water was mixed in a header tank to the desired temperature and pCO_2 and then flowed (grey arrows) into a PVC pipe that fed both the respiration chambers and the water bath used to maintain temperature. Water passed through a flow meter (black circle) and an optical oxygen electrode (black rectangle) before entering the chamber. Each chamber contained a single crab (C) and at least one chamber per assay was empty (E). We note that the order of the treatments in each trial was randomized. Water flowed out of the respirometry chamber through another optical oxygen electrode before going into the drain.

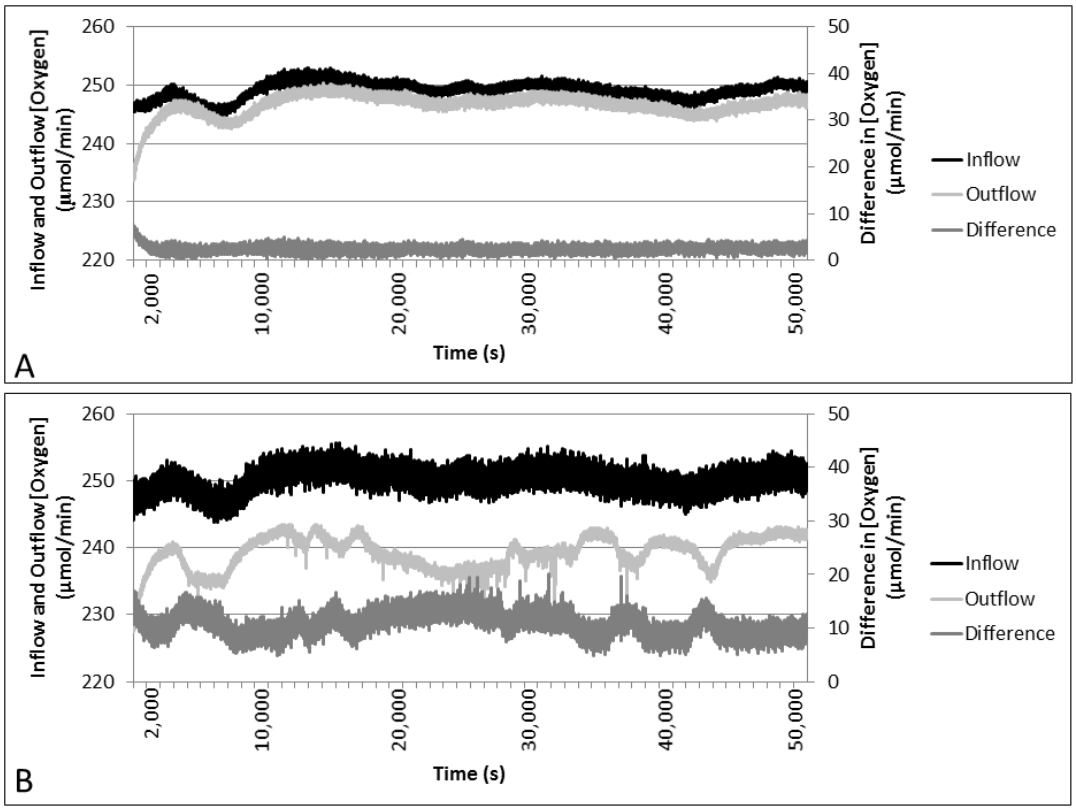

Figure 4.2. Example of data from an overnight respiration trial of an empty chamber (A) and a chamber with a single juvenile blue crab (B).

Chapter 5: An analysis of changes in the overwintering behavior of blue crab in the Maryland portion of the Chesapeake Bay based on historical and future predicted temperature trends in the region.

Abstract

Understanding how increases in water temperature may effect dormancy period duration is important for the effective conservation and management of blue crab in the face of a warming climate. Daily air and water temperature observations collected at the Chesapeake Biological Laboratory (CBL) from 1938-2016 were used to develop a harmonic model that characterized the dynamics of air and water temperature in the Patuxent River, MD, a tributary of the Chesapeake Bay. These data were used to estimate a transfer function which was used in concert with downscaled global climate model (GCM) air temperature projections to forecast water temperature in the Maryland portion of the Chesapeake Bay through the year 2100. The forecast from the downscaled GCM was compared to a conservative estimate of water temperature based on an extension of the current trend in the observational data. For both forecasts, the length of the overwintering period was determined as the number of days below the critical temperature for growth (9°C) in a given year. Downscaled GCM model data predicted a shortening of the current overwintering period from 117±15.5 days to 56±15.1 days. Direct extension of the trend in the observational data predicted a 3 week shortening of the overwintering period to 89.7±0.97 days. This analysis indicates that the length of the overwintering period will be shorter under future warming conditions, and may be absent in warmer years in the Maryland portion of the Chesapeake Bay by the year 2100. The

shortening of the overwintering period has both ecological and economic implications for blue crab in Maryland and the mid-Atlantic region as a whole.

Introduction

Dormancy is a life history strategy employed by a variety of species to survive periods of environmental hardship and can be initiated by external cues or by an internal physiological response (Cáceres, 1997). Environmental variability within a species' range may cause interannual variability in the proportion of the population undergoing dormancy. The blue crab, *Callinectes sapidus*, is an ecologically and economically valuable marine crustacean that is found along the western Atlantic coast from Central America through Massachusetts and through the Gulf of Mexico (Williams, 1984; Hines, 2007; Kennedy et al., 2007). The life history of the blue crab is temperature dependent such that stage duration, and therefore growth rate and maturation, are determined by external temperature (Smith and Chang, 2007). The latitudinal cline in temperature present throughout the blue crab range creates predictable variability in stage duration, growth rates and the likelihood of winter dormancy (Hines et al., 2010). For example, due to colder temperatures, stage duration of blue crab in the northern latitudes is longer than the stage duration of crabs at southern latitudes. Due to slower growth rates, individuals at northern latitudes take significantly longer to recruit to the fishery and reach sexual maturity than individuals from southern latitudes. At southern latitudes, growth rates are faster, allowing for life stage milestones, such as maturity, to be reached in a shorter period of time (Hines et al., 2010). In addition to causing changes to growth rate and

maturation schedules, variability in external temperature likely affects the probability of and duration of dormancy in blue crab.

The critical temperature for growth in blue crab is approximately 9°C, below which metabolic rates decline and dormancy is induced, referred to as overwintering in this species (Smith and Chang, 2007). Since the length of the overwintering period in blue crab is temperature-dependent, the large range of temperature within the geographic range of blue crab causes variability in overwintering behavior. Individuals that live in the central portion of the range, below approximately 32°N latitude, are not exposed to water temperatures below 9°C and therefore do not display overwintering behavior (Fogarty and Lipcius, 2007; Hines, 2007). Conversely, overwintering behavior is reliably displayed by individuals that live in the northern portion of the range, above approximately 38°N latitude (Fogarty and Lipcius, 2007; Hines, 2007). Individuals that live between 32°N and 38°N latitude are thought to be behaviorally flexible such that they overwinter during cold years but can grow throughout the winter in warm years (Rock, pers. comm. 2017; Whitaker, pers. comm. 2017). This variation in overwintering behavior further increases the latitudinal variability in the stage durations and maturation schedules of blue crab. The intensity and duration of overwintering behavior has been directly linked the winter survival of blue crab (Bauer and Miller, 2010) and therefore changes to overwintering behavior may effect blue crab population dynamics.

Anthropogenic release of fossil fuels since the Industrial Revolution has caused an increase in the concentration of carbon dioxide (CO_2) in the atmosphere (Doney and Schimel, 2007). Since 1990, the International Panel on Climate Change (IPCC) has been using models of climate processes to predict future changes in climate on a global scale

(Stocker et al., 2013). Complex global climate models (GCM) have been developed that incorporate appropriate physical processes, weather patterns, and sub-grid-scale stochastic processes. These models have predicted that atmospheric levels of CO_2 will continue to increase to over 1,000 ppm by the end of the 21st century (Caldeira and Wickett, 2003; Orr et al., 2005; Stocker et al., 2013). Due to the greenhouse effect, this increase in CO_2 has caused an increase in atmospheric and, consequently, ocean temperatures, which are predicted to warm by 2.6-4.8°C by the year 2100 (Stocker et al., 2013). Understanding how current warming trends will affect the life history of marine species is critical to predicting the fate of these ecosystems in the face of a changing climate.

Despite the robust nature of GCMs, many questions regarding the effects of climate change require forecasts at regional, rather than global scales. Downscaling of GCM predictions has been proposed to address this issue as a way to understand how GCM predictions may play out in specific environments (Taylor et al., 2012; Maloney et al., 2014). Downscaled models use predictions from an ensemble of runs of a single GCM to yield a model that has more accurate physical forcing and weather data at a smaller geographic scale than the GCMs. It is important to note that the downscaled information can be no more reliable than the climate model simulation that underlies it and therefore downscaled GCM results should be taken with caution. Long-term, high-resolution local environmental data can aid in understanding the climate dynamics of local systems and can improve downscaled GCM projections.

The realization of future climate from any run of a single GCM depends on the specific modeling assumptions, the model's initial conditions, and the model forcings. To

reduce these sources of uncertainty, a standard set of modeling conditions have been used by the World Climate Research Programme's Working Group on Coupled Models, Coupled Model Intercomparison Project (CMIP), to facilitate inter-model comparisons (CMIP; Taylor et al., 2012). Currently in its fifth phase, CMIP5 requires participating research groups to perform an ensemble of GCM simulations. Each element of the ensemble is run under identical experimental conditions, but with different initial conditions which produce different climate trajectories with different, yet equally likely results. The CMIP5 project allows researchers to select an ensemble of a specific GCM for examination or create a multi-model, multi-ensemble analysis in order to account for known GCM biases.

The goal of this study was to explore how predicted changes in water temperature in the Patuxent River, MD, a tributary of the Chesapeake Bay, may impact the overwintering behavior of blue crab in that system. Due to the important ecological and economic role of blue crab, changes in overwintering behavior may directly impact blue crab survival and growth and could have widespread impacts throughout its range. Commercial and recreational crabbing is prohibited in the Chesapeake Bay during winter (generally December – March) in all three management jurisdictions to protect overwintering crabs. This seasonal closure provides a *de facto* marine protected area for blue crab during winter months and is important for the sustainable management of blue crab in the mid-Atlantic. Predicted increases in temperature may increase the length of the growing season of crabs in the Chesapeake Bay and could initiate changes to the length of the wintertime closure of crab fishing in the Chesapeake Bay. Understanding

the ecological and economic impacts of changes to the length of the wintertime closure is critical to the ongoing sustainable management of this species in the region.

To determine how blue crab overwinter behavior may change in a warming climate, we accessed a high-resolution, long-term environmental dataset to inform a model that utilized downscaled global climate model data from the CMIP5 project to predict water temperature in the Patuxent River, Chesapeake Bay. Water temperature predictions were then used to estimate the length of the overwintering period of blue crab in the Maryland portion of the Chesapeake Bay through the year 2100.

Methods

Observational data collection and processing

Air and water temperatures have been observed since 1938 at the end of a 274m pier located at the Chesapeake Biological Laboratory, located in Solomons, MD. Observations were taken manually at noon using a research grade mercury thermometer from 1938-2012. From 2012 to 2016, water temperatures were recorded every 15 minutes via YSI EX02 multi-parameter sonde (Yellow Springs Instruments, Inc., Yellow Springs, OH). Air temperature data were recorded every 15 minutes via Davis Vantage Pro 2 (model 6152) meteorological station (Davis Instruments, Haywood, CA). Although largely complete, missing values were replaced with nearby observational data from the Chesapeake Bay Program (CBP) and the National Oceanic and Atmospheric Administration (NOAA) buoy system. The relationship between CBL pier data and other sources (CBP and NOAA) was assessed using major axis (MA) regression. The slope, intercept, R^2 value, and P-value of the MA regression of the CBL pier data with the other

sources were analyzed to evaluate the suitability of the replacement data, and to determine if the replacement data may introduce new patterns in the CBL data.

Prediction of future water temperature

Future water temperatures in the Patuxent River were predicted from both an extension of patterns in current observations and from projections of down-scaled global climate models. Climate models directly forecast air temperature only, and thus a transfer function that estimated water temperatures expected given air temperatures was developed. The process of predicting water temperature from air temperature projections and then applying the temperature data to estimate crab overwintering behavior is summarized in Figure 5.1. Specific statistical details of the calibration and validation of the models and the development of the transfer function are provided in Appendix A, and are only summarized here.

Harmonic regression models were fit to observed air and water temperature to characterize the seasonal cycle of air and water temperature (Miller et al., 1995). Following appropriate calibration and validation, these models were used to develop a transfer function that estimated water temperature from air temperature (Cho and Lee, 2011; Appendix A).

The transfer function was used to predict daily future water temperature from two data sources: extension of the observational data trend (extended data) and the downscaled CMIP5 data (downscaled CMIP5). The least squares parameter estimates of the transfer function were used directly to extend patterns in the observed data from 2016-2100. Future predicted air temperature data were accessed from downscaled GCM

data available on the CMIP3 and CMIP5 downscaled climate and hydrology projections website (http://gdo-dcp.ucllnl.org/downscaled_cmip_projections/dcpInterface.html#Welcome). Data 42 ensemble runs for the east coast basin near Solomons, Maryland were downloaded for January 1950-December 2099. Daily minimum and maximum 1/8 degree downscaled bias-corrected constructed analogs, version 2 (BCCAv2) projected surface air temperature were downloaded for representative concentration pathway (RCP) 8.5. Groups and ensemble runs are shown in Table 5.1. Daily minimum and maximum values from each of the 42 ensemble members were averaged to create a single daily observation of air temperature.

Uncertainties in forecasted temperatures were calculated differently for the extended data and the downscaled CMIP5 projections. For the extended data, we resampled regression parameters from a normal distribution defined by the mean and variance of the original parameter estimate, generating 50 new forecasted temperature projections. This created 50 separate projected temperature time series, and we subsequently calculated the daily mean (± SD) temperature. For the downscaled CMIP5 projections, we carried forward the predictions of air temperature from each of the 42 individual ensemble members and then calculated mean (±SD) daily temperature.

Determination of changes in overwintering period of crab

The number of days when water temperature was predicted to be below the critical temperature for growth (9°C; Brylawski and Miller, 2006) were determined for the two sources of time series of predicted water temperature (extended data and downscaled CMIP5). The number of days below 9°C represents the length of the

overwintering period of crab in any given year. Winter years were determined as the bridge of calendar years (e.g.: winter 1990-1991) since wintertime temperatures occur between calendar years in the Chesapeake Bay. Changes in the length of the overwintering period with year were examined using linear regression. The length of the overwintering period predicted by the extended data was compared to the length of the overwintering period predicted by the downscaled CMIP5 data in four year groups (2017-2030, 2031-2050, 2051-2070, 2071-2100) using a t-test. Data processing and analyses were conducted in R (version 3.2.2 -- R Core Team, 2015) using R-Studio (version 0.98.1103) using an alpha level of $P < 0.05$ for all analyses.

Results

Observational data

Missing values in CBL air temperature data were filled in using data from NOAA buoys at Cove Point (buoy number 8577018, COVM2) and Solomons (buoy number 8577330, SLIM2) and missing values in CBL water temperature were filled in using data from CBP water quality stations LE1.3 and LE1.4 and NOAA Solomons buoy (buoy number 8577330, SLIM2). Major axis regression of the CBL data with all other sources indicated a high degree of similarity between data sources (Figure 5.2). Table 5.2 summarizes the intercept, slope, R^2 value, and P-value of each relationship examined. We filled data gaps representing 11.7% and 8.3% of the total CBL air and water temperature time series, respectively.

Prediction of future water temperature

The transfer function estimated from the observed data was used to predict water temperatures based on projected air temperature from the extension of the current trend and the downscaled CMIP5 model data. The climatology of water temperature in the reference period (1961-1990) and the prediction period (2070-2099) for both data sources indicates that the temperature during the average year in the prediction period will be significantly warmer than the temperature during the average year in the reference period (Figure 5.3). The warming trend observed using the downscaled CMIP5 data was more drastic than the trend from extended data (Figure 5.4).

Impact of predicted changes in water temperature on blue crab overwintering period

The number of days below 9°C was quantified for each year of observational data and for both sources of time series predicted data (extended trend and downscaled CMIP5 data). Despite the addition of supplementary data sources to fill in missing observational data from the CBL pier, the annual data from 1977-2005 contained less than 300 daily observations of temperature. The lack of a complete register of daily temperatures made calculations of the number of days less than 9°C threshold for these years unreliable. Therefore, these years were excluded from the analysis of the trend in the length of past overwintering periods.

The length of the overwintering period in the observational data ranged from 145 days in 1943 to 75 days in 2012 with an average length of 117.0 ± 15.5 days (standard deviation). Despite high interannual variability, a significant decline in the length of the

overwintering period with time was observed in this dataset (P<0.05, R^2=0.37; Figure 5.5).

The extended data predicted the overwintering period declined to 89.7±0.97 days by the year 2100 while the downscaled CMIP5 model data predicted the overwintering period declined to 56±15.1 days by the year 2100. The length of the overwintering period estimated from the extension of the current trend began to diverge from the length of the overwintering period estimated from the downscaled CMIP5 data by 2040 (Figure 5.5). After this point, the length of the overwintering period estimated from the downscaled CMIP5 data drastically declined relative to the length of the overwintering period estimated from the extended data. The t-test indicated that the estimated length of the overwintering period was significantly different between the two data sources in each year group examined (2017-2030, 2031-2050, 2051-2070, 2071-2100; P<0.05 for all year groups).

The number of days below 9°C at various locations along the east coast of North America from 2012-2016 were collected from the NOAA National Buoy Data Center and are shown in Figure 5.6. A linear regression of these data with latitude provided estimates of the latitude equivalent to the number of days below 9°C predicted by the extension of the observational data and the downscaled CMIP5 data. According to the regression of the number of days below 9°C and latitude, the number of days below 9°C in the year 2100 predicted by the extended data is equivalent to the current number of days below 9°C in Newport News, Virginia and the number of days below 9°C in the year 2100 predicted by the downscaled CMIP5 model data is equivalent to the current number of days below 9°C in Morehead City, North Carolina.

Discussion

Our analyses indicate that water temperature in the Maryland portion of the Chesapeake Bay will be significantly warmer in the future. Average annual temperature in the reference period (1961-1990) was 15.1°C, and the average year in the prediction period (2070-2099) will be significantly warmer than the average year in the prediction period under both warming scenarios. The extension of the current empirical trend indicates an average annual temperature increase of 2.4°C during the prediction period and the downscaled CMIP5 model predictions indicate an annual temperature increase of 4.1°C in the prediction period.

We observed a significant negative trend in the number of days below 9°C with time in the historical water temperature data from the CBL pier. Using the first order harmonic transfer function, we determined that this shortening trend will continue in the future under both a conservative estimate of future warming using the extended trend of historical data and a more aggressive RCP 8.5 warming scenario from the downscaled CMIP5 data. The number of days below 9°C was predicted to decline from an average of 115 days to 89 days based on the conservative estimate of the change in future water temperature and to 56 days long based on the downscaled CMIP5 model outputs.

The overwintering behavior of blue crab is a response to a seasonal decrease in environmental temperature (Tankersley and Forward, 2007). Blue crab occurs along the eastern coast of North America and the overwintering behavior of individuals is latitude-dependent, with crabs at higher latitudes overwintering for a longer time than crabs at lower latitudes. Blue crab in the Chesapeake Bay region and to the north are known to

overwinter and crab from Georgia to the south do not overwinter at all (Fogarty and Lipcius, 2007; Hines, 2007). Overwintering behavior is variable by year in the area between Virginia and Georgia; during cold winters individuals may overwinter for a short time but warmer winters allow for year round growth in this region (Rock, pers. comm. 2017; Whitaker, pers. comm. 2017). The inconsistent nature of overwintering behavior of crabs from Virginia to Georgia is driven by the temperature regime in a given year; overwintering occurs in cold years but may not occur in years with milder winters. Therefore, the probability of overwintering at a given location along the east coast of the US is determined not only by latitude, but by other factors including the severity of winter. This concept is illustrated in Figure 5.7, which displays the number of days below 9°C at various latitudes along the US east coast together with a hypothetical logistic curve that describes the likely pattern in the probability of overwintering at a given latitude, based on knowledge of local crab behavior. For example, overwintering does not occur at lower latitudes near Florida and Georgia. Conversely, overwintering occurs each year at higher latitudes near Maryland and Delaware. The dashed grey bands are possible 95% confidence intervals around the logistic curve and represent the inconsistent nature of overwintering behavior at these middle latitudes.

Our analyses predict that by the year 2100, the temperature regime of the Chesapeake Bay will likely resemble the current regimes present in the Sounds of North Carolina. Therefore, it is reasonable to assume that the future overwintering behavior of blue crab in Chesapeake Bay will change to resemble the behavior currently observed in the Sounds of North Carolina and further south. Under current conditions, overwintering behavior in the Maryland portion of the Chesapeake Bay is consistent; crab always

overwinter and the length of the overwintering period is determined by the severity of winter. In contrast, blue crab populations in North Carolina overwinter inconsistently; in colder years individuals display overwintering behavior but in warmer years they may display year round growth if water temperatures never fall below 9°C. Assuming similar variability in the length of winter in the future as was observed in the past, the probability of crab overwintering in Maryland will decline and in some years individuals may not overwinter in this region at all. We infer from our analyses that blue crab overwintering behavior in the Maryland portion of the Chesapeake Bay will change from a pattern in which blue crab overwinter consistently to one in which overwintering of blue crab occurs inconsistently, sensitive to winter severity.

The shortening and inconsistent nature of the overwintering period predicted by our analysis will lengthen the crab growing season in the Maryland portion of the Chesapeake Bay in the future and this has implications for the ecology of blue crab in the Chesapeake Bay. Additionally, predicted increases in temperature will not occur without other environmental changes resultant from climate change, including increases in atmospheric, and therefore oceanic pCO_2. Although blue crab growth is not expected to be directly affected by increasing pCO_2,(Ries et al., 2009; Glandon and Miller, 2017), bivalve prey of crab are expected to experience decreased calcification and growth as water acidifies in the future (Waldbusser et al., 2011b). The increase in energetic demands as a result of the predicted increase in the growing season combined with an increase in the vulnerability of crab prey in the more acidic water of the future may cause crab to target weaker prey items more heavily and could have ecosystem-wide effects. Additionally, there is evidence that the increased growth rates associated with increased

temperature may come at a cost to blue crab carapace integrity (Glandon et al., *submitted*). In order to sustain increased growth rates when exposed to high temperature, the percent of high-magnesium calcite in the carapace declined, which has been associated with declines in carapace strength in other invertebrates (Jordaens et al., 2006; Amato et al., 2008; Fabritius et al., 2012). As described above, the physiological response of blue crab to climate change stressors is complex and has been shown to be life-stage dependent in other species (Small et al., 2010; Carter et al., 2013). Despite the fact that the adult life stage is targeted by the fishery, effects of climate change on adult blue crab have yet to be determined. Taken together, these tradeoffs underscore the complexities involved in predicting the population level impacts of climate change, even on a single, well-studied species such as the blue crab.

The predicted changes to the temperature regime in the Maryland portion of the Chesapeake Bay also have implications for the management of the blue crab fishery in this region. Currently, crabbing is currently prohibited during the overwintering period (December-March) in all three management jurisdictions in the Chesapeake Bay. This creates a *de facto* closed season for crab during the winter. The positive response of the population to the closure of a winter fishing in the lower Bay in 2008 shows the role of this closed season in maintaining the Chesapeake Bay crab population at sustainable levels (Miller et al., 2011). However, the increase in temperature and subsequent increase in wintertime crab activity may serve to increase the length of crabbing season, as is currently the practice in the lower latitude states where crab are active year-round. Although it is possible that the increases in growth rate resulting from increased temperature could sustain a longer crab fishery, the predicted inconsistent nature of

overwintering behavior in the Maryland portion of the Chesapeake Bay in the future complicates the wintertime management of this species. Reductions to, or elimination of, the wintertime closure in response to increases in average wintertime activity might drastically reduce population levels during cold years when crab will likely still overwinter. Therefore, despite the warming trends observed in this study, until crabs are consistently active year-round, the elimination of the wintertime fishery closure may put immobile, overwintering crab at great risk during cold years.

The conclusions from this study are limited to a small spatial area and a single definition of overwintering. Our analysis focused on the Maryland portion of the Chesapeake Bay because of the substantial time series of temperature data available for that area. However, the warming trend observed in our analysis will be similar to the trend in the mid-Atlantic region and likely the eastern US coast (Najjar et al., 2009). Therefore blue crab will be exposed to warming temperatures throughout its range during the next century and the predicted changes in Chesapeake Bay blue crab behavior in Maryland may also be observed at other locations along the US east coast. Additionally, the overwintering period was defined in this study simply as the total number of days below 9°C, despite known fluctuations in daily temperature and therefore crab behavior, as well as a possible lag in crab overwintering behavior at the temperature threshold of 9°C. A more realistic representation of the true start of the overwintering period of crab in the fall could be after the water temperature is below 9°C for a given period of time (e.g.: one week), such that the temperature would be long enough for crab metabolic rate to slow and burial would be required for survival, which would delay the true start of overwintering in the fall. Similarly, individuals likely do not come out of the sediments

on the first day above 9°C, lengthening the true period of time crab are buried in the spring. We did not examine the variability in warming trends by season in this study, but seasonal variation of the impact of warming could affect fall and spring temperatures differently and might change the length of the overwintering period of blue crab in the future. Further analysis of the predicted changes in the temperature regime could determine changes in the seasonality of winter (a later start to winter, for example) and are an important area for future research.

Understanding the physiological effects of climate change throughout the life cycle of the blue crab could greatly improve the ability of our model to predict population level effects of warming in this system. Possible effects of climate change on adult crab have important implications for fisheries management. Significant carry-over effects have been observed in embryonic and larval Tanner crab, *Chionoecetes bairdi*, from adults exposed to acidified conditions over two brood cycles (Long et al., 2016a) and would be important to quantify in concert with possible generational effects of increased temperature. Although downscaled GCM data are preferable to GCM output for this study, downscaling has inherent limitations and biases. Improvement of downscaling approaches would benefit the analysis in this study and would allow for more accurate predictions regarding the behavior of this ecologically and economically important species in the future.

Table 5.1. Research groups that contributed model ensembles to the downscaled CMIP5 data used in this study.

Group	Number of Ensemble Members (RCP 8.5)
Commonwealth Scientific and Industrial Research Organization and Bureau of Meteorology, Australia	1
Beijing Climate Center, China Meteorological Administration	1
Canadian Center for Climate Modelling and Analysis	5
National Center for Atmospheric Research	2
Community Earth System Model Contributors	1
Centre National de Recherches Meteorologiques/Centre Europeen de Recherche et Formation Avancee en Calcul Scientifique	1
Commonwealth Scientific and Industrial Research Organization, Queensland Climate Change Centre of Excellence	10
NOAA Geophysical Fluid Dynamics Laboratory	2
Institute for Numerical Mathematics	1
Institute Pierre-Simon Laplace	5
Japan Agency for Marine-Earth Science and Technology, Atmosphere and Ocean Research Institute (The University of Tokyo), and National Institute for Environmental Studies	2
Atmosphere and Ocean Research Institute (The University of Tokyo), National Institute for Environmental Studies, and Japan Agency for Marine-Earth Science and Technology	3
Max Planck Institute for Meteorology	4
Meteorological Research Institute	1
Norwegian Climate Centre	1

Table 5.2. Intercept, slope, R^2 value and *P*-value of the major axis regression of CBL temperature with temperature observations from other sources. CBL: Chesapeake Biological Lab, CP: NOAA Cove Point buoy, Sol: NOAA Solomons buoy, CBP: Chesapeake Bay Program buoy.

Relationship	**Intercept**	**Slope**	**R^2 value**	***P*-value**
CBL Air – CP Air	0.562	0.942	0.947	0.01
CBL Air – Sol Air	0.415	0.948	0.949	0.01
CBL Water – CBP Water	-0.338	0.963	0.984	0.01
CBL Water – Sol Water	0.026	0.990	0.995	0.01

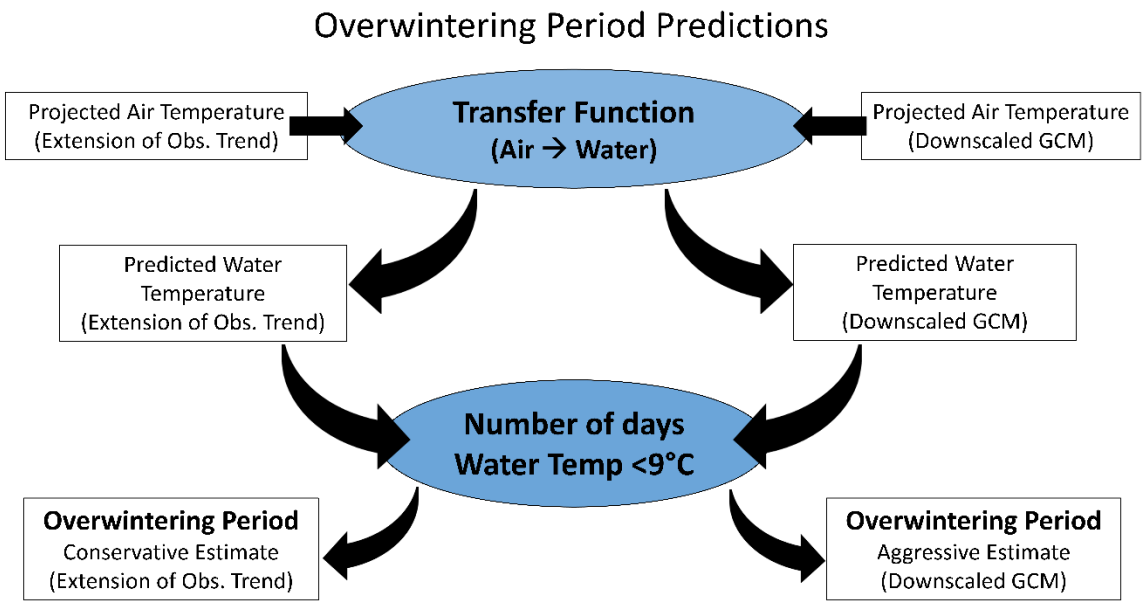

Figure 5.1. Schematic of the utilization of the transfer function to predict the length of the overwintering period of blue crab through the year 2100. Daily projected air temperature was accessed from two sources: the extension of the observational trend of increasing water temperature in the historical data and downscaled CMIP5 model projections. The projected air temperature time series were used with the transfer function to predict future water temperature based on those two data sources. The number of days below 9°C for each source of future temperature data was used as a proxy for the overwintering period of crabs under both the conservative (extension of the observational data trend) and the aggressive (downscaled GCM) warming scenarios.

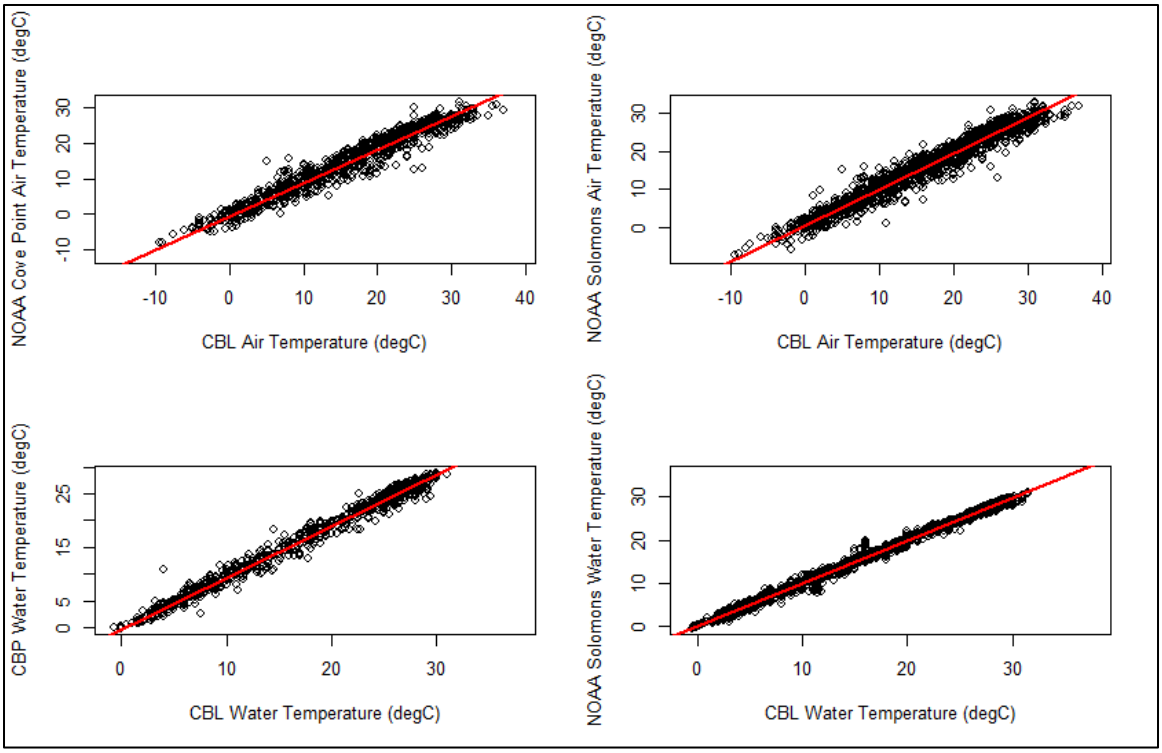

Figure 5.2. Temperature observations at the Chesapeake Biological Lab (CBL) by temperature observations from other the National Oceanic and Atmospheric Administration (NOAA) and the Chesapeake Bay Program (CBP). The red line in each plot indicates a major axis regression of CBL temperature observations with temperature observations from other sources. All relationships had a high degree of similarity (R^2>0.94 and P<0.01 for all regressions).

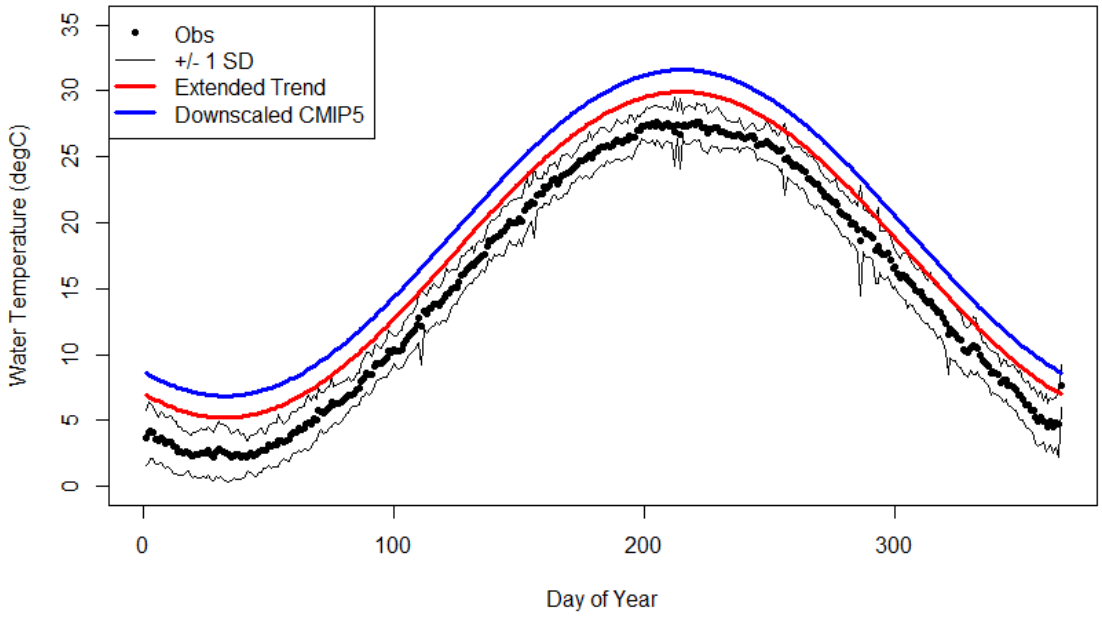

Figure 5.3. Climatology of observed historical water temperature (Obs) in the reference period (1961-1990) and the predicted future water temperature (red and blue lines) in the prediction period (2070-2099). Both the conservative estimate of the increase in water temperature (Extended Trend, red line) and the predicted water temperature from the downscaled CMIP5 models (Downscaled CMIP5, blue line) predict the average year in the prediction period will be warmer than the average year in the reference period.

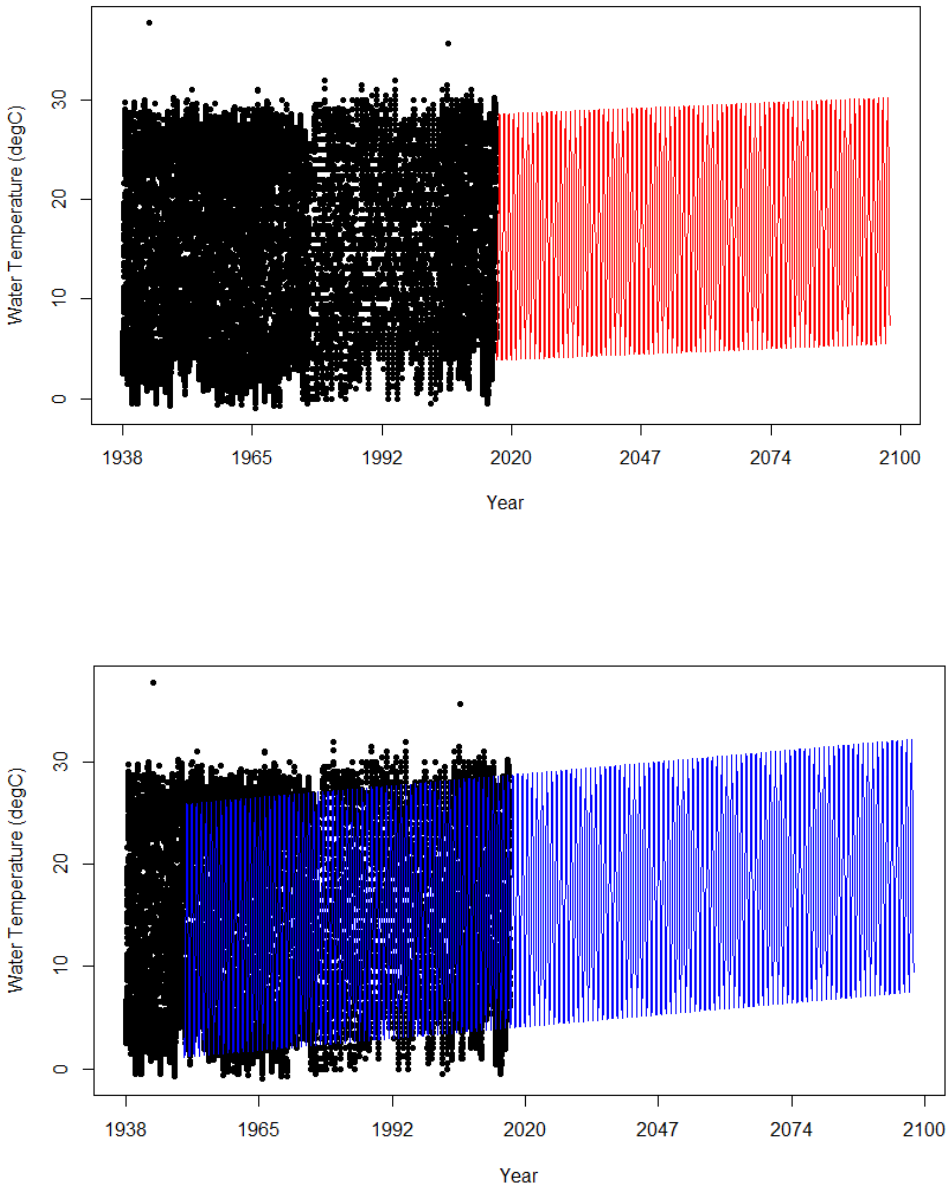

Figure 5.4. Historical observed water temperature (black dots) and predicted water temperature using the extended trend in the observational data (upper panel, red line) and the trend in the downscaled CMIP5 model air temperature from 1950-2099 (lower panel, blue line).

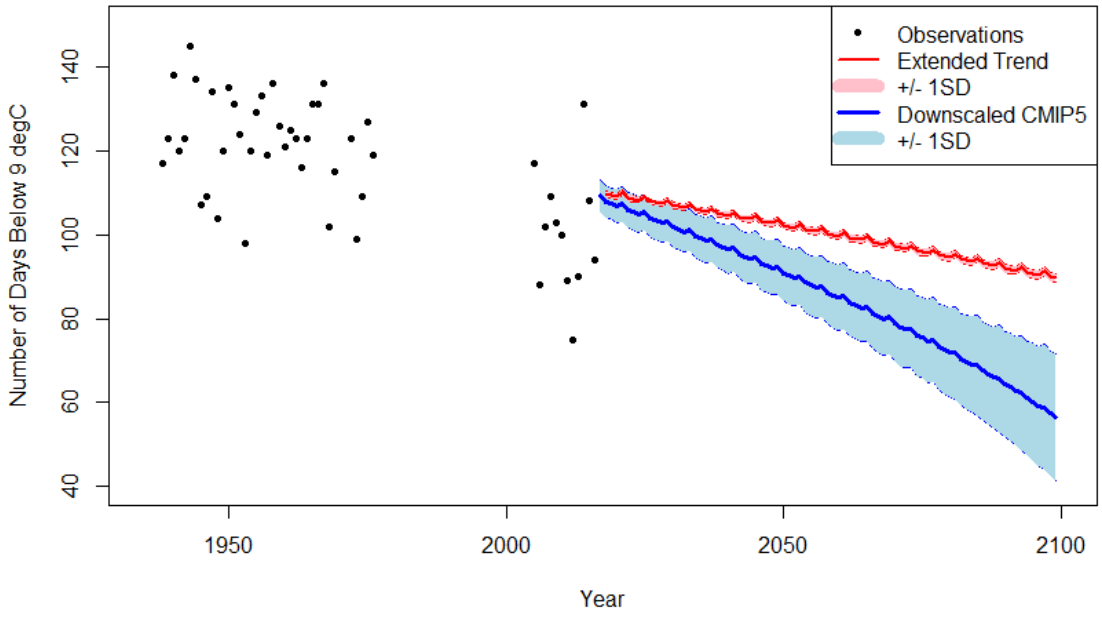

Figure 5.5. Length of crab overwintering period (expressed as the number of days below 9°C in a given year) for observational data (black dots) and predicted data from two sources (conservative estimate (Extended Trend) in red and downscaled CMIP5 model data (Downscaled CMIP5) in blue). Years with less than 300 observations of water temperature were excluded from this analysis. Error bands for predictions represent one standard deviation.

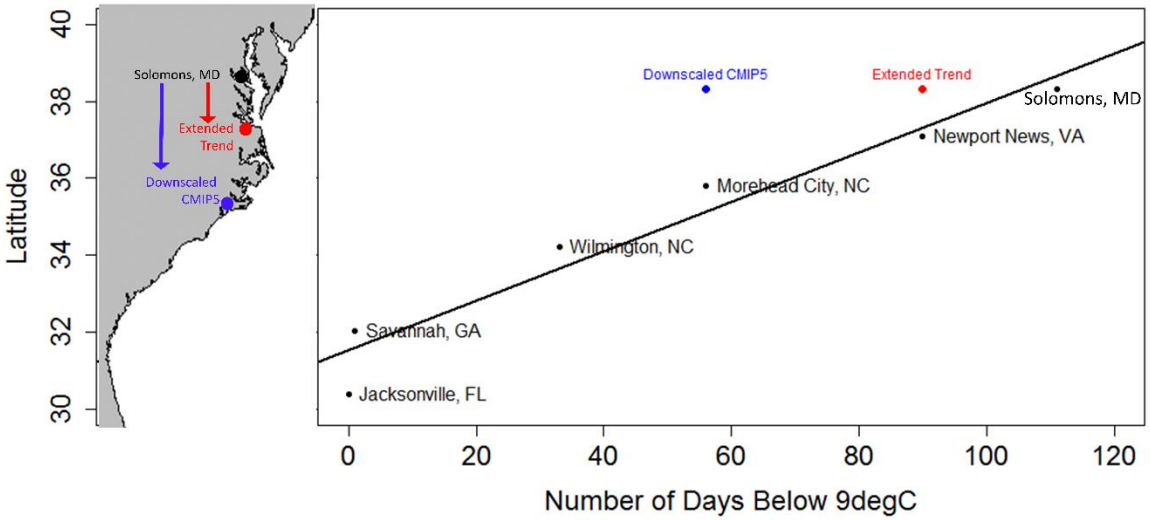

Figure 5.6. Number of days below 9°C by latitude at different locations along the east coast of the US from 2012-2016. Points in black indicate observational data from the CBL Pier and the National Data Buoy Center. The black line is the linear regression of the observations with latitude. The red and blue points indicates the location equivalent to number of days below 9°C predicted by the extended trend and the downscaled CMIP5 data, respectively. Direct extension of trend in the observational data predicted a shortening of the overwintering period from 117±15.5 days to 90±0.93 days and the downscaled CMIP5 model data predicted a shortening of the current overwintering period to 56±14.7 days.

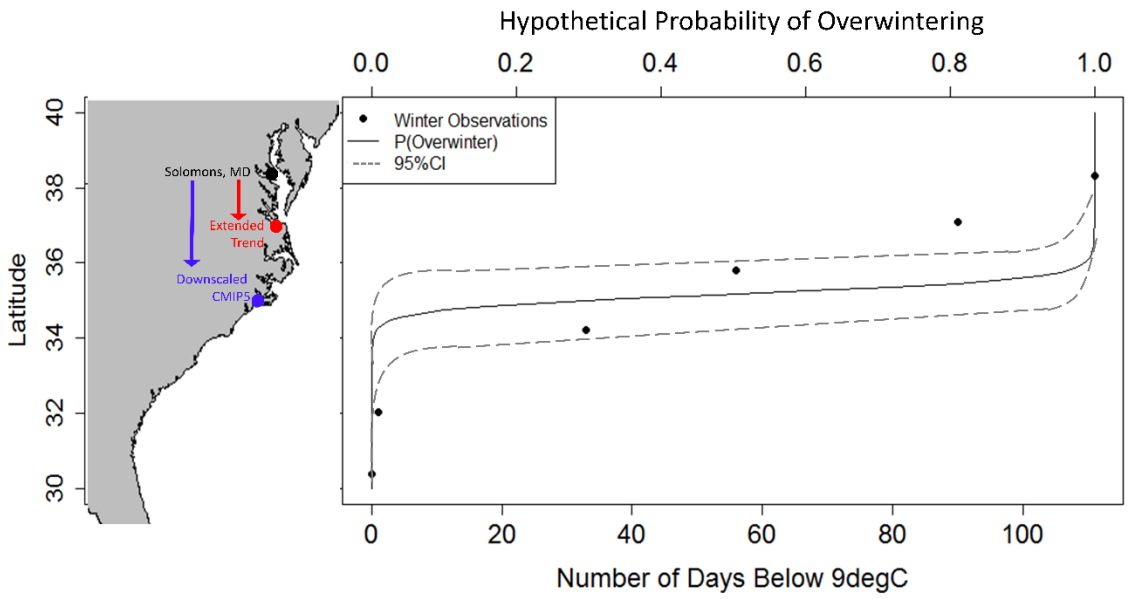

Figure 5.7. The number of days below 9°C at various latitudes along the US east coast (black dots) and a logistic curve representing the hypothetical probability of blue crab overwintering at a given latitude, based on knowledge of local crab behavior. The dashed grey bands are 95% confidence intervals around the logistic curve and represent the inconsistent nature of blue crab overwintering behavior at these middle latitudes. The extension of the trend in the observational data predicts that the length of winter in the year 2100 will be equivalent to the current length of winter in Newport News, VA and is shown as the red dot on the map. The downscaled CMIP5 model data predicts that the length of winter in the year 2100 will be equivalent to the current length of winter in Morehead City, NC and is shown as the blue dot on the map.

Appendix A: Model calibration and validation and development of the transfer function.

Estimation of the harmonic model and transfer function

The calibration, validation and estimation of the transfer function are summarized in Figure 5.8. Harmonic models were developed to characterize the seasonal cycle of air and water temperature known to exist in mid-latitude estuaries. These models were used to develop a transfer function that estimated water temperature from air temperature.

The available time series of air and water temperatures were divided into calibration (C) and validation (V) datasets. Harmonic regressions were fit to both the air and water temperature calibration data sets separately. The ability of the harmonic function to predict the two individual calibration data sets (air and water temperature) was then assessed using the independent validation data set. Subsequently, the air and water harmonic models were used to develop a transfer function that estimated water temperature from air temperature, and the ability of the transfer function to predict water temperature was then assessed using the independent validation data set. Following the methods of Cho and Lee (2011),

Equation 1a: $AT_E(t_i) = B_{0A} + B_{1A}t_i + \sum_{m=1}^{M}[CA_m^C \cos(w_m t_i) + SA_m^C \sin(w_m t_i)]$

Equation 1b: $WT_E(t_i) = B_{0W} + B_{1W}t_i + \sum_{m=1}^{M}[CW_m^C \cos(w_m t_i) + SW_m^C \sin(w_m t_i)]$

where A refers to air, W to water, T to temperature, B_{0A} and B_{0W} are the intercepts of the linear regression of temperature with time, B_{1A} and B_{1W} are the slopes of the linear regression of temperature with time, M is the maximum order of the harmonic

function, CA_m, CW_m and SA_m, SW_m are the harmonic cosine and sine coefficients of the order m, and superscript C denotes the calibration stage. For harmonic regression, the frequency term is expressed as $w_m = 2\pi m/365$.

The observational data were split into consecutive calibration and validation sets in order to determine the most parsimonious model for the data. The size of the calibration set was determined by the root mean squared error (RMSE, Equation 2) of the predicted values (T_E) compared to the observed values (T_O) in both the calibration for air and water temperature.

Equation 2: RMSE = $\sqrt{\frac{\sum_{i=1}^{N}[T_E(t_i) - T_O(t_i)]^2}{N-1}}$

Once the size of the calibration set was determined, information theoretic statistical criteria (AIC and ΔAIC) were used to determine the most parsimonious harmonic order for both air and water temperature in the calibration data. The ability of the most parsimonious models of air and water temperature to predict temperature in the validation set was evaluated using RMSE.

Validation of the air-water temperature transfer function

The ratio of the coefficients of the water temperature calibration model to the coefficients of the air temperature calibration model were utilized to predict water temperature in the validation set by the following equation:

Equation 3: $WT_E(t_i) = B_0 + B_1 t_i + \sum_{m=1}^{M}[cr_m CA_m^V \cos(w_m t_i) + sr_m SA_m^V \sin(w_m t_i)]$

where B_0 is the intercept of the relationship used to characterize the change in daily temperature, B_1 is the slope of the relationship used to characterize the change in daily temperature, and cr_m and sr_m are CW_m^C / CA_m^C and SW_m^C / SA_m^C.

Model calibration and validation results

The performance of models when the calibration set consisted of 50% and 70% of the observational air and water temperature data was evaluated over model orders 1-40 using root mean squared error (RMSE). The calibration model was marginally better (lower RMSE) at predicting the calibration data for both air and water temperature when the calibration set consisted of 50% of the data (Figure 5.9). However, the calibration model was considerably better at predicting the validation data for both air and water temperature when the calibration set consisted of 70% of the data (Figure 5.9). Therefore, the determination of model order was conducted on the calibration set which consisted of 70% of the data.

ΔAIC values for alternative models indicated that a 22nd order harmonic was the most parsimonious model for air temperature and a 17th order harmonic was the most parsimonious model for water temperature. However, the high frequency of the observational data may have caused the high order harmonic to over-fit the data, as shown in the example in Figure 5.10. Due to possible over-fitting of the data, future temperature predictions were conducted using 1st, 10th, and 20th order harmonics and results were examined for significant differences in the temperature predictions by model order. Results indicated no significant difference in future water temperature predictions by model orders examined.

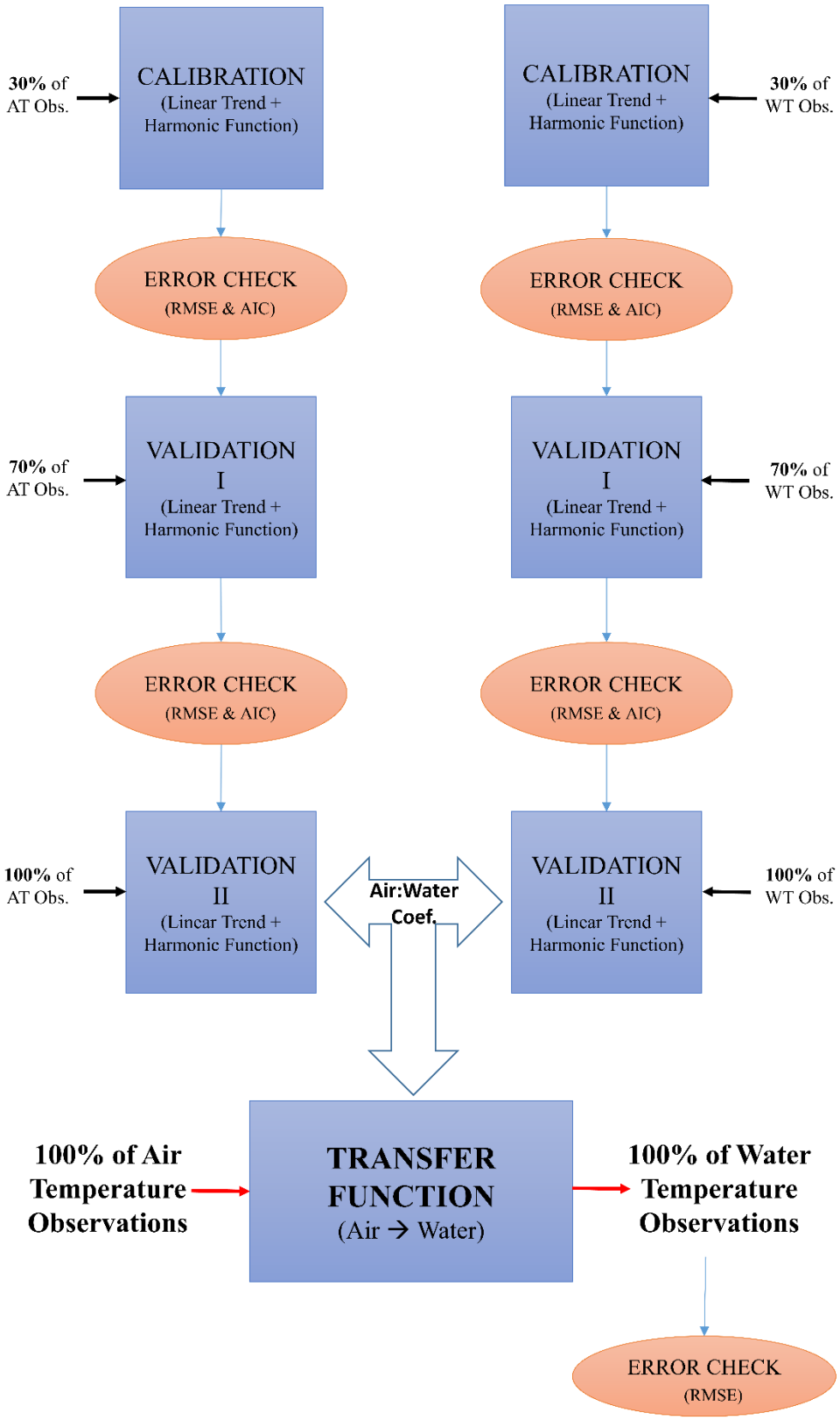
30% of AT Obs.
CALIBRATION (Linear Trend + Harmonic Function)
CALIBRATION (Linear Trend + Harmonic Function)
30% of WT Obs.
ERROR CHECK (RMSE & AIC)
ERROR CHECK (RMSE & AIC)
70% of AT Obs.
VALIDATION I (Linear Trend + Harmonic Function)
VALIDATION I (Linear Trend + Harmonic Function)
70% of WT Obs.
ERROR CHECK (RMSE & AIC)
ERROR CHECK (RMSE & AIC)
100% of AT Obs.
VALIDATION II (Linear Trend + Harmonic Function)
Air:Water Coef.
VALIDATION II (Linear Trend + Harmonic Function)
100% of WT Obs.
100% of Air Temperature Observations
TRANSFER FUNCTION (Air → Water)
100% of Water Temperature Observations
ERROR CHECK (RMSE)

Figure 5.8. Flow chart outlining the procedure for calibrating and validating the air and water temperature models that led to the development of the transfer function. Air and water temperature models were calibrated separatelying using 30% of the observational data and validated separately using 70% of the observational data. Model performance was checked using RMSE and the most parsimonious model order was determined using AIC. After calibration and validation, model coefficients were determined using 100% of the air and water temperature observations (Validation II). The ratio of the air coefficients to the water coefficients was used to develop the transfer function, which converted air temperature to water temperature. The performance of the transfer function in predicting water temperature in the observational dataset was evaluated using RMSE. AT: air temperature, WT: water temperature.

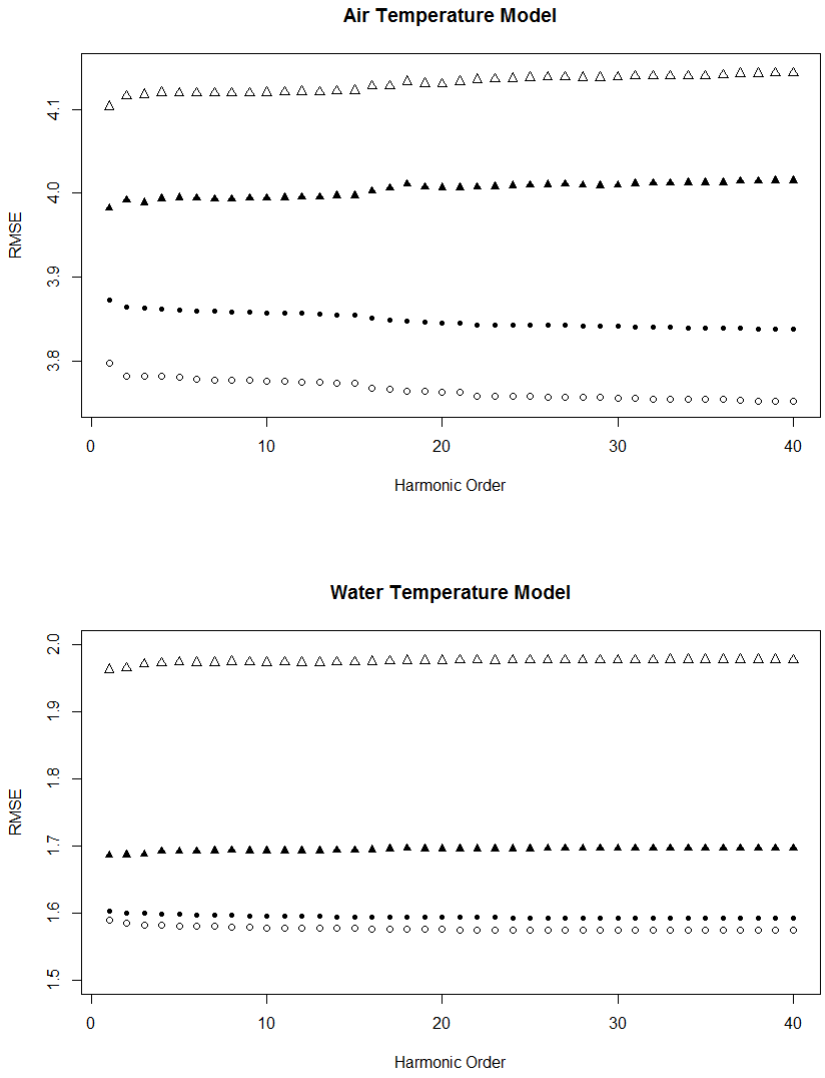

Figure 5.9. Root mean squared error (RMSE) of the air temperature model (upper panel) and the water temperature model (lower panel) for calibration and validation over 40 harmonic orders. Open symbols represent model results when 50% of the data were used in the calibration set, closed symbols represent model results when 70% of the data were used in the calibration set. Circles represent model results when predicting calibration data and triangles represent model results when predicting validation data.

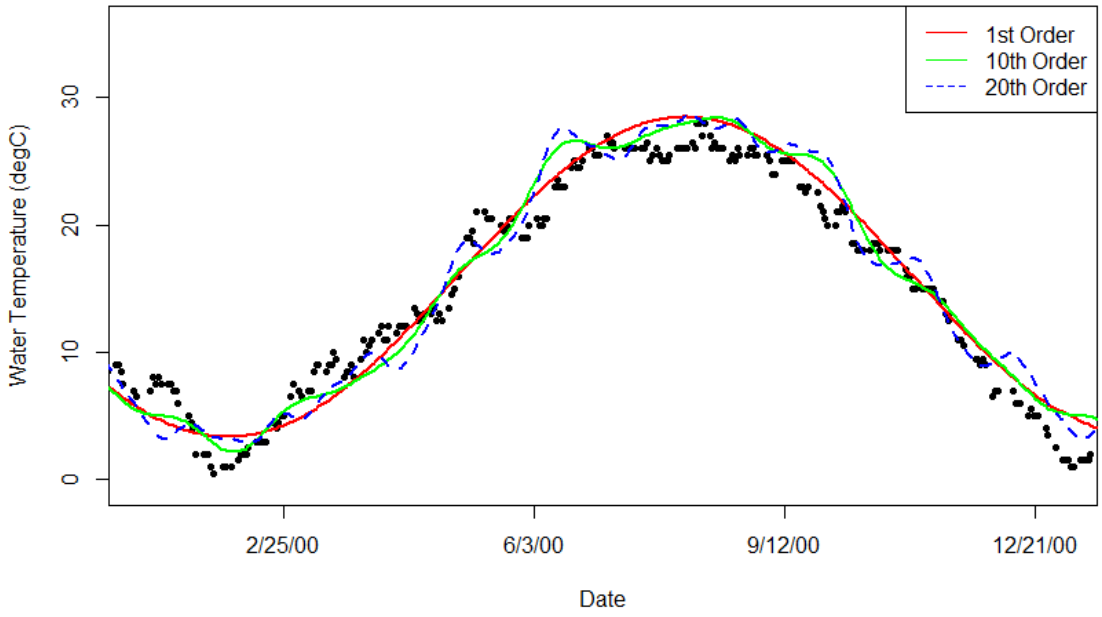

Figure 5.10. Example of 1st, 10th, and 20th order harmonic model fit to observational data from the year 2000. Although the ΔAIC analysis indicated that 17th and 22nd order models were the most parsimonious for air and water temperature, respectively, the higher order harmonics are clearly over-fitting the observational data.

Chapter 6: Discussion

The results of my dissertation indicate that juvenile blue crab, *Callinectes sapidus,* are well equipped physiologically to adapt to a warmer, more acidic climate in the future. Their tolerance to the highly variable estuarine system as well as the intense physiological regulation of the internal environment both contribute to the resilience observed during my experiments. My dissertation contributes to the body of knowledge on the effects of climate change on estuarine crustaceans by quantifying the organismal and population level impact of increased temperature and pCO_2 on juvenile blue crab in the Chesapeake Bay. I determined crab growth, metabolism, energy storage, and carapace properties in response to increases in water temperature and pCO_2 and used these data to draw physiological insights and possible population-level responses from a model that described potential effects of warming temperatures on crab overwintering behavior.

Growth is a comprehensive response which integrates energy allocation and metabolism into a single measure each time a crab molts. Additionally, changes in individual growth can be manifested throughout ontogeny as well as through populations (Miller, 2001; Brylawski and Miller, 2006). Considering the broad applicability of changes in growth throughout the crab life cycle, I designed a laboratory system that allowed me to quantify the effects of experimentally manipulated temperature and pH on crab growth and food consumption. Given the known effects of changes in water chemistry on growth and calcification in calcifying invertebrates (Pörtner, 2002), I then determined changes in the thickness and chemistry of the carapaces of crabs exposed to increased temperature and pCO_2

during the growth experiment. Although the metabolic response of poikilotherms to increased temperature is well characterized, the effects of increased pCO_2 and the interaction between increased temperature and pCO_2 on metabolic rate are less well understood. Therefore, I quantified the oxygen consumption rate of crabs after exposure to increased temperature and pCO_2. I also utilized the physiological data I had collected to hypothesize how increased temperatures might affect blue crab at the population-level through changes in overwintering behavior. To achieve this goal, I modeled the relationship between air and water temperature in the Patuxent River, Chesapeake Bay using a 78-year observational dataset. I then used this relationship combined with projected air temperature from downscaled global climate models to determine possible changes to the overwintering behavior of blue crab in the future and discuss population and fisheries impacts of these changes.

The results of the growth experiment (Chapter 2) showed that juvenile blue crab responded predictably to increases in temperature and were tolerant of high levels of pCO_2 (Glandon and Miller, 2017). During this experiment, crabs were exposed to one of four treatment combinations of temperature and pCO_2 in a 2x2 factorial design for a period of two molts (approximately 30 days). Treatment levels were chosen to reflect current average summer conditions in the Chesapeake Bay and future predicted levels in the year 2100 (Temperature treatments: 26°C and 32°C, pCO_2 treatments: 800 and 8,000 µatm). As a result of the highly productive estuarine environment, there are large daily and seasonal fluctuations present in pH in the Chesapeake Bay; pH is regularly above 8.3 during the daytime due to high levels of photosynthesis and can drop below 7 at night as a result of high levels of cellular

respiration (Breitburg et al., 2015). Given the goal of creating realistic conditions in the laboratory setup, the high pCO_2 treatment was determined with this variability in mind. The data from the experiment indicated no temperature x pCO_2 interaction. Crabs grew significantly faster and ate significantly more food in the warm temperature treatment compared to the ambient temperature treatment. Also, there was no effect of increased pCO_2 on crab growth or food consumption.

The lack of a significant pCO_2 effect was likely caused by two factors: the natural high variability of pCO_2 in the estuarine environment and the superb ability of crab to regulate their internal chemistry (Towle et al., 1976; Henry and Wheatly, 1992; Towle and Burnett, 2007). High natural productivity in the Chesapeake Bay ecosystem creates large variation and intense cycling of pCO_2 on a daily basis (Cai et al., 2011). Additionally, there is a strong latitudinal gradient of pCO_2 in the Chesapeake Bay and therefore blue crab are exposed to a range of pCO_2 levels as they migrate from the mouth into the estuary as juveniles (MdDNR, 2017). Crabs are prolific in the highly variable environment described above due to their excellent ability to regulate their internal conditions. Blue crab are known to be efficient osmoregulators and highly regulate their internal pH during the molt process (Mangum et al., 1985). The ability of crabs to live in the highly variable estuarine environment may have contributed to the lack of a significant pCO_2 effect on crab growth and consumption during the growth experiment.

Although there was no effect of increased pCO_2 on crab growth and food consumption, the robustness of other physiological parameters, such as carapace properties or oxygen consumption rate, may have declined in order to maintain

growth and food consumption in the face of increased pCO_2 during the growth experiment. The thickness and chemistry of the carapaces of the crabs from the growth experiment was determined in Chapter 3 to quantify the effect of increase temperature and pCO_2 on the blue crab carapace (Glandon et al., *submitted*). Carapace thickness aids in its protective ability and carapace calcium content has been correlated to shell strength in a variety of invertebrate species (Jordaens et al., 2006; Amato et al., 2008; Fabritius et al., 2012). There was a counteractive effect of temperature and pCO_2 on the percent of high-magnesium calcite (HMC) in crab carapaces; increased temperature caused a significant increase in percent HMC in the carapace while increased pCO_2 caused a significant decrease in the percent HMC in the carapace. The decrease in HMC was coupled with a decrease in carapace thickness in response to increased temperature in this study. These results combined with the increase in growth observed in the growth study suggest that carapace thickness and chemistry may decline in order to support increased growth as temperatures increase.

The response of the crab carapace to increased pCO_2 was less clear; although there was a significant decline in the percent HMC, this was coupled with a significant increase in the ratio of magnesium to calcium in the carapaces of these individuals. Since the incorporation of magnesium increases the solubility of the calcium carbonate (Berner, 1975), increases in the ratio of magnesium to calcium in the carapace may be an indicator of stress and could be used to gauge the condition of animals exposed to external stressors, as has been suggested for other calcifying organisms such as bivalves and foraminifera (Lorens and Bender, 1980; Toler et al.,

2001). The relative importance of the amount of HMC versus the ratio of magnesium to calcium in the HMC in crabs is not known and is an important area of future study.

The final physiological metric determined in my dissertation was the metabolic response of juvenile crab to increased temperature and pCO_2 (Chapter 4). Juvenile blue crab were exposed to temperature and pCO_2 treatments similar to those in the growth study for a period of two molts after which the oxygen consumption rate of individual crab were determined in a flow-through respiration system. Given the growth response observed in previous work (Chapter 2) combined with the known effect of temperature on the metabolic rate of blue crab (Leffler, 1972; Booth and McMahon, 1992), I expected temperature to have a significant positive effect on oxygen consumption rate and there to be no effect of increased pCO_2 on the oxygen consumption rate of crabs. Although the temperatures tested in this study did not elicit a significant difference in mean oxygen consumption rate, Q_{10} values indicated that oxygen consumption rates did predictably rise with temperature. However, the small temperature range (5°C) I tested and the high individual variability of oxygen consumption rates may have compromised my ability to detect statistically significant differences in mean metabolic rate by temperature treatment. It is also possible that the crabs had acclimated to the increase in temperature through being exposed to high temperature for approximately 30 days prior to metabolic measurement, as has been observed in other species such including fish (Fangue et al., 2014), zooplankton (Zeis et al., 2004), and salamanders (Brown and Fitzpatrick, 1981). Although oxygen consumption rate measurements throughout the acclimation period would be necessary to determine if the crabs had truly acclimated to experimental conditions.

Additionally, increased pCO_2 had no significant effect on the mean oxygen consumption rate of crabs.

The results from the first three experiments of my dissertation highlight the resilience of juvenile blue crab to increased temperature and pCO_2. Crabs responded in a predictable, positive way to a large increase in environmental temperature by increasing growth rates and food consumption. Although changes in carapace thickness and chemistry were observed in response to increased temperature in Chapter 3, the biological significance of these effects are not known. Crabs were highly tolerant of extremely high levels of pCO_2 in these experiments. Growth, food consumption, and oxygen consumption rates were maintained when crabs were exposed to a ten-fold increase in pCO_2 (800 to 8,000 µatm). Although increased pCO_2 had no effect on the thickness of crab carapaces, an increase in the percent of HMC in the carapaces was observed, suggesting a possible positive effect of increased pCO_2 on crab carapaces. The environmental factors in my laboratory experiments were manipulated much faster than the projected rate of change of temperature and pCO_2 in the future. Given the rapid increase in temperature and pCO_2 experienced by the individuals in my experiments, the physiological responses observed in Chapters 2-4 of my dissertation indicate that juvenile blue crab will be tolerant of increases in temperature and pCO_2 predicted to occur in the Chesapeake Bay through the next century.

The results from the physiological metrics measured during my dissertation research highlight the importance of measuring multiple parameters in multi-stressor studies. The results from Chapter 2 tell a relatively simple story; crab growth and

food consumption were predictably affected by increased temperature while no effect of increased pCO_2 was observed in either response. However, the carapace and metabolic data add complexity to the response of juvenile blue crab to climate change stressors. Carapace thickness and percent HMC declined in crabs exposed to high temperatures while carapace percent HMC increased in response to increased pCO_2. Although not statistically significant, antagonistic effects of increased temperature and pCO_2 were observed in crab carapace percent HMC and oxygen consumption rate. The percent HMC and oxygen consumption rate of crabs exposed to ambient temperature/ambient pCO_2 conditions was similar to the percent HMC and oxygen consumption rate of crab exposed to high temperature/high pCO_2. The balance of chemical and kinetic processes may contribute to the antagonistic effect of temperature and pCO_2 observed in the carapace data. Temperature and pCO_2 may act antagonistically to reduce metabolic scope (the threshold identified by the onset of anaerobic metabolism) of blue crab exposed to the combination of both stressors, as has been observed in adult edible crab, *Cancer pagurus*, (Metzger et al., 2007) and adult spider crab, *Maja squinado* (Frederich and Pörtner, 2000), emphasizing the importance of multi-stressor studies that quantify secondary effects.

The significant effect of temperature on crab growth observed in the laboratory experiments was the motivation for the final component of my dissertation research; to determine population level impacts of increased temperature on blue crab in the Chesapeake Bay. I utilized a high-resolution, long term dataset of air and water temperature collected at the Chesapeake Biological Laboratory (CBL) to characterize the relationship between air and water temperature in the Patuxent River, Chesapeake

Bay. These data were used to estimate a transfer function that could forecast water temperature from air temperature. Downscaled phase 5 Coupled Model Intercomparison Project (CMIP5) air temperature predictions were utilized in concert with the observational harmonic model to predict water temperature in the Maryland portion of the Chesapeake Bay through the year 2100. Using a cutoff temperature of 9°C, below which blue crab are known to cease growth processes (Smith, 1997), I quantified changes in the length of the overwintering period of crabs based on two future climate scenarios. I found that the probability of crabs overwintering in the Chesapeake Bay will decline as temperatures warm through the end of the century. In contrast to the consistent nature of overwintering currently displayed by blue crab in the Chesapeake Bay, overwintering will be more inconsistent as the temperature warms in the mid-Atlantic region, and may be absent in warmer years. The increase in the length of the growing season resulting from increases in temperature in the region has ecological and management implications. Increased crab growth rates will also cause increases in food consumption, placing additional stress on the prey of crabs that may be weakened by increasing pCO_2 concomitant with increasing temperatures. Increased wintertime blue crab activity may cause reductions to or elimination of the winter blue crab fishery. The Chesapeake Bay blue crab population may not be able to sustain this additional exploitation given the stochastic nature of future overwintering behavior, especially during cold years when growth may be unusually slow.

The results of my dissertation underscore the resilience of estuarine species, in this case juvenile blue crab, to projected future climatic changes. Given the variability

in environmental conditions in the estuary, the plasticity of the physiological response of crab to increased temperature and pCO_2 was not unexpected. However, the excellent ability of blue crab to regulate their internal chemistry (Towle et al., 1976; Henry and Kormanik, 1985; Mangum et al., 1985) may also contribute to the lack of observed effects of changes in environmental chemistry on crab physiology. Hemolymph pH was not quantified in this study, but understanding the effects of increased environmental pCO_2 on blue crab hemolymph chemistry is an important next step in determining the effects of environmental change on blue crab. Additionally, quantifying the effects of the observed changes in blue crab growth and carapace structure and chemistry on juvenile blue crab fitness would further place these findings in greater ecological context.

I focused on the juvenile life stage because it is known to be important in regulating overall crab population dynamics (Miller, 2001). Juveniles are the first complete life stage of blue crab to live entirely in the estuary, and the frequency of molting in juveniles allowed me to measure responses in a tractable period. However important the juvenile life stage may be, my study does not address impacts of climate change stressors in other life stages of blue crab. The larval and megalopal blue crab life stages are oceanic; an environment with consistency relative to the estuary and where individuals may be more susceptible to small changes in temperature and pCO_2. Additionally, my research did not quantify effects of increased temperature and pCO_2 on mating and reproduction, since juveniles do not display these behaviors. Adult female blue crab display a terminal molt, only after which are they receptive to receiving sperm from a male. The successful completion

of this molt is therefore critical to reproduction in this species. The effect of acidification on shell chemistry observed in my study may be amplified in adults and could impact mating success. Also, the allocation of energetic resources to reproduction in adults may be a cost of exposure to increased temperature or pCO_2 that I did not quantify. These costs could be manifested throughout generations of crab exposed to changing climate conditions. For example, Long et al. (2016a) observed negative carryover effects of decreased pH on Tanner crab, *Chionoecetes bairdi*, larvae hatched from females that had brooded eggs in low pH treatments for two years in contrast to resilience of larvae from females that were not acclimated to low pH. However, a similar effect was not observed in juvenile blue king crab, *Paralithodes platypus* (Long et al., 2016b). A comprehensive ontogenetic examination of the effects of increased temperature and pCO_2 on blue crab would more accurately quantify possible effects of climate change on this species overall.

Understanding the tolerance of individual species to environmental stressors is an important first step in quantifying the impact of environmental change on ecosystems. However, determining the effects of these stressors on multiple species as well as on the interactions among species is critical to determine ecosystem level impacts. Certain species, such as diatoms and fleshy algae, have been shown to be resistant to increases in pCO_2, while other groups, such as calcifying corals and mollusks, display decreased survival, growth, and calcification in the face of decreased pH/increased pCO_2 (Kroeker et al., 2013). Although the data from my dissertation indicate that blue crab likely fall into the "resistant" group, important prey items of crab, such as oysters and clams, may be "climate change losers"

(Waldbusser et al., 2011b; Parker et al., 2012). The documented negative effects of pCO_2 on blue crab prey may limit the ability of blue crab to maintain the increased growth and food consumption rates observed in the growth study. It is difficult to predict how species interactions may change in the face of a changing climate, underscoring the need for multi-species experiments to determine these effects.

Another important multi-species interaction in the Chesapeake Bay is between blue crab and commercial and recreational fishermen. The management of the Chesapeake Bay blue crab fishery is currently based upon a 3-month closure to protect overwintering blue crab. The results of the modeling study (Chapter 5) indicate a shortening and possible elimination of the blue crab overwintering period in the Chesapeake Bay during warm years. This extension of the growing season combined with the observed increase in growth rate in response to increasing temperatures may cause individuals will mature faster than the current management scheme predicts and therefore recruiting to the fishery in a shorter period of time. Possible impacts on the management of blue crab from this work are changes to the maturity schedule of crab in current management models and revision of the timing of the closure of the Chesapeake Bay blue crab fishery. The scale of the ecological and economic impacts of changes to the biology and management of blue crab as a response to climate change will depend on the rate of change of the climate and the ability of crab and the Chesapeake Bay ecosystem as a whole to acclimate and adapt to these changes.

The data from my dissertation indicate that blue crab are likely well equipped to continue to be a successful member of the estuarine food web in the face of a

changing climate. Increased temperature caused a significant increase in crab growth rate and food consumption, but at a cost to carapace thickness and chemistry. Crabs were resilient to exposure to extremely high levels of pCO_2; there was no significant effect of increased pCO_2 on crab growth rate, food consumption, or oxygen consumption rate. Although there was a significant increase in percent HMC in response to increased pCO_2, this was paired with an increase in the concentration of magnesium, complicating the overall effect of increased pCO_2 on the carapace. The temperatures tested in this study did not elicit a significant difference in mean oxygen consumption rate, however Q_{10} values indicate that oxygen consumption rates did predictably rise with temperature. The climate model indicated that the temperature regime of the northern Chesapeake Bay will become significantly warmer through the next century and this warming will cause inconsistency in blue crab overwintering behavior through increases in blue crab growth rates and concurrent decreases in stage duration. The physiological and population level responses observed in this study underscore the resilience of blue crab to predicted future climate changes in the mid-Atlantic region.

Bibliography

Aldridge, J. B., and Cameron, J. N. 1982. Gill morphometry in the blue crab, *Callinectes sapidus* Rathbun (Decapoda Brachyura). Crustaceana, 43: 297-305.

Amato, C. G., Waugh, D. A., Feldmann, R. M., and Schweitzer, C. E. 2008. Effect of calcification on cuticle density in decapods: a key to lifestyle. Journal of Crustacean Biology, 28: 587-595.

Anthony, K. R., Kline, D. I., Diaz-Pulido, G., Dove, S., and Hoegh-Guldberg, O. 2008. Ocean acidification causes bleaching and productivity loss in coral reef builders. Proceedings of the National Academy of Sciences, 105: 17442-17446.

Aumann, C. A., Eby, L. A., and Fagan, W. F. 2006. How transient patches affect population dynamics: the case of hypoxia and blue crabs. Ecological Monographs, 76: 415-438.

Baird, D., and Ulanowicz, R. E. 1989. The seasonal dynamics of the Chesapeake Bay ecosystem. Ecological Monographs: 329-364.

Bauer, L. J., and Miller, T. J. 2010. Temperature-, salinity-, and size-dependent winter mortality of juvenile blue crabs (*Callinectes sapidus*). Estuaries and Coasts, 33: 668-677.

Beaupre, S., and Dunham, A. 1995. A comparison of ratio-based and covariance analyses of a nutritinal data set. Functional Ecology: 876-880.

Beniash, E., Ivanina, A., Lieb, N. S., Kurochkin, I., and Sokolova, I. M. 2010. Elevated level of carbon dioxide affects metabolism and shell formation in oysters *Crassostrea virginica*. Marine Ecology Progress Series, 419: 95-108.

Berge, J. A., Bjerkeng, B., Pettersen, O., Schaanning, M. T., and Øxnevad, S. 2006. Effects of increased sea water concentrations of CO_2 on growth of the bivalve *Mytilus edulis* L. Chemosphere, 62: 681-687.

Berner, R. 1975. The role of magnesium in the crystal growth of calcite and aragonite from sea water. Geochimica et Cosmochimica Acta, 39: 489-504.

Bibby, R., Cleall-Harding, P., Rundle, S., Widdicombe, S., and Spicer, J. 2007. Ocean acidification disrupts induced defences in the intertidal gastropod *Littorina littorea*. Biology Letters, 3: 699-701.

Blackford, J., and Gilbert, F. 2007. pH variability and CO_2 induced acidification in the North Sea. Journal of Marine Systems, 64: 229-241.

Boesch, D., Dawson, F., Summers, R., Busalacchi, A., Boicourt, W., Cahoon, D., Coale, F., et al. 2008. Global Warming and the Free State. Report of the Scientific and Technical Working Group, Marlyand Commission on Climate Change. University of Maryland Center for Environmental Science, Cambridge, Maryland.

Booth, C. E., and McMahon, B. R. 1992. Aerobic capacity of the blue crab, *Callinectes sapidus*. Physiological zoology, 65: 1074-1091.

Boyd, P. W., and Hutchins, D. A. 2012. Understanding the responses of ocean biota to a complex matrix of cumulative anthropogenic change. Marine Ecology Progress Series, 470: 125-135.

Boßelmann, F., Romano, P., Fabritius, H., Raabe, D., and Epple, M. 2007. The composition of the exoskeleton of two crustacea: The American lobster Homarus americanus and the edible crab Cancer pagurus. Thermochimica Acta, 463: 65-68.

Breitburg, D. L., Salisbury, J., Bernhard, J. M., Cai, W.-J., Dupont, S., Doney, S. C., Kroeker, K. J., et al. 2015. And on top of all that…: Coping with ocean acidification in the midst of many stressors by denise. Oceanography, 28.

Breteler, W. K. 1975. Food consumption, growth and energy metabolism of juvenile shore crabs, *Carcinus maenas*. Netherlands Journal of Sea Research, 9: 255-272.

Brezonik, P., and Arnold, W. 2011. Solubility: Reactions of solid phases with water. *In* Water Chemistry, pp. 364-405. Ed. by P. Brezonik, and W. Arnold. Oxford University Press, New York, New York.

Browman, H. I. 2016. Applying organized scepticism to ocean acidification research. ICES Journal of Marine Science: Journal du Conseil, 73: 529-536.

Brown, A. C., and Terwilliger, N. B. 1999. Developmental changes in oxygen uptake in *Cancer magister* (Dana) in response to changes in salinity and temperature. Journal of Experimental Marine Biology and Ecology, 241: 179-192.

Brown, A. V., and Fitzpatrick, L. C. 1981. Metabolic acclimation to temperature in the Ozark salamander *Plethodon dorsalis angusticlavius*. Comparative Biochemistry and Physiology Part A: Physiology, 69: 499-503.

Brylawski, B. J., and Miller, T. J. 2006. Temperature-dependent growth of the blue crab (*Callinectes sapidus*): a molt process approach. Canadian Journal of Fisheries and Aquatic Sciences, 63: 1298-1308.

Byrne, M., and Przeslawski, R. 2013. Multistressor impacts of warming and acidification of the ocean on marine invertebrates' life histories. Integrative and Comparative Biology, 53: 582-596.

Cai, W.-J., Hu, X., Huang, W.-J., Murrell, M. C., Lehrter, J. C., Lohrenz, S. E., Chou, W.-C., et al. 2011. Acidification of subsurface coastal waters enhanced by eutrophication. Nature Geoscience, 4: 766-770.

Cai, W.-J., and Wang, Y. 1998. The chemistry, fluxes, and sources of carbon dioxide in the estuarine waters of the Satilla and Altamaha Rivers, Georgia. Limnology and Oceanography, 43: 657-668.

Caldeira, K., and Wickett, M. E. 2003. Anthropogenic carbon and ocean pH. Nature, 425: 365-365.

Cameron, J. N. 1985. Molting in the blue crab. Scientific American, 252: 102-109.

Campanati, C., Yip, S., Lane, A., and Thiyagarajan, V. 2015. Combined effects of low pH and low oxygen on the early-life stages of the barnacle *Balanus amphitrite*. ICES Journal of Marine Science: Journal du Conseil, 73: 791-802.

Carter, H. A., Ceballos-Osuna, L., Miller, N. A., and Stillman, J. H. 2013. Impact of ocean acidification on metabolism and energetics during early life stages of the intertidal porcelain crab *Petrolisthes cinctipes*. The Journal of Experimental Biology, 216: 1412-1422.

Checkley, D. M., Dickson, A. G., Takahashi, M., Radich, J. A., Eisenkolb, N., and Asch, R. 2009. Elevated CO_2 enhances otolith growth in young fish. Science, 324: 1683-1683.

Chen, P.-Y., Lin, A. Y.-M., McKittrick, J., and Meyers, M. A. 2008. Structure and mechanical properties of crab exoskeletons. Acta Biomaterialia, 4: 587-596.

Cho, H.-Y., and Lee, K.-H. 2011. Development of an air–water temperature relationship model to predict climate-induced future water temperature in estuaries. Journal of Environmental Engineering, 138: 570-577.

Coen, L. D., Brumbaugh, R. D., Bushek, D., Grizzle, R., Luckenbach, M. W., Posey, M. H., Powers, S. P., et al. 2007. Ecosystem services related to oyster restoration. Marine Ecology Progress Series, 341: 303-307.

Coffey, W. D., Nardone, J. A., Yarram, A., Long, W. C., Swiney, K. M., Foy, R. J., and Dickinson, G. H. 2017. Ocean acidification leads to altered micromechanical properties of the mineralized cuticle in juvenile red and blue king crabs. Journal of Experimental Marine Biology and Ecology, 495: 1-12.

Comeau, S., Jeffree, R., Teyssié, J.-L., and Gattuso, J.-P. 2010. Response of the Arctic pteropod *Limacina helicina* to projected future environmental conditions. PLOS ONE, 5: e11362.

Cooley, S. R., and Doney, S. C. 2009. Anticipating ocean acidification's economic consequences for commercial fisheries. Environmental Research Letters, 4.

Cornwall, C. E., and Hurd, C. L. 2015. Experimental design in ocean acidification research: problems and solutions. ICES Journal of Marine Science: Journal du Conseil, 73: 572-581.

Costlow, J., and Bookhout, C. 1965. The effect of environmental factors on larval development of crabs. *In* Biological Problems in Water Pollution, 3rd Seminar. US Public Health Service, Publication 999-WP-25, pp. 77-86. Ed. by C. M. Tarzwell.

Crain, C. M., Kroeker, K., and Halpern, B. S. 2008. Interactive and cumulative effects of multiple human stressors in marine systems. Ecology Letters, 11: 1304-1315.

Cunningham, S. C., Smith, A. M., and Lamare, M. D. 2015. The effects of elevated pCO_2 on growth, shell production and metabolism of cultured juvenile abalone, *Haliotis iris*. Aquaculture Research: 2375-2392.

Cáceres, C. E. 1997. Dormancy in invertebrates. Invertebrate biology: 371-383.

Davis, M. 2000. The combined effects of temperature and salinity on growth, development, and survival for tropical gastropod veligers of *Strombus gigas*. Journal of Shellfish Research, 19: 883-890.

De Jonge, V. 1974. Classification of brackish coastal inland waters. Aquatic Ecology, 8: 29-39.

Deng, W., Liu, Y., Wei, G., Li, X., Tu, X., Xie, L., Zhang, H., et al. 2010. High-precision analysis of Sr/Ca and Mg/Ca ratios in corals by laser ablation inductively coupled plasma optical emission spectrometry. Journal of Analytical Atomic Spectrometry, 25: 84-87.

Dickson, A., Afghan, J., and Anderson, G. 2003. Reference materials for oceanic CO_2 analysis: a method for the certification of total alkalinity. Marine Chemistry, 80: 185-197.

Dickson, A. G. 1990. Standard potential of the reaction -$AgCl_{(S)}^{+1}/2H^{-2}_{(G)}$ = $Ag_{(S)}$+$HCl_{(AQ)}$ and the standard acidity constant of the ion HSO_4^- in synthetic sea water from 273.15-K to 318.15-K. Journal of Chemical Thermodynamics, 22: 113-127.

Dickson, A. G., Sabine, C. L., and Christian, J. R. 2007. Guide to best practices for ocean CO_2 measurements.

Dillaman, R., Hequembourg, S., and Gay, M. 2005. Early pattern of calcification in the dorsal carapace of the blue crab, *Callinectes sapidus*. Journal of Morphology, 263: 356-374.

Dissanayake, A., and Ishimatsu, A. 2011. Synergistic effects of elevated CO_2 and temperature on the metabolic scope and activity in a shallow-water coastal decapod (*Metapenaeus joyneri*; Crustacea: Penaeidae). ICES Journal of Marine Science: Journal du Conseil: fsq188.

Dittel, A. I., Hines, A. H., Ruiz, G. M., and Ruffin, K. K. 1995. Effects of shallow water refuge on behavior and density-dependent mortality of juvenile blue crabs in Chesapeake Bay. Bulletin of Marine Science, 57: 902-916.

Dodd, L. F., Grabowski, J. H., Piehler, M. F., Westfield, I., and Ries, J. B. 2015. Ocean acidification impairs crab foraging behaviour. Proceedings of the Royal Society of London B, 282: 20150333.

Doney, S. C., Ruckelshaus, M., Duffy, J. E., Barry, J. P., Chan, F., English, C. A., Galindo, H. M., et al. 2012. Climate change impacts on marine ecosystems. Marine Science, 4.

Doney, S. C., and Schimel, D. S. 2007. Carbon and climate system coupling on timescales from the Precambrian to the Anthropocene. Annual Reviews of Environment and Resources, 32: 31-66.

Duarte, C. M., Hendriks, I. E., Moore, T. S., Olsen, Y. S., Steckbauer, A., Ramajo, L., Carstensen, J., et al. 2013. Is ocean acidification an open-ocean syndrome? Understanding anthropogenic impacts on seawater pH. Estuaries and Coasts, 36: 221-236.

Edmond, J. M. 1970. High precision determination of titration alkalinity and total carbon dioxide content of sea water by potentiometric titration. *In* Deep Sea Research and Oceanographic Abstracts, pp. 737-750. Elsevier.

Eggleston, D. B., Lipcius, R. N., and Hines, A. H. 1992. Density-dependent predation by blue crabs upon infaunal clam species with contrasting distribution and abundance patterns. pp. 55-68. Marine Ecology Progress Series.

Epifanio, C. E. 2007. Biology of larvae. *In* The blue crab, *Callinectes sapidus*, pp. 513-533. Ed. by V. S. Kennedy, and L. E. Cronin. Maryland Sea Grant College, College Park, MD.

Eriksson, S., and Edlund, A.-M. 1977. On the ecological energetics of 0-group *Carcinus maenas* (L.) from a shallow sandy bottom in Gullmar Fjord, Sweden. Journal of Experimental Marine Biology and Ecology, 30: 233-248.

Etherington, L. L., and Eggleston, D. B. 2003. Spatial dynamics of large-scale, multistage crab (*Callinectes sapidus*) dispersal: determinants and consequences for recruitment. Canadian Journal of Fisheries and Aquatic Sciences, 60: 873-887.

Fabritius, H.-O., Karsten, E. S., Balasundaram, K., Hild, S., Huemer, K., and Raabe, D. 2012. Correlation of structure, composition and local mechanical properties in the dorsal carapace of the edible crab *Cancer pagurus*. Zeitschrift für Kristallographie-Crystalline Materials, 227: 766-776.

Fabry, V. J. 2008. Marine calcifiers in a high-CO_2 ocean. Science, 320: 1020-1022.

Fangue, N. A., Wunderly, M. A., Dabruzzi, T. F., and Bennett, W. A. 2014. Asymmetric thermal acclimation responses allow sheepshead minnow *Cyprinodon variegatus* to cope with rapidly changing temperatures. Physiological and Biochemical Zoology, 87: 805-816.

Feely, R., Doney, S., and Cooley, S. 2009. Ocean acidification: Present conditions and future changes in a high-CO_2 world. Oceanography, 22: 36-47.

Feely, R. A., Alin, S. R., Newton, J., Sabine, C. L., Warner, M., Devol, A., Krembs, C., et al. 2010. The combined effects of ocean acidification, mixing, and respiration on pH and carbonate saturation in an urbanized estuary. Estuarine Coastal and Shelf Science, 88: 442-449.

Feely, R. A., Sabine, C. L., Lee, K., Berelson, W., Kleypas, J., Fabry, V. J., and Millero, F. J. 2004. Impact of anthropogenic CO_2 on the $CaCO_3$ system in the oceans. Science, 305: 362-366.

Findlay, H. S., Wood, H. L., Kendall, M. A., Spicer, J. I., Twitchett, R. J., and Widdicombe, S. 2011. Comparing the impact of high CO_2 on calcium carbonate structures in different marine organisms. Marine Biology Research, 7: 565-575.

Fogarty, M. J., and Lipcius, R. N. 2007. Population dynamics and fisheries. *In* The blue crab, *Callinectes sapidus*., pp. 711-755. Ed. by V. S. Kennedy, and L. E. Cronin. Maryland Sea Grant College, College Park, Maryland.

Fox, J., and Weisberg, S. 2010. An R companion to applied regression, Sage.

Frederich, M., and Pörtner, H. O. 2000. Oxygen limitation of thermal tolerance defined by cardiac and ventilatory performance in spider crab, *Maja squinado*. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 279: R1531-R1538.

Freeman, J. A., Kilgus, G., Laurendeau, D., and Perry, H. M. 1987. Postmolt and intermolt molt cycle stages of *Callinectes sapidus*. Aquaculture, 61: 201-209.

Frieder, C. A., Applebaum, S. L., Pan, T.-C. F., Hedgecock, D., and Manahan, D. T. 2016. Metabolic cost of calcification in bivalve larvae under experimental ocean acidification. ICES Journal of Marine Science, 74: 941-954.

Fuiman, L. A., and Cowan, J. H. 2003. Behavior and recruitment success in fish larvae: repeatability and covariation of survival skills. Ecology, 84: 53-67.

Gazeau, F., Quiblier, C., Jansen, J. M., Gattuso, J. P., Middelburg, J. J., and Heip, C. H. 2007. Impact of elevated CO_2 on shellfish calcification. Geophysical Research Letters, 34.

Gillooly, J. F., Brown, J. H., West, G. B., Savage, V. M., and Charnov, E. L. 2001. Effects of size and temperature on metabolic rate. Science, 293: 2248-2251.

Glandon, H. L., Kilbourne, K. H., Schijf, J., and Miller, T. J. *submitted*. Counteractive effects of increased temperature and pCO_2 on the carapace thickness and

chemistry of juvenile blue crab, *Callinectes sapidus*, from the Patuxent River, Chesapeake Bay. Journal of Experimental Marine Biology and Ecology.

Glandon, H. L., and Miller, T. J. 2017. No effect of high pCO_2 on juvenile blue crab, *Callinectes sapidus*, growth and consumption despite positive responses to concurrent warming. ICES Journal of Marine Science: Journal du Conseil, 74: 1201-1209.

Green, J. P., and Neff, M. R. 1972. A survey of the fine structure of the integument of the fiddler crab. Tissue and Cell, 4: 137-171.

Gutermuth, F. B., and Armstrong, D. A. 1989. Temperature-dependent metabolic response of juvenile Dungeness crab *Cancer magister* Dana: ecological implications for estuarine and coastal populations. Journal of Experimental Marine Biology and Ecology, 126: 135-144.

Hadfield, M., and Strathmann, M. 1996. Variability, flexibility and plasticity in life histories of marine invertebrates. Oceanologica Acta, 19: 323-334.

Hamilton, M. A., Rode, P. W., Merchant, M. E., and Sneddon, J. 2008. Determination and comparison of heavy metals in selected seafood, water, vegetation and sediments by inductively coupled plasma-optical emission spectrometry from an industrialized and pristine waterway in Southwest Louisiana. Microchemical Journal, 88: 52-55.

Harding, J., and Mann, R. 2001. Oyster reefs as fish habitat: Opportunistic use of restored reefs by transient fishes. Journal of Shellfish Research, 20: 951-959.

Hays, G. C., Richardson, A. J., and Robinson, C. 2005. Climate change and marine plankton. Trends in Ecology & Evolution, 20: 337-344.

Heerebout, G. 1970. A classification system for isolated brackish inland waters, based on median chlorinity and chlorinity fluctuation. Netherlands Journal of Sea Research, 4: 494-503.

Hendriks, I. E., Duarte, C. M., and Alvarez, M. 2010. Vulnerability of marine biodiversity to ocean acidification: A meta-analysis. Estuarine Coastal and Shelf Science, 86: 157-164.

Henry, R. P. 1996. Multiple roles of carbonic anhydrase in cellular transport and metabolism. Annual Review of Physiology, 58: 523-538.

Henry, R. P., and Cameron, J. N. 1983. The role of carbonic anhydrase in respiration, ion regulation and acid-base balance in the aquatic crab *Callinectes sapidus* and the terrestrial crab *Gecarcinus lateralis*. Journal of Experimental Biology, 103: 205-223.

Henry, R. P., and Kormanik, G. A. 1985. Carbonic anhydrase activity and calcium deposition during the molt cycle of the blue crab *Callinectes sapidus*. Journal of Crustacean Biology: 234-241.

Henry, R. P., and Wheatly, M. G. 1992. Interaction of respiration, ion regulation, and acid-base balance in the everyday life of aquatic crustaceans. American Zoologist, 32: 407-416.

Hines, A. 2007. Ecology of juvenile and adult blue crabs. *In* The blue crab *Callinectes sapidus*, pp. 565-654. Ed. by V. S. Kennedy, and L. E. Cronin. Maryland Sea Grant College, College Park, MD.

Hines, A. H., Haddon, A. M., and Wiechert, L. A. 1990. Guild structure and foraging impact of blue crabs and epibenthic fish in a subestuary of Chesapeake Bay. Marine Ecology Progress Series, 67: 105-126.

Hines, A. H., Johnson, E. G., Darnell, M. Z., Rittschof, D., Miller, T. J., Bauer, L. J., Rodgers, P., et al. 2010. Predicting effects of climate change on blue crabs in Chesapeake Bay. Biology and management of exploited crab populations under climate change. Fairbanks: Alaska Sea Grant, University of Alaska Fairbanks: 109-127.

Hines, A. H., and Ruiz, G. M. 1995. Temporal variation in juvenile blue crab mortality: nearshore shallows and cannibalism in Chesapeake Bay. Bulletin of Marine Science, 57: 884-901.

Hoegh-Guldberg, O., Mumby, P., Hooten, A., Steneck, R., Greenfield, P., Gomez, E., Harvell, C., et al. 2007. Coral reefs under rapid climate change and ocean acidification. Science, 318: 1737-1742.

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

Howland, R., Tappin, A., Uncles, R., Plummer, D., and Bloomer, N. 2000. Distributions and seasonal variability of pH and alkalinity in the Tweed Estuary, UK. Science of the Total Environment, 251: 125-138.

Hu, M. Y., Casties, I., Stumpp, M., Ortega-Martinez, O., and Dupont, S. 2014. Energy metabolism and regeneration are impaired by seawater acidification in

the infaunal brittlestar *Amphiura filiformis*. Journal of Experimental Biology, 217: 2411-2421.

Hurlbert, S. H. 1984. Pseudoreplication and the design of ecological field experiments. Ecological Monographs, 54: 187-211.

Iglesias-Rodriguez, M. D., Halloran, P. R., Rickaby, R. E., Hall, I. R., Colmenero-Hidalgo, E., Gittins, J. R., Green, D. R., et al. 2008. Phytoplankton calcification in a high-CO_2 world. Science, 320: 336-340.

Jivoff, P., Hines, A. H., and Quankenbush, L. S. 2007. Reproduction biology and embryonic development. *In* The blue crab, *Callinectes sapidus*, pp. 255-298. Ed. by V. S. K. a. L. E. Cronin. Maryland Sea Grant College, College Park, MD.

Jordaens, K., De Wolf, H., Vandecasteele, B., Blust, R., and Backeljau, T. 2006. Associations between shell strength, shell morphology and heavy metals in the land snail *Cepaea nemoralis* (Gastropoda, Helicidae). Science of the Total Environment, 363: 285-293.

Kalber, F. 1970. Osmoregulation in decapod larvae as a consideration in culture techniques. Helgoländer wissenschaftliche Meeresuntersuchungen, 20: 697.

Kennedy, V. S., Oesterling, M., and Van Engel, W. A. 2007. History of blue crab fisheries on the U.S. Atlantic and Gulf Coasts. *In* The blue crab *Callinectes sapidus* pp. 655-710. Ed. by V. S. Kennedy, and L. E. Cronin. Maryland Sea Grant College, College Park, MD.

Keppel, A. G., Breitburg, D. L., Wikfors, G. H., Burrell, R. B., and Clark, V. M. 2015. Effects of co-varying diel-cycling hypoxia and pH on disease

susceptibility in the eastern oyster, *Crassostrea virginica*. Marine Ecology Progress Series, 538: 169.

Kinne, O. 1964. Non-genetic adaptation to temperature and salinity. Helgoländer wissenschaftliche Meeresuntersuchungen, 9: 433-458.

Kleypas, J. A., Buddemeier, R. W., Archer, D., Gattuso, J.-P., Langdon, C., and Opdyke, B. N. 1999. Geochemical consequences of increased atmospheric carbon dioxide on coral reefs. Science, 284: 118-120.

Kroeker, K. J., Kordas, R. L., Crim, R., Hendriks, I. E., Ramajo, L., Singh, G. S., Duarte, C. M., et al. 2013. Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. Global Change Biology, 19: 1884-1896.

Kroeker, K. J., Kordas, R. L., Crim, R. N., and Singh, G. G. 2010. Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. Ecology Letters, 13: 1419-1434.

Kunitake, M. E., Baker, S. P., and Estroff, L. A. 2012. The effect of magnesium substitution on the hardness of synthetic and biogenic calcite. MRS Communications, 2: 113-116.

Landes, A., and Zimmer, M. 2012. Acidification and warming affect both a calcifying predator and prey, but not their interaction. Marine Ecology Progress Series, 450: 1-10.

Lannig, G., Eilers, S., Poertner, H. O., Sokolova, I. M., and Bock, C. 2010. Impact of ocean acidification on energy metabolism of oyster, *Crassostrea gigas*-

changes in metabolic pathways and thermal response. Marine Drugs, 8: 2318-2339.

Lefevre, S. 2016. Are global warming and ocean acidification conspiring against marine ectotherms? A meta-analysis of the respiratory effects of elevated temperature, high CO_2 and their interaction. Conservation Physiology, 4: cow009.

Leffler, C. 1972. Some effects of temperature on the growth and metabolic rate of juvenile blue crabs, *Callinectes sapidus*, in the laboratory. Marine Biology, 14: 104-110.

Lewis, E., and Wallace, D. 1998. Program Developed for CO_2 System Calculations (Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Dept. of Energy, Oak Ridge, TN). ORNL/CDIAC-105.

Lipcius, R. N., Eggleston, D. B., Heck, K. L., Seitz, R. D., and van Montfrans, J. 2007. Ecology of postlarval and young juvenile blue crabs. *In* The blue crab, *Callinectes sapidus*. Ed. by V. S. Kennedy, and L. E. Cronin. Maryland Sea Grant College, College Park, MD.

Lipcius, R. N., Stockhausen, W. T., Seitz, R. D., and Geer, P. J. 2003. Spatial dynamics and value of a marine protected area and corridor for the blue crab spawning stock in Chesapeake Bay. Bulletin of Marine Science, 72: 453-469.

Lischka, S., Büdenbender, J., Boxhammer, T., and Riebesell, U. 2010. Impact of ocean acidification and elevated temperatures on early juveniles of the polar shelled pteropod *Limacina helicina*: mortality, shell degradation, and shell growth. Biogeosciences Discussions, 7: 8177-8214.

Long, W. C., Swiney, K. M., and Foy, R. J. 2013a. Effects of ocean acidification on the embryos and larvae of red king crab, *Paralithodes camtschaticus*. Marine Pollution Bulletin, 69: 38-47.

Long, W. C., Swiney, K. M., and Foy, R. J. 2016a. Effects of high pCO_2 on Tanner crab reproduction and early life history, Part II: carryover effects on larvae from oogenesis and embryogenesis are stronger than direct effects. ICES Journal of Marine Science: Journal du Conseil, 73: 836-848.

Long, W. C., Swiney, K. M., Harris, C., Page, H. N., and Foy, R. J. 2013b. Effects of ocean acidification on juvenile red king crab (*Paralithodes camtschaticus*) and Tanner crab (*Chionoecetes bairdi*) growth, condition, calcification, and survival. PLOS ONE, 8: e60959.

Long, W. C., Van Sant, S. B., Swiney, K. M., and Foy, R. J. 2016b. Survival, growth, and morphology of blue king crabs: effect of ocean acidification decreases with exposure time. ICES Journal of Marine Science: Journal du Conseil: fsw197.

Lorens, R. B., and Bender, M. L. 1980. The impact of solution chemistry on *Mytilus edulis* calcite and aragonite. Geochimica et Cosmochimica Acta, 44: 1265-1278.

Maloney, E. D., Camargo, S. J., Chang, E., Colle, B., Fu, R., Geil, K. L., Hu, Q., et al. 2014. North American climate in CMIP5 experiments: Part III: Assessment of twenty-first-century projections. Journal of Climate, 27: 2230-2270.

Mangum, C. 1992. Physiological aspects of molting in the blue crab *Callinectes sapidus*. American Zoologist, 32: 459-469.

Mangum, C., McMahon, B., Defur, P., and Wheatly, M. 1985. Gas exchange, acid-base balance, and the oxygen supply to the tissues during a molt of the blue crab *Callinectes sapidus*. Journal of Crustacean Biology: 188-206.

Mansour, R. A., and Lipcius, R. N. 1991. Density-dependent foraging and mutual interference in blue crabs preying upon infaunal clams. Marine Ecology Progress Series, 72: 239-246.

McBryan, T., Anttila, K., Healy, T., and Schulte, P. 2013. Responses to temperature and hypoxia as interacting stressors in fish: implications for adaptation to environmental change. Integrative and Comparative Biology, 53: 648-659.

McDonald, M. R., McClintock, J. B., Amsler, C. D., Rittschof, D., Angus, R. A., Orihuela, B., and Lutostanski, K. 2009. Effects of ocean acidification over the life history of the barnacle *Amphibalanus amphitrite*. Mar Ecol Prog Ser, 385: 179-187.

MdDNR 2017. Eyes on the Bay. Maryland Department of Natural Resources.

MdOA. 2015. Task Force to Study the Impact of Ocean Acidification on State Waters: Report to the Governor and the Maryland General Assembly. 46 pp.

Meseck, S. L., Alix, J. H., Swiney, K. M., Long, W. C., Wikfors, G. H., and Foy, R. J. 2016. Ocean acidification affects hemocyte physiology in the Tanner crab (*Chionoecetes bairdi*). PLOS ONE, 11: e0148477.

Metzger, R., Sartoris, F. J., Langenbuch, M., and Pörtner, H. O. 2007. Influence of elevated CO_2 concentrations on thermal tolerance of the edible crab *Cancer pagurus*. Journal of Thermal Biology, 32: 144-151.

Michaelidis, B., Ouzounis, C., Paleras, A., and Pörtner, H. O. 2005. Effects of long-term moderate hypercapnia on acid-base balance and growth rate in marine mussels *Mytilus galloprovincialis*. Marine Ecology Progress Series, 293: 109-118.

Miller, A. W., Reynolds, A. C., Sobrino, C., and Riedel, G. F. 2009. Shellfish face uncertain future in high CO_2 world: influence of acidification on oyster larvae calcification and growth in estuaries. PLoS One, 4: e5661.

Miller, T., Wilberg, M., Colton, A., Davis, G., Sharov, A., Lipcius, R., Ralph, G., et al. 2011. Stock assessment of blue crab in Chesapeake Bay, 2011. University of Maryland Center for Environmental Science Chesapeake Biological Laboratory, Solomons, MD.

Miller, T. J. 2001. Matrix-based modeling of blue crab population dynamics with applications to the Chesapeake Bay. Estuaries, 24: 535-544.

Miller, T. J., Herra, T., and Leggett, W. C. 1995. An individual-based analysis of the variability of eggs and their newly hatched larvae of Atlantic cod (*Gadus morhua*) on the Scotian Shelf. Canadian Journal of Fisheries and Aquatic Sciences, 52: 1083-1093.

Mojica Prieto, F. J., and Millero, F. J. 2002. The values of pK 1+ pK 2 for the dissociation of carbonic acid in seawater. Geochimica et Cosmochimica Acta, 66: 2529-2540.

Moore, P. J., Thompson, R. C., and Hawkins, S. J. 2011. Phenological changes in intertidal con-specific gastropods in response to climate warming. Global Change Biology, 17: 709-719.

Morse, J. W., Andersson, A. J., and Mackenzie, F. T. 2006. Initial responses of carbonate-rich shelf sediments to rising atmospheric pCO_2 and "ocean acidification": role of high Mg-calcites. Geochimica et Cosmochimica Acta, 70: 5814-5830.

Mutlu, C., Türkmen, M., Türkmen, A., and Tepe, Y. 2011. Comparison of metal concentrations in tissues of blue crab, *Callinectes sapidus* from Mediterranean lagoons. Bulletin of Environmental Contamination and Toxicology, 87: 282-286.

Najjar, R., Patterson, L., and Graham, S. 2009. Climate simulations of major estuarine watersheds in the Mid-Atlantic region of the US. Climatic Change, 95: 139-168.

Najjar, R. G., Pyke, C. R., Adams, M. B., Breitburg, D., Hershner, C., Kemp, M., Howarth, R., et al. 2010. Potential climate-change impacts on the Chesapeake Bay. Estuarine, Coastal and Shelf Science, 86: 1-20.

Navarro, J. M., Duarte, C., Manríquez, P. H., Lardies, M. A., Torres, R., Acuña, K., Vargas, C. A., et al. 2016. Ocean warming and elevated carbon dioxide: multiple stressor impacts on juvenile mussels from southern Chile. ICES Journal of Marine Science: Journal du Conseil: fsv249.

Neufeld, D., and Cameron, J. 1994. Mechanism of the net uptake of water in moulting blue crabs (*Callinectes sapidus*) acclimated to high and low salinities. The Journal of Experimental Biology, 188: 11-23.

Neufeld, G. J., Holliday, C. W., and Pritchard, J. B. 1980. Salinity adaption of gill Na, K-ATPase in the blue crab, *Callinectes sapidus*. Journal of Experimental Zoology Part A: Ecological Genetics and Physiology, 211: 215-224.

Newell, R., and Branch, G. 1980. The influence of temperature on the maintenance of metabolic energy balance in marine invertebrates. Advances in Marine Biology, 17: 329-396.

Nienhuis, S., Palmer, A. R., and Harley, C. D. 2010. Elevated CO_2 affects shell dissolution rate but not calcification rate in a marine snail. Proceedings of the Royal Society of London B: Biological Sciences, 277: 2553-2558.

O'Connor, M. I., Piehler, M. F., Leech, D. M., Anton, A., and Bruno, J. F. 2009. Warming and resource availability shift food web structure and metabolism. PLoS biology, 7: e1000178.

Orr, J. C., Fabry, V. J., Aumont, O., Bopp, L., Doney, S. C., Feely, R. A., Gnanadesikan, A., et al. 2005. Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. Nature, 437: 681-686.

Orth, R. J., Carruthers, T. J., Dennison, W. C., Duarte, C. M., Fourqurean, J. W., Heck, K. L., Hughes, A. R., et al. 2006. A global crisis for seagrass ecosystems. Bioscience, 56: 987-996.

Pan, T.-C. F., Applebaum, S. L., and Manahan, D. T. 2015. Experimental ocean acidification alters the allocation of metabolic energy. Proceedings of the National Academy of Sciences, 112: 4696-4701.

Pane, E. F., and Barry, J. P. 2007. Extracellular acid-base regulation during short-term hypercapnia is effective in a shallow-water crab, but ineffective in a deep-sea crab. Marine Ecology Progress Series, 334: 1-9.

Parker, L. M., Ross, P. M., O'Connor, W. A., Borysko, L., Raftos, D. A., and Poertner, H.-O. 2012. Adult exposure influences offspring response to ocean acidification in oysters. Global Change Biology, 18: 82-92.

Parry, G. 1966. Osmotic adaptation in fishes. Biological Reviews, 41: 392-440.

Pershing, A. J., Alexander, M. A., Hernandez, C. M., Kerr, L. A., Le Bris, A., Mills, K. E., Nye, J. A., et al. 2015. Slow adaptation in the face of rapid warming leads to collapse of the Gulf of Maine cod fishery. Science, 350: 809-812.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., and R Core Team 2015. *nlme: Linear and Nonlinear Mixed Effects Models*., pp. R package version 3.1-122.

Poehlman, E. T., and Toth, M. J. 1995. Mathematical ratios lead to spurious conclusions regarding age-and sex-related differences in resting metabolic rate. The American Journal of Clinical Nutrition, 61: 482-485.

Pörtner, H. 2001. Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. Naturwissenschaften, 88: 137-146.

Pörtner, H.-O. 2002. Climate variations and the physiological basis of temperature dependent biogeography: systemic to molecular hierarchy of thermal tolerance in animals. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 132: 739-761.

Pörtner, H.-O. 2010. Oxygen-and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. The Journal of Experimental Biology, 213: 881-893.

Pörtner, H. O., Langenbuch, M., and Michaelidis, B. 2005. Synergistic effects of temperature extremes, hypoxia, and increases in CO_2 on marine animals: From Earth history to global change. Journal of Geophysical Research: Oceans, 110.

Pörtner, H. O., Langenbuch, M., and Reipschläger, A. 2004. Biological impact of elevated ocean CO_2 concentrations: lessons from animal physiology and earth history. Journal of Oceanography, 60: 705-718.

Pörtner, H. O., Peck, L., and Somero, G. 2007. Thermal limits and adaptation in marine Antarctic ectotherms: an integrative view. Philosophical Transactions of the Royal Society B: Biological Sciences, 362: 2233-2258.

R Core Team 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Raabe, D., Sachs, C., and Romano, P. 2005. The crustacean exoskeleton as an example of a structurally and mechanically graded biological nanocomposite material. Acta Materialia, 53: 4281-4292.

Rasband, W. 2012. ImageJ. US National Institutes of Health, Bethesda, Maryland, USA.

Reeder, R. 1983. Crystal chemistry of the rhombohedral carbonates. *In* Carbonates: Mineralogy and Chemistry, pp. 1-47. Ed. by R. Reeder. Mineralogical Society of America, Reviews in Mineralogy.

Rice, J. A., Miller, T. J., Rose, K. A., Crowder, L. B., Marschall, E. A., Trebitz, A. S., and DeAngelis, D. L. 1993. Growth rate variation and larval survival: inferences from an individual-based size-dependent predation model. Canadian Journal of Fisheries and Aquatic Sciences, 50: 133-142.

Richardson, A., and Poloczanska, E. 2008. Ocean Science: Under-resourced, under threat. Science, 320: 1294-1295.

Richardson, A. J., and Schoeman, D. S. 2004. Climate impact on plankton ecosystems in the Northeast Atlantic. Science, 305: 1609-1612.

Riebesell, U., Zondervan, I., Rost, B., Tortell, P. D., Zeebe, R. E., and Morel, F. M. 2000. Reduced calcification of marine plankton in response to increased atmospheric CO_2. Nature, 407: 364-367.

Ries, J. B. 2011. Skeletal mineralogy in a high-CO_2 world. Journal of Experimental Marine Biology and Ecology, 403: 54-64.

Ries, J. B., Cohen, A. L., and McCorkle, D. C. 2009. Marine calcifiers exhibit mixed responses to CO_2-induced ocean acidification. Geology, 37: 1131-1134.

Rock, J. pers. comm. 2017. Biologist Supervisor North Carolina Department of Marine Fisheries.

Roer, R., and Dillaman, R. 1984. The structure and calcification of the crustacean cuticle. American Zoologist, 24: 893-909.

Roer, R. D. 1980. Mechanisms of resorption and deposition of calcium in the carapace of the crab Carcinus maenas. The Journal of Experimental Biology, 88: 205-218.

Roer, R. D., and Dillaman, R. M. 1993. Molt-related change in integumental structure and function. *In* The crustacean integument. Morphology and biochemistry, pp. 1-37. Ed. by M. N. Horst, and J. A. Freeman. CRC Press, Inc., Boca Raton, FL.

Sampou, P., and Kemp, W. 1994. Factors regulating plankton community respiration in Chesapeake Bay. Marine Ecology Progress Series, 110.

Sanders, H. 1968. Benthic marine diversity and the stability-time hypothesis. *In* Brookhaven Symposia in Biology, pp. 71-81.

Schrag, D. P. 1999. Rapid analysis of high-precision Sr/Ca ratios in corals and other marine carbonates. Paleoceanography, 14: 97-102.

Schram, J. B., Schoenrock, K. M., McClintock, J. B., Amsler, C. D., and Angus, R. A. 2015. Testing Antarctic resilience: the effects of elevated seawater temperature and decreased pH on two gastropod species. ICES Journal of Marine Science: Journal du Conseil, 73: 739-752.

Secor, D. H., Dean, J. M., and Laban, E. H. 1992. Otolith removal and preparation for microstructural examination. pp. 19-57. Ed. by D. K. Stevenson, and S. E. Campana. Canadian Special Publication of Fisheries and Aquatic Sciences, Ottowa, Canada.

Sigwart, J. D., Lyons, G., Fink, A., Gutowska, M. A., Murray, D., Melzner, F., Houghton, J. D., et al. 2015. Elevated pCO_2 drives lower growth and yet increased calcification in the early life history of the cuttlefish *Sepia officinalis* (Mollusca: Cephalopoda). ICES Journal of Marine Science, 73: 970-980.

Small, D., Calosi, P., White, D., Spicer, J. I., and Widdicombe, S. 2010. Impact of medium-term exposure to CO_2 enriched seawater on the physiological functions of the velvet swimming crab *Necora puber*. Aquatic Biology, 10: 11-21.

Small, D. P., Calosi, P., Boothroyd, D., Widdicombe, S., and Spicer, J. I. 2015. Stage-specific changes in physiological and life-history responses to elevated temperature and pCO_2 during the larval development of the European lobster *Homarus gammarus* (L.). Physiological and Biochemical Zoology, 88: 494-507.

Smith, S. G. 1997. Models of crustacean growth dynamics. University of Maryland at College Park.

Smith, S. G., and Chang, E. S. 2007. Molting and growth. *In* The blue crab, *Callinectes sapidus*, pp. 197-254. Ed. by V. S. Kennedy, and L. E. Cronin. Maryland Sea Grant College, College Park, Maryland.

Soetaert, K., Hofmann, A. F., Middelburg, J. J., Meysman, F. J., and Greenwood, J. 2007. The effect of biogeochemical processes on pH. Marine Chemistry, 105: 30-51.

Spicer, J. I., Raffo, A., and Widdicombe, S. 2007. Influence of CO_2-related seawater acidification on extracellular acid-base balance in the velvet swimming crab *Necora puber*. Marine Biology, 151: 1117-1125.

Stocker, T., Qin, D., Plattner, G., Tignor, M., Allen, S., Boschung, J., Nauels, A., et al. 2013. IPCC, 2013: Summary for Policymakers. In Climate Change 2013: The Physical Science Basis.

Stocker, T. F. 2015. The silent services of the world ocean. Science (New York, NY), 350: 764-765.

Swarzenski, C. M., Doyle, T. W., Fry, B., and Hargis, T. G. 2008. Biogeochemical response of organic-rich freshwater marshes in the Louisiana delta plain to chronic river water influx. Biogeochemistry, 90: 49-63.

Swiney, K. M., Long, W. C., and Foy, R. J. 2015. Effects of high pCO_2 on Tanner crab reproduction and early life history—Part I: long-term exposure reduces hatching success and female calcification, and alters embryonic development. ICES Journal of Marine Science: Journal du Conseil, 73: 825-835.

Tankersley, R. A., and Forward, R. B. 2007. Environmental physiology. *In* The blue crab, *Callinectes sapidus*, pp. 451-484. Ed. by V. S. Kennedy, and L. E. Cronin. Maryland Sea Grant College, College Park, Maryland.

Tanner, C. A., Burnett, L. E., and Burnett, K. G. 2006. The effects of hypoxia and pH on phenoloxidase activity in the Atlantic blue crab, *Callinectes sapidus*. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 144: 218-223.

Taylor, E., Whiteley, N., and Wheatly, M. 1991. Respiratory gas exchange and the regulation of acid-base status in decapodan crustaceans. *In* Physiological strategies for gas exchange and metabolism., pp. 79-104. Ed. by A. J. Woakes, M. K. Grieshaber, and C. R. Bridges. Cambridge University Press, Cambridge, UK.

Taylor, H. H., and Taylor, E. W. 1992. Gills and lungs: The exchange of gases and ions. *In* Microscopic anatomy of invertebrates, pp. 203-293. Ed. by F. W. Harrison, and A. G. Humes. Wiley-Liss, New York.

Taylor, K. E., Stouffer, R. J., and Meehl, G. A. 2012. An overview of CMIP5 and the experiment design. Bulletin of the American Meteorological Society, 93: 485-498.

Todgham, A. E., and Stillman, J. H. 2013. Physiological responses to shifts in multiple environmental stressors: relevance in a changing world. Integrative and Comparative Biology, 53: 539-544.

Toler, S. K., Hallock, P., and Schijf, J. 2001. Mg/Ca ratios in stressed foraminifera, *Amphistegina gibbosa*, from the Florida Keys. Marine Micropaleontology, 43: 199-206.

Towle, D. W., and Burnett, L. E. 2007. Osmoregulatory, digestive, and respiratory physiology. *In* The blue crab, *Callinectes sapidus*, pp. 419-449. Ed. by V. S. Kennedy, and L. E. Cronin. Maryland Sea Grant College, College Park, MD.

Towle, D. W., and Holleland, T. 1987. Ammonium ion substitutes for K+ in ATP-dependent Na+ transport by basolateral membrane vesicles. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 252: R479-R489.

Towle, D. W., Palmer, G. E., and Harris, J. L. 1976. Role of gill Na+- K+-dependent ATPase in acclimation of blue crabs (*Callinectes sapidus*) to low salinity. Journal of Experimental Zoology Part A: Ecological Genetics and Physiology, 196: 315-321.

Uppström, L. R. 1974. The boron/chlorinity ratio of deep-sea water from the Pacific Ocean. *In* Deep Sea Research and Oceanographic Abstracts, pp. 161-162. Elsevier.

Vicente, J., Silbiger, N. J., Beckley, B. A., Raczkowski, C. W., and Hill, R. T. 2015. Impact of high pCO_2 and warmer temperatures on the process of silica biomineralization in the sponge *Mycale grandis*. ICES Journal of Marine Science: Journal du Conseil, 73: 704-714.

Vincent, J. F. 2002. Arthropod cuticle: a natural composite shell system. Composites Part A: Applied Science and Manufacturing, 33: 1311-1315.

Wagner, C. M., and Austin, H. M. 1999. Correspondence between environmental gradients and summer littoral fish assemblages in low salinity reaches of the Chesapeake Bay, USA. Marine Ecology Progress Series, 177: 197-212.

Waldbusser, G. G., Powell, E. N., and Mann, R. 2013. Ecosystem effects of shell aggregations and cycling in coastal waters: an example of Chesapeake Bay oyster reefs. Ecology, 94: 895-903.

Waldbusser, G. G., and Salisbury, J. E. 2014. Ocean acidification in the coastal zone from an organism's perspective: multiple system parameters, frequency domains, and habitats. Annual Review of Marine Science, 6: 221-247.

Waldbusser, G. G., Steenson, R. A., and Green, M. A. 2011a. Oyster shell dissolution rates in estuarine waters: effects of pH and shell legacy. Journal of Shellfish Research, 30: 659-669.

Waldbusser, G. G., Voigt, E. P., Bergschneider, H., Green, M. A., and Newell, R. I. E. 2011b. Biocalcification in the Eastern oyster (*Crassostrea virginica*) in

relation to long-term trends in Chesapeake Bay pH. Estuaries and Coasts, 34: 221-231.

Walther, K., Anger, K., and Poertner, H. O. 2010. Effects of ocean acidification and warming on the larval development of the spider crab *Hyas araneus* from different latitudes (54 degrees vs. 79 degrees N). Marine Ecology Progress Series, 417: 159-170.

Walther, K., Sartoris, F. J., Bock, C., and Poertner, H. O. 2009. Impact of anthropogenic ocean acidification on thermal tolerance of the spider crab *Hyas araneus*. Biogeosciences, 6: 2207-2215.

Waugh, D. A., Feldmann, R. M., Burrell, J. L., Hull, A. L., Hein, K., and Schweitzer, C. E. 2009. Ontogenetic variations in cuticle morphology in the blue crab *Callinectes sapidus* Rathbun, 1896. Journal of Crustacean Biology, 29: 141-156.

Werner, E. E. 1986. Amphibian metamorphosis: growth rate, predation risk, and the optimal size at transformation. The American Naturalist, 128: 319-341.

Wheatly, M. G. 1985. Free amino acid and inorganic ion regulation in the whole muscle and hemolymph of the blue crab *Callinectes sapidus* Rathburn in relation to the molting cycle. Journal of Crustacean Biology: 223-233.

Whitaker, D. pers. comm. 2017. Assistant Deputy Director, South Carolina Department of Natural Resources.

Whiteley, N. M. 2011. Physiological and ecological responses of crustaceans to ocean acidification. Marine Ecology Progress Series, 430: 257-271.

Wilbur, H. M., and Collins, J. P. 1973. Ecological aspects of amphibian metamorphosis. Science, 182: 1305-1314.

Williams, A. B. 1984. Shrimps, lobsters, and crabs of the Atlantic coast of the eastern United States, Maine to Florida, Smithsonian Institution Press, Washington, D.C. 550 pp.

Williams, A. B. 2007. Systematics and evolution. *In* The blue crab, *Callinectes sapidus*, pp. 1-21. Ed. by V. S. Kennedy, and L. E. Cronin. Maryland Sea Grant College, College Park, MD.

Wingate, R. L., and Secor, D. H. 2008. Effects of winter temperature and flow on a summer-fall nursery fish assemblage in the Chesapeake Bay, Maryland. Transactions of the American Fisheries Society, 137: 1147-1156.

Yao, W., and Byrne, R. H. 1998. Simplified seawater alkalinity analysis: Use of linear array spectrometers. Deep Sea Research Part I: Oceanographic Research Papers, 45: 1383-1392.

Zeis, B., Maurer, J., Pinkhaus, O., Bongartz, E., and Paul, R. J. 2004. A swimming activity assay shows that the thermal tolerance of *Daphnia magna* is influenced by temperature acclimation. Canadian Journal of Zoology, 82: 1605-1613.

Zhang, H., Shin, P. K., and Cheung, S. G. 2015. Physiological responses and scope for growth in a marine scavenging gastropod, *Nassarius festivus* (Powys, 1835), are affected by salinity and temperature but not by ocean acidification. ICES Journal of Marine Science: Journal du Conseil, 73: 814-824.