The effect of increasing acidity and temperature on an early life stage crustacean, *Callinectes sapidus*

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Dedication

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Thesis Abstract

The Atlantic blue crab is an important keystone and commercial species within the Chesapeake Bay, with current management practices successfully maintaining the population. However, future environmental conditions caused by increasing carbon dioxide from anthropogenic sources may impact these gains. In other decapods early life stages are most vulnerable to acidification, however little research has explored the response of larval blue crabs. With continued absorption of excess CO_2 into surface waters, the pH of these waters continue to decrease and may affect blue crab larval development. I determined the effects of increased acidity (pH 7.8, 7.4, & 6.8) on morphology, mortality, development, and protein and lipid content of embryos and larval blue crabs over a period of 16 and 7 days, respectively. Embryonic development is delayed, whereas larval survival and lipid content declines with increasing acidity. Morphology and protein content were unaffected in larvae, with the exception of shortened dorsal spine length at the highest acidity. Combining increased temperatures (23º, 26º, & 28º C) with acidification caused further declines in survival and lipid content, with new losses in protein content and swimming activity. No changes were observed in morphology or calcium content. Unless blue crabs are able to adapt at a rapid rate, these results denote negative outcomes for future populations and illustrate the need to explore mitigation strategies along with continued studies on adaptation.

Chapter One:

Introduction

Exposing early life stage blue crabs (*Callinectes sapidus*) to acidity and temperature requires developing foundational knowledge of their life history, habitat, physiology, calcification process, and responses to other pollutants in their environment. Beginning with life history, this chapter provides the introductory framework upon which subsequent chapters build in relation to embryonic and larval responses to two changing environmental parameters. Based on these concepts, I have found increased acidity alone and the combination of increased acidity with increased temperature to negatively affect survival and development in their early life stages.

Callinectes sapidus Life History:

Adults are sexually dimorphic, with males reaching maturity between 18-19th instars and females reaching sexual maturity at their terminal molt between 18-20th instars (Newcombe *et al,*. 1949a; Van Engel, 1958), or 12 months in warm water and 18 months in cool water. Size and sexual maturity are strongly influenced by environmental factors such as salinity, temperature, food, and quality of food in both sexes (Newcombe *et al.,* 1949b; Leffler, 1972; Millikin *et al.,* 1980; Cadman & Weinstein, 1988). Although males mate multiple times throughout their lives (Van Engel, 1958; Fischler, 1965), females only mate during their terminal pubertal molt (Van Engel, 1958) and store the ejaculate from one or more partners (Jivoff, 1997). After courtship, which is initiated primarily through chemical cues (Teytaud, 1971; Gleeson, 1980; Gleeson, 1991; Bushmann, 1999), mated females migrate to the lower Chesapeake Bay spawning

grounds (Aguilar *et al.,* 2005) and males remain in the upper Chesapeake with lower salinity (Hines *et al.,* 1987).

Female migration brings them to the mainstem of the Chesapeake Bay for egg production and incubation (Aguilar *et al.,* 2005), with subsequent migration leading to the Chesapeake's mouth or just outside to the ocean (Tagatz, 1968; Tankersley *et al.*, 1998) for egg hatching. Excellent swimmers, females swim vertically into nocturnal and strong ebb-tide currents (Tankersley *et al.*, 1998), which help to minimize near-shore planktivorous predation by swiftly drawing larvae off-shore (Morgan & Christy, 1995, 1997).

Brood production is strongly influenced by temperature, salinity, and female size (Costlow & Bookhout, 1959; Davis, 1965; Jivoff *et al.*, 2007), with females in the temperate Chesapeake Bay typically producing 1-3 broods per season (Hines *et al.,* 2003). Broods are carried between 14-17 days and are consistently aerated, which provides an added benefit of reducing parasite infestation (Kuris, 1991; Oh & Hartnoll, 1999; Levi *et al.*, 1999). Fungal infections are particularly prevalent, with up to 95% of all broods infected and 35-50% of each brood infested with fungi (Bland & Amerson, 1974). Increased salinity increases infestation rates (Millikin & Williams, 1980; Overstreet, 1982) and egg loss is common and potentially catastrophic (Wickham, 1986). To countermeasure, blue crabs exhibit extraordinarily high fecundity and produce broods between 700,000 to 6,000,000 eggs (Van Engel, 1958; Prager *et al.,* 1990; Zohar *et al.,* 2008). This strategy differs from other crustaceans and is successful because blue crabs produce unusually small eggs in comparison to other crustaceans (Davis, 1965).

During embryonic development, the chorion increases water volume by 18% (Davis, 1965), rapidly increasing in volume near hatching time to rupture the inner membrane through prezoea movement (Jivoff *et al.,* 2007). After rupture, stage I zoea are strong enough swimmers to maintain their position in the upper water column (Epifanio, 1995; Whitney & Garvine, 2005). Larval development occurs in coastal shelf waters (Provenzano *et al.,* 1983; Epifanio *et al.,* 1984) and distribution is highly dependent on upwelling, downwelling, and wind patterns that ultimately result in patchy larval distribution (Epifanio, 2007). Total development from stage I to stage VII zoea requires 3-4 weeks (Kennedy, 2007; Epifanio 2019), with the subsequent megalopae stage length highly dependent on a variety of environmental and chemical cues (Epifanio 2007; Epifanio & Cohen, 2016).

Upon megalopae recruitment and metamorphosis through advective transport (Forward & Tankersley, 2001), settlement is distributed through a variety of habitats. These include seagrass beds, macroalgal patches, marsh fringes, and oyster reefs, all of which offer good substrata with cover from predators and high prey abundance (Epifanio, 2019). Once juveniles reach 20-30 mm they begin migrating from nursery habitat (Lipcius *et al.,* 2007) to disperse throughout the estuary, continuing to molt toward sexual maturity. Mature crabs generally prefer habitat that provides plenty of cover to avoid numerous predators or conspecifics, as blue crabs exhibit strong cannibalistic behavior (Moksnes *et al.,* 1997). Such habitat is also available outside of the Chesapeake Bay, and reproductive populations of blue crabs are found from New York to the northern coasts of South America, with additional populations found throughout the Caribbean islands

(Zohar *et al.,* 2008).

Ecological Role of *Callinectes sapidus*:

In many ecosystems, apex predator populations have consistently diminished (Estes *et al.,* 2011), primarily due to strong fishing pressures from humans, which have been classified as the top predator for many ecosystems (Castilla, 1999). These pressures have had a two-fold effect, with both bottom up and top down controls in estuaries affected (Kemp *et al.,* 2005; Miller *et al.,* 2005). Eutrophication from changing land use has been exacerbated by the loss of filter feeders in the Chesapeake Bay from intensive oyster harvesting (Jackson *et al.,* 2001), while declining blue crab populations from similar harvest pressures likely caused large salt marsh die-offs, with subsequent conversion to mud flats (Silliman & Bertness, 2002). Additionally, the removal of apex predators has altered trophic food webs, with invertebrate predators now of primary importance in continental coastal ecosystems (Baum & Worm, 2009; Boudreau & Worm, 2012). Blue crabs are particularly described as keystone species due to their out-sized economic importance (Miller *et al.,* 2005) and their ability to alter habitat structure (Silliman & Bertness, 2002). They may also help the Chesapeake Bay resist invasive species (DeRivera *et al.,* 2005), although this does have mixed success (Roudez *et al.,* 2008).

Blue crabs serve as an important predator on bivalve communities (Arnold, 1984), crustaceans (Hill & Weissburg, 2013), scallops (Carroll *et al.,* 2015), small fishes (Kneib, 1982), and snails (West & Williams, 1986). As opportunistic predators, they will consume any prey available (Hines, 2007; Mancinelli *et al.,* 2017). Additionally, blue

crabs are highly important prey items for fishes, birds, mammals, and cephalopods (Boudreau & Worm, 2012). Reducing their population could exacerbate trophic cascades (Johnson *et al.*, 2014) and reduce environmental resilience (Lipcius & Stockausen, 2002), given their importance as both predator and prey.

Overall, blue crabs provide services ranging from habitat structural stability (Silliman & Bertness, 2002), trophic stability (Johnson *et al.*, 2014), and economic stability (Miller *et al.*, 2005) to the Chesapeake Bay region. Their current importance in the Chesapeake is likely due to human pressures that removed other predators from the area, such as sharks, gray whales, giant sturgeon, rays, and others (Jackson *et al.*, 2001). Although this heavy trophic reliance on large invertebrates such as the blue crab is relatively new, it emphasizes the need for better conservation strategies to prevent further trophic down-grading (Estes *et al.*, 2011).

Ion Regulation and Calcification in Crustaceans:

In general, decapods are strong ion regulators and actively transport bicarbonate into their hemolymph to maintain acid-base balance. However, it can be difficult to describe a generic calcification process due to the great habitat diversity in which crustaceans are found. Estuarine species like *Callinectes sapidus* are daily exposed to wide ranges in temperature, pH, and salinity, which enable adults to respond relatively well to increasing acidification. However, it is unknown whether larvae are also capable of internal regulation, because early life stages develop in more stable ocean conditions outside of the estuary (Whiteley, 2011). With this in mind, it is still necessary to understand adult regulatory processes in order to determine if similar processes are

employed in larvae.

Most adult decapods regulate hemolymph pH by controlling concentrations of haemocyanin proteins and bicarbonate ions. Approximately 93% of this bicarbonate is derived from surrounding ocean water (Cameron, 1985) through energetically expensive active transport, using the branchial Na^+/K^+-ATPase system. Estimates wildly range from 2.8-40% of total expenditure (Whiteley, 2011). Although some organisms can transfer ion regulation to a less expensive ion transporter (Pörtner *et al.,* 2000), it is unknown whether decapods can do the same (Whiteley, 2011). They can temporarily tolerate increased haemolymph acidity, however it is costly and can reduce oxygen affinity to respiratory pigments, severely disrupting oxygen delivery (Whiteley, 2011).

The cuticle structure of crustaceans is quite complex and composed of four distinct layers: epicuticle, exocuticle, endocuticle, and membranous layer (Taylor *et al*., 2015). The inner and outer layers do not contain elements susceptible to acidity (Travis, 1955a, 1955b; Green & Neff, 1972), but the two middle layers contain chitin-protein fibers with calcium carbonate layered in between to form a Bouligand structure (Bouligand, 1970). This calcium carbonate can be found as calcite, amorphous calcium carbonate, or Mg-calcite (Travis, 1963; Huner *et al.,* 1979; Roer & Dillaman, 1984; Dillaman *et al.,* 2005). Amorphous calcium carbonate gradually undergoes transformation into Mg-calcite or calcite (Andersson *et al.,* 2008) and calcium content may compose up to 22% of the middle layers (Vigh & Dendinger, 1982). Solubility is greatest with amorphous calcium carbonate and least with pure calcite, with calcite solubility increasing with greater magnesium incorporation (Andersson *et al.,* 2008).

Cuticle calcification relies on bicarbonate, unlike other calcifiers, which utilize carbonate (Cameron, 1985). Rather than relying on haemolymph acid-base balance, synthesis occurs within the exoskeletal compartment where pH is kept more basic to facilitate calcification (Cameron & Wood, 1985). Acidification could directly impact calcification rates through two pathways. Firstly, exoskeletal compartment pH could decrease, reducing calcium carbonate precipitation (Whiteley, 2011). Secondly, post-molt calcification could be compromised, which heavily relies on gills transporting calcium and bicarbonate from surrounding water (Neufield & Cameron, 1992; Wheatly, 1997). Inhibiting either pathway would delay exoskeleton hardening and increase predation vulnerability when they are immobile (Whiteley, 2011). *Callinectes sapidus* already experiences delayed calcification rates from hypercapnic events, slowing from 14 to 28 days (Whiteley, 2011). Additionally, crabs utilized metabolic bicarbonate rather than external bicarbonate, which slowed calcification rates significantly (Cameron, 1985). Even so, medium-term studies suggest adults are able to compensate well under high CO_2 conditions, indicating the unaffected epicuticle may provide a good barrier for calcification sites (Ries *et al.,* 2009).

Historical Ocean Acidity and the Chemistry of Acidification:

Excluding the recent past, carbon dioxide concentrations have been reasonably steady over the past 300 million years (Palmer *et al.,* 1998; Sabine *et al.,* 2004; Honisch *et al.,* 2008). Small fluctuations occurred over millennia and were primarily due to tectonic and biological inputs (Caldeira & Wickett, 2003). The greatest exception occurred during the current Cenozoic era (Pearson & Palmer, 2000) and developed

during the Paleocene-Eocene Thermal Maximum (PETM). Over a period of less than 10,000 years, CO_2 increased by a rate of 0.2 GtC/yr, causing catastrophic extinction of 35-50% across all benthic invertebrate species (Kurihara, 2008). As a result, global average temperatures rose to reach approximately 8° C warmer than present conditions. By 24 million years ago, ocean pH and atmospheric CO_2 re-stabilized to near present-day values (Pearson & Palmer, 2000).

Presently, anthropogenic rates of CO_2 emissions are 8 GtC/yr, 16 times higher than during the PETM (Gibbs *et al.*, 2006). Accordingly, current climate models predict temperature and pH changes over the next few centuries will be significantly faster than historic rates (Caldeira & Wickett, 2003), strongly and negatively influencing ocean pH (Marshall *et al.*, 2008). Approximately 26% of fossil fuel outputs have been absorbed by oceans (Le Quere *et al.*, 2012), which by the year 2300 may reduce average global ocean pH to as low as pH 7.4 (Caldeira & Wickett, 2003). This would be lower than any other global average pH in the past 25 million years (Widdicombe & Spicer, 2008).

When carbon dioxide enters seawater, it reacts with water to form carbonic acid, which dissociates into hydrogen, bicarbonate, and carbonate (Eq. 1).

$$CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow H^+ + HCO_3^- + CO_3^{2-} \quad \text{(Eq. 1)}$$

Bicarbonate and carbonate are important for regulating ocean acidity, in conjunction with a separate boron buffering system. Between these two systems, ocean acidity is currently maintained at approximately pH 8.06. The ocean prior to the Industrial Revolution is estimated to have been at pH 8.2 (Raven *et al.*, 2005), approximately reflecting a 30% increase in acidification since that time.

As carbon dioxide increases in the mixed layer, bicarbonate and carbonate concentrations shift to a new equilibrium with more bicarbonate and less carbonate, buffering the extra hydrogen ions produced in (Eq. 1). In certain systems like the Chesapeake Bay, this may result in sudden acidification events as buffering capacity is reached (Cai *et al.*, 2017). Organisms relying on carbonate uptake for shell synthesis are most impacted by these changes (Ries *et al*., 2009).

Crustacean Responses to Changing Environmental Conditions:

In other invertebrates, responses to increasing acidity and temperature are varied. Several reviews have highlighted the negative, neutral, or positive changes across differing species (Kurihara, 2008, Kroeker *et al*., 2013), collating single-species studies into a comprehensive whole. This section emphasizes the response of decapod crustaceans to increased acidity and temperature, narrowing to crabs and early life stages in particular. For a more thorough summary on economic and environmental impacts across marine invertebrates, please reference Appendices I & II.

Geographically, the most sensitive species are concentrated at high latitudes due to more rapid changes in carbonate chemistry (Kurihara, 2008), with early life stages likely to be most affected, although this depends on species' adaptability (Kurihara, 2008; Ross *et al*., 2011). Adaptation rates are generally unknown, but organisms with extremely short generation times are expected to adapt more quickly. Longer generation times, including decapod crustaceans, may slow adaptation rates. At least one study predicts invertebrate extinction rates between 15-37% by 2050 (Thomas *et al.*, 2004), although this estimate will likely change with more refined adaptation studies.

Although the Chesapeake Bay is not located in a high latitude, it is expected to be extremely sensitive to any changes in chemistry. It must balance interactions between riverine input, eutrophication, respiration, decomposition, and current dynamics (Najjar *et al.,* 2010; Cai *et al.,* 2011). This highly productive system supports a significant volume of marine calcifiers, dramatically lowering natural carbonate concentrations (Cai & Wang, 1998). As a result of these interactions, the Chesapeake Bay could suddenly shift to severely acidic conditions throughout the estuary that would initiate a cascade of calcium carbonate dissolution (Cai *et al*., 2017). Such an outcome would have significant ramifications for its diverse calcifying populations.

In shrimp and prawns, acidification primarily affects metabolic rates (Furtado *et al.*, 2017; Augusto *et al.,* 2018), with one species' metabolism declining by 30% at pH 7.3 (Augusto *et al.,* 2018). Such declines may impact immunosuppression and their ability to fight off disease (Furtado *et al.,* 2017). Growth, hatching success, calcification rates, and survival can also be affected (Wickins, 1984; Kurihara, 2008; Taylor *et al.*, 2015; Zheng *et al.,* 2015). Warming temperatures likely reduce health outcomes more than acidification alone, although in one shrimp species increased acidity mitigated its effects (Arnberg *et al.*, 2013).

Although there are relatively few published studies on lobster outcomes, the few existing studies provide contradictory results. One commercially important species (*Nephrops norvegicus*) experiences oxidative stress and immunosuppression in acidic conditions, with no effect on survival (Styf *et al.,* 2013; Hernroth *et al.,* 2015). Warming temperatures do not exacerbate any high acidity responses (Styf *et al.,* 2013). In another

commercially valuable species (*Homarus gammarus*), calcium content was reduced up to 50%, although length and survival were unaffected (Arnold *et al.,* 2009). In contradiction, another study did find reduced growth along with increased oxidative stress in the same species (Rato *et al.,* 2017). A near cousin, *Homarus americanus*, exhibited delayed development to subsequent molting stage, reduced carapace length, and increased mortality in its final larval stage (Keppel *et al.,* 2012), which was substantiated in later studies (McLean *et al.,* 2018; Niemisto, 2019). These outcomes, variable from its European cousin, appear to be population dependent responses (Niemisto, 2019) and may explain further contradictions found throughout lobster studies (McLean *et al.,* 2018).

Within crab species, outcomes of commercially valuable species dominate acidification and multi-stressor studies (Walther *et al.,* 2010; Long *et al.,* 2013a, 2013b, 2017; Giltz & Taylor, 2017; Gravinese, 2018; Bednarsek *et al.,* 2020; Tomasetti *et al.,* 2018). The cumulative worth of US crab fisheries sensitive to ocean acidification surpasses $400 million annually (Gravinese, 2018; Tomasetti *et al.,* 2018; Bednarsek *et al*., 2020), with the blue crab fishery alone one of the most valuable fisheries in the world (Eggleston *et al*., 2008).

Briefly mentioned earlier, juvenile and adult crabs have broadly been considered immune to acidification (Pane & Berry, 2007; Spicer *et al.,* 2007; Small *et al.,* 2010). However more recent studies detect increased calcification rates (Ries, 2011), delayed post-molt regrowth (Whiteley, 2011), and increased locomotory fatigue (Stover *et al.,* 2013). Increasing temperature alongside acidity reveals unaffected oxygen consumption and growth (Glandon *et al.,* 2017; Glandon *et al.,* 2019), although higher temperatures do

decrease intermolt period and increase food consumption (Glandon *et al.,* 2017). These two changes are expected, as increased temperatures quicken maturation (Fisher, 1999).

Early life stage crustaceans are far more susceptible to increased acidity and this is likely because they develop in more stable ocean environments that require less flexibility than estuarine and near-shore systems occupied by adult stages (Tomasetti *et al.,* 2018). Early life stages in many species respond with reduced lipids (Walther *et al*., 2010; Carter *et al.,* 2013; Long *et al*., 2013a), cardiac performance (Ceballos-Osuna *et al*., 2013), developmental rates (Walther *et al.,* 2010; Long *et al.,* 2017), size (Walther *et al.,* 2010; Long *et al.*, 2013b, 2017; Giltz & Taylor, 2017), and altered calcification rates (Long *et al.,* 2013a, 2013b; Bednarsek *et al*., 2020). However, morphological changes are highly variable, with some species undergoing no change (Long *et al.,* 2013a; Ceballos-Osuna *et al.,* 2013). Embryonic hatching success declined in the Florida stone crab (*Menippe mercenaria*) but was highly variable (Gravinese, 2018), similar to highly variable hatching success in *Petrolisthes cinctipes* (Ceballos-Osuna *et al*., 2013).

Beyond these sub-lethal outcomes, reduced survival has been evidenced in several species (Long *et al*., 2013a; Long *et al*., 2013b, 2017; Tomasetti *et al.,* 2018), with blue crab survival in Louisiana dropping by 23% (Giltz & Taylor, 2017). However, not all species experience reduced survival (Schiffer *et al*., 2013), and geographically distinct populations reveal markedly divergent responses to combined temperature and acidity changes (Walther *et al*., 2010). In addition to population differences, responses can widely vary between broods (Carter *et al.,* 2013; Ceballos-Osuna *et al.,* 2013; Gravinese, 2018) and could point to future adaptability (Waldbusser *et al.,* 2011; Long *et al.,* 2017),

but more studies are certainly required.

Although responses have been observed in other populations of early life stage blue crabs, none have yet evaluated the Chesapeake Bay population, which may differ as found in other species (Walther *et al*., 2010). Even so, the effects of acidification and temperature are likely to be negative. It is unlikely they will be able to internally compensate for increased acidity alone or increased temperatures and acidity because they develop in stable environmental conditions (Whiteley, 2011). Based on responses across other species, I expect calcification processes to be affected in their exocuticle and endocuticle, lipids and protein will decrease as their metabolic rates are affected, locomotory fatigue may occur, and survival will be diminished. I will also evaluate how acidification affects blue crab embryos, which has yet to be determined, and which will likely delay development and reduce hatching success.

Literature Cited

Aguilar, R., A. H. Hines, T. G. Wolcott, D. L. Wolcott, M. A. Kramer, & R. N. Lipcius. 2005. The timing and route of movement and migration of post-copulatory female blue crabs, *Callinectes sapidus* Rathbun, from the upper Chesapeake Bay. *Journal of Experimental Marine Biology and Ecology*, 319:117-128. doi: 10.1016/j.jembe.2004.08.030

Andersson, A. J., F. T. Mackenzie, & N. R. Bates. 2008. Life on the margin: implications of ocean acidification on Mg-calcite, high latitude and cold-water marine calcifiers. *Marine Ecological Progress Series*, 373:265–273. doi: 10.3354/meps07639

Arnberg, M., P. Calosi, J. I. Spicer, A. H. S. Tandberg, M. Nilsen, S. Westerlund, & R. K. Bechmann. 2013. Elevated temperature elicits greater effects than decreased pH on the development, feeding and metabolism of northern shrimp (*Pandalus borealis*) larvae. *Marine Biology*, 160:2037-2048. doi: 10.1007/s00227-012-2072-9

Arnold, W. S. 1984. The effects of prey size, predator size, and sediment composition on the rate of predation of the blue crab, *Callinectes sapidus* Rathbun, on the hard clam, *Mercenaria mercenaria* (Linne). *Journal of Experimental Marine Biology and Ecology*, 80:207-219.

Arnold, K. E., H. S. Findlay, J. I. Spicer, C. L. Daniels, & D. Boothroyd. 2009. Effect of CO_2-related acidification on aspects of the larval development of the European lobster, *Homarus gammarus* (L.). *Biogeosciences*, 6:1747-1754. doi: 10.5194/bg-6-1747-2009

Augusto, A., A. C. Ramaglia, & P. V. Mantoan. 2018. Effect of carbon dioxide-induced water acidification and seasonality on the physiology of the sea-bob shrimp *Xiphopenaeus kroyeri* (Decapoda, Penaeidae). *Crustaceana*, 91:947-960. doi: 10.1163/15685403-00003807

Baum, J. K., & B. Worm. 2009. Cascading top-down effects of changing oceanic predator abundances. *Journal of Animal Ecology*, 78:699-714. doi: 10.1111/j.1365-2656.2009.01531.x

Bednarsek, N., R. A. Feely, M. W. Beck, S. R. Alin, S. A. Siedlecki, P. Calosi, E. L. Norton, C. Saenger, J. Strus, D. Greeley, N. P. Nezlin, M. Roethler, & J. I. Spicer. 2020. Exoskeleton dissolution with mechanoreceptor damage in larval Dungeness crab related to severity of present-day ocean acidification vertical gradients. *Science of the Total Environment*. https://doi.org/10.1016/j.scitotenv.2020.136610

Bland, C. E. & H. V. Amerson. 1974. Occurrence and distribution in North Carolina waters of *Lagenidium callinectes* Couch, a fungal parasite of blue crab ova. *Chesapeake Science*, 15:232-235.

Boudreau, S. A., & B. Worm. 2012. Ecological role of large benthic decapods in marine ecosystems: a review. *Marine Ecology Progress Series*, 469:195-212. doi: 10.3354/meps09862

Bouligand, Y. 1970. Aspects ultrastructuraux de la calcification chez les Crabes. 7e Congrès int. Microsc. Électr., Grenoble, France 3:105–106.

Bushmann, E. J. 1999. Concurrent signals and behavioral plasticity in blue crab (*Callinectes sapidus* Rathbun) courtship. *Biological Bulletin*, 197:63-71.

Cadman, L. R., & M. P. Weinstein. 1988. Effects of temperature and salinity on the growth of laboratory-reared juvenile blue crabs *Callinectes sapidus* Rathbun. *Journal of Experimental Marine Biology and Ecology*, 121:193-207.

Cai, W.-J., and Y. Wang. 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.

Cai, W.-J., X. Hu, W.-J. Huang, M. C. Murrell, J. C. Lehrter, S. E. Lohrenz, W.-C. Chou, W. Zhai, J. T. Hollibaugh, Y. Wang, P. Zhao, X. Guo, K. Gunderson, M. Dai, & G.-C. Gong. 2011. Acidification of subsurface coastal waters enhanced by eutrophication. *Nature Geoscience*, 4:766-770. doi: 10.1038/ngeo1297

Cai, W.-J., W.-J. Huang, G. W. Luther III, D. Pierrot, M. Li, J. Testa, M. Xue, A. Joesoef, R. Mann, J. Brodeur, Y.-Y. Xu, B. Chen, N. Hussain, G. G. Waldbusser, J. Cornwall, & W. M. Kamp. 2017. Redox reactions and weak buffering capacity lead to acidification in the Chesapeake Bay. *Nature Communications*, 8:369. doi: 10.1038/s41467-017-00417-7

Caldeira, K., & M. E. Wickett. 2003. Anthropogenic carbon and ocean pH. *Nature*. 425:365, doi:10.1038/425365a.

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

Cameron, J. N., & C. M. Wood. 1985. Apparent H^+ excretion and CO_2 dynamics accompanying carapace mineralization in the blue crab (*Callinectes sapidus*) following moulting. *Journal of Experimental Biology*, 114:181-196.

Carter, H. A., L. Ceballos-Osuna, N. A. Miller, & J. H. Stillman. 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. doi: 10.1242/jeb.078162

Carroll, J. M., L. J. Jackson, & B. J. Peterson. 2015. The effect of increasing habitat complexity on Bay scallop survival in the presence of different decapod crustacean predators. *Estuaries and Coasts*, 38:1569-1579. doi: 10.1007/s12237-014-9902-6.

Castilla, J. C. 1999. Coastal marine communities: trends and perspectives from human-exclusion experiments. *Trends in Ecology and Evolution*, 14:280–283.

Ceballos-Osuna, L., H. A. Carter, N. A. Miller, & J. H. Stillman. 2013. Effects of ocean acidification on early life-history stages of the intertidal porcelain crab *Petrolisthes cinctipes*. *Journal of Experimental Biology*, 216:1405-1411. doi: 10.1242/jeb.078154

Costlow, J. D. & C. G. Bookhout. 1959. The larval development of *Callinectes sapidus* Rathbun reared in the laboratory. *Biological Bulletin*, 116:373-396.

Davis, C. C. 1965. A study of the hatching process in aquatic invertebrates: XX. The blue crab, *Callinectes sapidus*, Rathbun, XXI. The nemertean, *Carcinonemertes carcinophila* (Kolliker). *Chesapeake Science*, 6:201-208.

DeRivera, C. E., G. M. Ruiz, A. H. Hines, & P. Jivoff. 2005. Biotic resistance to invasion: native predator limits abundance and distribution of an introduced crab. *Ecology*, 86:3364–3376. doi: 10.1890/05-0479

Dillaman, R., S. Hequembourg, & M. Gay. 2005. Early pattern of calcification in the dorsal carapace of the blue crab, *Callinectes sapidus. Journal of Morphology*, 263:356-374. doi: 10.1002/jmor.10311

Eggleston, D. B., E. G. Johnson, G. T. Kellison, G. R. Plaia & C. L. Huggett. 2008. Pilot evaluation of early juvenile blue crab stock enhancement using a replicated BACI design. *Reviews in Fisheries Science and Aquaculture,* 16:91–100. doi: 10.1080/10641260701681664

Epifanio, C. E. 1995. Transport of blue crab (*Callinectes sapidus*) larvae in the waters off the Mid-Atlantic states. *Bulletin of Marine Scienc*e, 57:713–725.

Epifanio, C. E. 2007. Larval biology. In: Kennedy, V. S. & L. E. Cronin, editors. The blue crab *Callinectes sapidus*. College Park, MD: Maryland Sea Grant. pp. 513–533.

Epifanio, C. E. 2019. Early life history of the blue crab *Callinectes sapidus*: A review. *Journal of Shellfish Research*, 38:1-22. doi: 10.2983/035.038.0101

Epifanio, C. E. & J. H. Cohen. 2016. Behavioral adaptations in larvae of brachyuran crabs: a review. *Journal of Experimental Marine Biology and Ecology*, 482:85–105. doi: 10.1016/j.jembe.2016.05.006

Epifanio, C. E., C. C. Valenti & A. E. Pembroke. 1984. Dispersal and recruitment of blue crab larvae in Delaware Bay, U.S.A. *Estuarine, Coastal and Shelf Science*, 18:1–12.

Estes, J. A., J. Terborgh, J. S. Brashares, M. E. Power, J. Berger, W. J. Bond, S. R. Carpenter, T. E. Essington, R. D. Holt, J. B. C. Jackson, R. J. Marquis, L. Oksanen, T. Oksanen, R. T. Paine, E. K. Pikitch, W. J. Ripple, S. A. Sandin, M. Scheffer, T. W. Schoener, J. B. Shurin, A. R. E. Sinclair, M. E. Soule, R. Virtanen, & D. A. Wardle. 2011. Trophic downgrading of planet Earth. *Science*, 333: 301-306. doi: 10.1126/science.1205106.

Fischler, K. J. 1965. The use of catch-effort, catch-sampling, and tagging data to estimate a population of blue crabs. *Transactions of the American Fisheries Society*, 94:287-310.

Fisher, M. R. 1999. Effect of temperature and salinity on size at maturity of female blue crabs. *Transactions of the American Fisheries Society*, 128:499-506.

Forward, R. B., Jr. & R. A. Tankersley. 2001. Selective tidal-stream transport of marine animals. *Oceanography and Marine Biology- An Annual Review*, 39:305–353.

Furtado, P. S., C. A. P. Gaona, F. P. Serra, L. H. Poersch, & W. Wasielesky Jr. 2017. Acute toxicity of carbon dioxide to juvenile marine shrimp *Litopenaeus vannamei* (Boone 1931). *Marine and Freshwater Behavior and Physiology*, 50:293-301. doi: 10.1080/10236244.2017.1371568

Gibbs, S. J., P. R. Bown, J. A. Sessa, T. J. Bralower, & P. A. Wilson. 2006. Nanoplankton extinction and origination across the Paleocene-Eocene Thermal Maximum. *Science*, 314:1770–1773. doi: 10.1126/science.1133902

Giltz, S. M. & C. M. Taylor. 2017. Reduced growth and survival in the larval blue crab *Callinectes sapidus* under predicted ocean acidification. *Journal of Shellfish Research*, 36:481-485. doi: 10.2983/035.036.0219

Glandon, H. L., & T. J. Miller. 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*, 74:1201-1209. doi: 10.1093/icesjms/fsw171

Glandon, H. L., K. T. Paynter, C. L. Rowe, & T. J. Miller. 2019. Resilience of oxygen consumption rates in the juvenile blue crab *Callinectes sapidus* to future predicted increases in environmental temperature and pCO_2 in the mesohaline Chesapeake Bay. *Journal of Shellfish Research*, 38:711-723. doi: 10.2983/035.038.0323

Gleeson, R. A. 1980. Pheromone communication in the reproductive behavior of the blue crab, *Callinectes sapidus. Marine Behaviour and Physiology*, 7:119-134.

Gleeson, R. A. 1991. Intrinsic factors mediating pheromone communication in the blue crab, *Callinectes sapidus*. Pages 17-32 in R. Bauer and J.W. Martin (eds.). Crustacean Sexual Biology. Columbia University Press, New York.

Gravinese, P. M. 2018. Ocean acidification impacts the embryonic development and hatching success of the Florida stone crab, *Menippe mercenaria*. *Journal of Experimental Marine Biology and Ecology*, 500:140-146. doi: 10.1016/j.jembe.2017.09.001

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

Hernroth, B., A.-S. Krang, & S. Baden. 2015. Bacteriostatic suppression in Norway lobster (*Nephrops norvegicus*) exposed to manganese or hypoxia under pressure of ocean acidification. *Aquatic Toxicology*, 159:217-224. doi: 10.1016/j.aquatox.2014.11.025

Hill, J. M., & M. J. Weissburg. 2013. Habitat complexity and predator size mediate interactions between intraguild blue crab predators and mud crab prey in oyster reefs. *Marine Ecology Progress Series*, 488:209-219. doi: 10.3354/meps10386

Hines, A. 2007. "Ecology of Juvenile and Adult Blue Crabs." The Blue Crab: *Callinectes sapidus*. Ed. V. S. Kennedy and L. E. Cronin. Maryland Sea Grant College, 565-654.

Hines, A. H., R. N. Lipcius, & A. M. Haddon. 1987. Population dynamics and habitat partitioning by size, sex, and molt stage of blue crabs *Callinectes sapidus* in a subestuary of central Chesapeake Bay. *Marine Ecology Progress Series*, 36:55-64.

Hines, A. H., P. R. Jivoff, E. J. Bushmann, J. van Montfrans, S. A. Reed, D. L. Wolcott & T. G. Wolcott. 2003. Evidence for sperm limitation in the blue crab, *Callinedes sapidus. Bulletin of Marine Science*, 72:287-310.

Honisch, B., T. Bickert, & N. G. Hemming. 2008. Modern and Pleistocene boron isotope composition of the benthic foraminifer *Cibicidoides wuellerstorfi*. *Earth and Planetary Science Letters*, 272:309-318. doi: 10.1016/j.epsl.2008.04.047

Huner, J. V., L. B. Colvin, & B. L. Reid. 1979. Whole-body calcium, magnesium and phosphorous levels of the California brown shrimp, *Penaeus californiensis* (Decapoda: Penaeidae) as functions of molt stage. *Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology*, 64:33–36.

Jackson, J. B. C., Kirby, M. X., Berger, W. H., Bjorndal, K. A., Botsford, L. W., Bourque, B. J., Bradbury, R. H., Cooke, R., Erlandson, J., Estes, J. A., Hughes, T. P., Kidwell, S., Lange, C. B., Lenihan, H. S., Pandolfi, J. M., Peterson, C. H., Steneck, R. S., Tegner, M. J., Warner, R. R. 2001. Historical overfishing and the recent collapse of coastal ecosystems. *Science*, 293: 629–631. doi: 10.1126/science.1059199

Jivoff, P. 1997. The relative roles of predation and sperm competition on the duration of the post-copulatory association between the sexes in the blue crab, *Callinedes sapidus. Behavioral Ecology and Sociobiology*, 40:175-185.

Jivoff, P., A. N. Hines & L. S. Quackenbush. 2007. Reproduction biology and embryonic development. In: Kennedy, V. S. & L. E. Cronin, editors. *The blue crab Callinectes sapidus*. College Park, MD: Maryland Sea Grant. pp. 255–298.

Johnson, K. D., J. H. Grabowski, & D. L. Smee. 2014. Omnivory dampens trophic cascades in estuarine

communities. *Marine Ecology Progress Series*, 507:197-206. doi: 10.3354/meps10815

Kemp, W. M., W. R. Boynton, J. E. Adolf, D. F. Boesch, W. C. Boicourt, G. Brush, J. C. Cornwell, T. R. Fisher, P. M. Gilbert, J. D. Hagy, L. W. Harding, E. D. Houde, D. G. Kimmel, W. D. Miller, R. I. E. Newell, M. R. Roman, E. M. Smith, & J. C. Stevenson. 2005. Eutrophication of Chesapeake Bay: historical trends and ecological interactions. *Marine Ecology Progress Series*, 303:1-29. doi:10.3354/meps303001

Kennedy, V. S. 2007. External anatomy of blue crab larvae. In: Kennedy, V. S. & L. E. Cronin, editors. The blue crab *Callinectes sapidus*. College Park, MD: Maryland Sea Grant. pp. 23–54.

Keppel, E. A., R. A. Scrosati, & S. C. Courtenay. 2012. Ocean acidification decreases growth and development in American lobster (*Homarus americanus*) larvae. *Journal of Northwest Atlantic Fisheries Science*, 44:61-66.

Kneib, R. T. 1982. The effects of predation by wading birds (Ardeidae) and blue crabs (*Callinectes sapidus*) on the population size structure of the common mummichog, *Fundulus heterclitus*. *Estuarine, Coastal and Shelf Science,* 14:159-165.

Kroeker, K. J., R. L. Kordas, R. Crim, I. E. Hendriks, L. Ramajos, G. S. Singh, C. M. Duartes, & J.-P. Gattuso. 2013. Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. *Global Change Biology*, 19:1884-1896, doi: 10.1111/gcb.12179

Kurihara, H. 2008. Effects of CO_2-driven ocean acidification on the early developmental stages of invertebrates. *Marine Ecology Progress Series*, 373:275-284. doi: 10.3354/meps07802

Kuris, A. M. 1991. A review of patterns and causes of crustacean brood mortality. Pages 117-141 in A. Wenner and A. Kuris (eds.). Crustacean Issues 7: Crustacean Egg Production. A.A. Balkema, Rotterdam.

Leffler, C. W. 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.

Le Quere, C., R. J. Andres, T. Boden, T. Conway, R. A. Houghton, J. I. House, G. Marland, G. P. Peters, G. van der Werf, A. Ahlstrom, R. M. Andrew, L. Bopp, J. G. Canadell, P. Ciais, S. C. Doney, C. Enright, P. Friedlingstein, C. Huntingford, A. K. Jain, C. Jourdain, E. Kato, R. F. Keeling, K. Klein Goldewijk, S. Levis, P. Levy, M. Lomas, B. Poulter, M. R. Raupach, J. Schwinger, S. Sitch, B. D. Stocker, N. Viovy, S. Zaehle, & N. Zeng. 2012. The global carbon budget, 1959-2011. *Earth System Science Data Discussions*, 5:1107-1157. doi:10.5194/essdd-5-1107-2012

Levi, T., A. Barki, G. Hulata and I. Karplus. 1999. Mother-offspring relationships in the red-claw crayfish *Cherax quadricarinatus. Journal of Crustacean Biology*, 19:477-484.

Lipcius, R., & W. Stockhausen. 2002. Concurrent decline of the spawning stock, recruitment, larval abundance, and size of the blue crab *Callinectes sapidus* in Chesapeake Bay. *Marine Ecology Progress Series*, 226:45–61. doi: 10.3354/meps226045

Lipcius, R. N., D. B. Eggleston, K. L. Heck, Jr., R. D. Seitz & J. van Montfrans. 2007. Ecology of postlarval and juvenile blue crabs. In: Kennedy, V. S. & L. E. Cronin, editors. The blue crab *Callinectes sapidus*. College Park, MD: Maryland Sea Grant. pp. 535–564.

Long, W. C., K. M. Swiney, & R. J. Foy. 2013a. Effects of ocean acidification on the embryos and larvae of red king crab, *Paralithodes camtschaticus. Marine Pollution Bulletin*, 69:38-47. doi: 10.1016/j.marpolbul.2013.01.011

Long, W. C., K. M. Swiney, C. Harris, H. N. Page, & R. J. Foy. 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:e6095. doi: 10.1371/journal.pone.0060959

Long, W. C., S. B. Van Sant, K. M. Swiney, & R. J. Foy. 2017. Survival, growth, and morphology of blue king crabs: effect of ocean acidification decreases with exposure time. *ICES Journal of Marine Science*, 74:1033-1041. doi: 10.1093/icesjms/fsw197

Mancinelli, G., M. T. Guerra, K. Alujevic, D. Raho, M. Zotti, & S. Vizzini. 2017. Trophic flexibility of the Atlantic blue crab *Callinectes sapidus* in invaded coastal systems of the Apulia region (SE Italy): A stable isotope analysis. *Estuarine, Shelf and Coastal Science,* 198:421-431. doi: 10.12681/mms.13886

Marshall, D. J., J. H. Santos, K. M. Y. Leung, & W. H. Chak. 2008. Correlations between gastropod shell dissolution and water chemical properties in a tropical estuary. *Marine Environmental Research*, 66:422-429. https://doi.org/10.1016/j.marenvres.2008.07.003

McLean, E. L., N. V. Katenka, & B. A. Seibel. 2018. Decreased growth and increased shell disease in early benthic phase *Homarus americanus* in response to elevated CO_2. *Marine Ecology Progress Series*, 596:113-126. doi: 10.3354/meps12586

Millikin, M. R., G. N. Biddle, T. C. Siewicki, A. R. Fortner & P. H. Fair. 1980. Effects of various levels of dietary protein on survival, molting frequency and growth of juvenile blue crabs (*Callinedes sapidus*). *Aquaculture*, 19:149-161.

Millikin, M. R. & A. B. Williams. 1980. Synopsis of biological data on the blue crab, *Callinedes sapidus* Rathbun. FAO Fisheries Synopsis 138, NOAA Technical Report NMFS 1. Washington, District of Columbia. 39 p.

Miller, T. J., S. J. D. Martell, D. B. Bunnell, G. Davis, L. Fegley, A. Sharov, C. Bonzek, D. Hewitt, J. Hoenig, & R. N. Lipcius. 2005. Stock assessment of the blue crab in Chesapeake Bay. University of Maryland Center for Environmental Science, Technical Report Series TS-487-05, Solomons. Available: hjort.cbl.umces.edu/crabs/Assessment05.html.

Moksnes, P-O., R. N. Lipcius, L. Pihl, & J. van Montfrans. 1997. Cannibal-prey dynamics in young juveniles and postlarvae of the blue crab. *Journal of Experimental Marine Biology and Ecology*, 215:157-187.

Morgan, S. G. & J. H. Christy. 1995. Adaptive significance of the timing of larval release by crabs. *American Naturalist,* 145:457-479.

Morgan, S. G. & J. H. Christy. 1997. Planktivorous fishes as selective agents for reproductive synchrony. *Journal of Experimental Marine Biology and Ecology*, 209:89-101.

Najjar, R. G., C. R. Pyke, M. B. Adams, D. Breitburg, C. Hershner, M. Kemp, R. Howarth, M. R. Mulholland, M. Paolisso, D. Secor, K. Sellner, D. Wardrop, & R. Wood. 2010. Potential climate-change impacts on the Chesapeake Bay. *Estuarine, Coastal and Shelf Science*, 86:1-20. doi: 10.1016/j.ecss.2009.09.026

Neufield, D. S. & J. N. Cameron. 1992. Postmoult uptake of calcium by the blue crab (Callinectes sapidus) in water of low salinity. Journal of Experimental Biology, 171:283–299.

Newcombe, C. L., F. Campbell, & A. M. Eckstine. 1949a. A study of the form and growth of the blue crab

Callinectes sapidus Rathbun. *Growth*, 13:71-96.

Newcombe, C. L., M. D. Sandoz, & R. Talbert-Rogers. 1949b. Differential growth and moulting characteristics of the blue crab, *Callinectes sapidus* Rathbun. *Journal of Experimental Zoology*, 110:113-152.

Niemisto, M. K. 2019. Response of early life stage *Homarus americanus* to ocean warming and acidification: an interpopulation comparison. *Electronic Theses and Dissertations*, 3076.

Oh, C.-W. & R. G. Hartnoll. 1999. Brood loss during incubation in *Philocheras trispinosus* (Decapoda) in Port Erin Bay, Isle of Man. *Journal of Crustacean Biology*, 19:467-476.

Overstreet, R. M. 1982. Metazoan symbionts of the blue crab. Pages 81-87 in H. M. Perry and W. A. Van Engel (eds.). Proceedings of the Blue Crab Colloquium, October 16-19, 1979. Gulf States Marine Fisheries Commission Publication 7. Ocean Springs, Mississippi.

Palmer, M. R., P. N. Pearson, & S. J. Cobb. 1998. Reconstructing past ocean pH-depth profiles. *Science*, 282:1468-1471.

Pane, E. F., & J. P. Barry. 2007. Extracellular acid–base regulation during short-term hypercapnia is effective in a shallow-water crab, but ineffective in a deep-sea crab. *Marine Ecological Progress Series*, 334:1–9. doi: 10.3354/meps334001

Pearson, P. N. & M. R. Palmer. 2000. Atmospheric carbon dioxide concentrations over the past 60 million years. *Nature*, 406:695-699. doi: 10.1038/35021000

Pörtner, H. O., C. Bock, & A. Reipschläger. 2000. Modulation of the cost of pH regulation during metabolic depression: a 31 P-NMR study in invertebrate (*Sipunculus nudus*) isolated muscle. *Journal of Experimental Biology*, 203:2417–2428.

Prager, M. H., J. R. McConaugha, C. M. Jones & P. J. Geer. 1990. Fecundity of blue crab, *Callinedes sapidus*, in Chesapeake Bay: Biological, statistical and management considerations. *Bulletin of Marine Science*, 46:170-179.

Provenzano, A. J., Jr., J. R. McConaugha, K. B. Philips, D. F. Johnson and J. Clark. 1983. Vertical distribution of first stage larvae of the blue crab, *Callinedes sapidus*, at the mouth of Chesapeake Bay. *Estuarine, Coastal and Shelf Science*, 16:489-499.

Rato, L. D., S. C. Novais, M. F. L. Lemos, L. M. F. Alves, & S. M. Leandro. 2017. *Homarus gammarus* (Crustacea: Decapoda) larvae under an ocean acidification scenario: responses across different levels of biological organization. *Comparative Biochemistry & Physiology- Part C: Toxicology and Pharmacology* 203: 29–38. doi: 10.1594/PANGAEA.892416

Raven, J. A., K. Caldeira, H. Elderfield, O. Hoegh-Guldberg, P. Liss, U. Riebesell, J. Shepherd, C. Turley & A. Watson. 2005. Ocean acidification due to increasing atmospheric carbon dioxide. Royal Society, London, UK. The Clyvedon Press Ltd.

Roer, R. & R. Dillaman. 1984. The structure and calcification of the crustacean cuticle. *American Zoology*, 24:893–909.

Ross, P. M. L. Parker, W. A. O'Connor, & E. A. Bailey. 2011. The impact of ocean acidification on reproduction, early development and settlement of marine organisms. *Water*, 3:1005-1030. doi: 10.3390/w3041005

Roudez, R. J., T. Glover, & J. S. Weis. 2008. Learning in an invasive and a native predatory crab. *Biological Invasions*, 10:1191-1196. doi: 10.1007/s10530-007-9195-9.

Ries, J. B., A. L. Cohen, & D. C. McCorkle. 2009. Marine calcifiers exhibit mixed responses to CO_2-induced ocean acidification. *Geology*, 37:1131-1134. doi: 10.1130/G30210A.1

Ries, J. B. 2011. Skeletal mineralogy in a high-CO_2 world. *Journal of Experimental Marine Biology and Ecology.* 403:54-64. doi:10.1016/j.jembe.2011.04.006

Sabine, C. L., R. A. Feely, N. Gruber, R. M. Key, K. Lee, J. L. Bullister, R. Wanninkhof, C. S. Wong, D. W. R. Wallace, B. Tilbrook, F. J. Millero, T.-H. Peng, A. Kozyr, T. Ono, & A. F. Rios. 2004. The oceanic sink for anthropogenic CO_2. *Science*, 305:367-371. doi: 10.1126/science.1097403

Small, D., P. Calosi, D. White, J. I. Spicer, & S. Widdicombe. 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. doi: 10.3354/ab00266

Silliman, B. R., & M. D. Bertness. 2002. A trophic cascade regulates salt marsh primary production. *Proceedings of the National Academy of Science, USA*, 99:10500–10505. doi: 10.1073/pnas.162366599

Spicer, J. I., A. Raffo, & S. Widdicombe. 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. doi: 10.1007/s00227-006-0551-6

Stover, K. K., K. G. Burnett, E. J. McElroy, & L. E. Burnett. 2013. Locomotory fatigue during moderate and severe hypoxia and hypercapnia in the Atlantic blue crab, *Callinectes sapidus*. *Biological Bulletin*, 224:68-78. doi: 10.1086/BBLv224n2p63

Styf, H. K., H. N. Skold, & S. P. Eriksson. 2013. Embryonic response to long-term exposure of the marine crustacean *Nephrops norvegicus* to ocean acidification and elevated temperature. *Ecology and Evolution*, 3:5055-5065. doi: 10.1002/ece3.860

Tagatz, M. E. 1968. Biology of the blue crab, *Callinectes sapidus* Rathbun, in the St. Johns River, Florida. *Fishery Bulletin*, 67:17-33.

Tankersley, R. A., M. G.Wieber, M. A. Sigala & K. A. Kachurak. 1998. Migratory behavior of ovigerous blue crabs *Callinectes sapidus* — evidence for selective tidal-stream transport. *Biological Bulletin*, 195:168-173.

Taylor, J. R. A., J. M. Gilleard, M. C. Allen, & D. D. Deheyn. 2015. Effects of CO_2-induced pH reduction on the exoskeleton structure and biophotonic properties of the shrimp *Lysmata californica*. *Nature Scientific Reports*, 5:10608. doi: 10.1038/srep10608

Teytaud, A. R. 1971. The laboratory studies of sex recognition in the blue crab *Callinectes sapidus* Rathbun. Sea Grant Technical Bulletin No. 15. University of Miami Sea Grant. Miami, Florida. 63 p.

Thomas, C. D., A. Cameron, R. E. Green, M. Bakkenes, L. J. Beaumont, Y. C. Collingham, B. F. N. Erasmus, M. F. de Siqueira, A. Grainger, L. Hannah, L. Hughes, B. Huntley, A. S. Van Jaarsveld, G. F. Midgley, L. Miles, M. A. Ortega-Huerta, A. T. Peterson, O. L. Philips, & S. E. Williams. 2004. Extinction risk from climate change. *Nature*, 427:145–148. doi: 10.1038/nature02121

Tomasetti, S. J., B. K. Morrell, L. R. Merlo, & C. J. Gobler. 2018. Individual and combined effects of low

dissolved oxygen and low pH on survival of early life stage blue crabs, *Callinectes sapidus. PLoS ONE*, 13:e0208629. doi: 10.1371/journal.pone.0208629

Travis, D. F. 1955a. The molting cycle of the spiny lobster, *Panulirus argus* Latreille. II. Pre-ecdysial histological and histochemical changes in the hepatopancreas and integumental tissues. *Biological Bulletin*, 108:88–112.

Travis, D. F. 1955b. The molting cycle of the spiny lobster, *Panulirus argus* Latreille. III. Physiological changes which occur in the blood and urine during the normal molting cycle. *Biological Bulletin*, 109:484–503.

Travis, D. F. 1963. Structural features of mineralization from tissue to macromolecular levels of organization in Decapod Crustacea. *Annals of the New York Academy of Science*, 109:177–245.

Van Engel, W. A. 1958. The blue crab and its fishery in Chesapeake Bay: reproduction, early development, growth and migration. *Commercial Fisheries Review*, 20:6-17.

Vigh, D. & J. Dendinger. 1982. Temporal relationships of postmolt deposition of calcium, magnesium, chitin and protein in the cuticle of the Atlantic blue crab, *Callinectes sapidus* Rathbun. *Comparative Biochemical Physiology*, 72:365–369.

Waldbusser, G. G., E. P. Voigt, H. Bergschneider, M. A. Green, & R. I. E. Newell. 2011. Biocalcification in the Eastern oyster (*Crassostrea virginica*) in relation to long-term trends in Chesapeake Bay pH. *Estuaries and Coasts*, 34:221-231. doi: 10.1007/s12237-010-9307-0

Walther, K., K. Anger & H.-O. Pörtner. 2010. Effects of ocean acidification and warming on the larval development of the spider crab *Hyas araneus* from different latitudes (54° vs. 79°N). *Marine Ecology Progress Series*, 417:159-170. doi: 10.3354/meps08807

West, D. L. & A. H. Williams. 1986. Predation by *Callinectes sapidus* (Rathbun) within *Spartina alterniflora* (Loisel). *Journal of Experimental Marine Biology and Ecology*, 100:75–95.

Wheatly, M. G. 1997. Crustacean models for studying calcium transport: the journey from whole organisms to molecular mechanisms. *Journal of the Marine Biological Association of the United Kingdom*, 77:107–125.

Whiteley, N. M. 2011. Physiological and ecological responses of crustaceans to ocean acidification. *Marine Ecology Progress Series*, 430:257-271. doi:10.3354/meps09185

Whitney, M. M. & R. W. Garvine. 2005. Wind influence on a coastal buoyant outflow. *Journal of Geophysical Research- Oceans*, 110:C03014. doi:10.1029/2003JC002261

Wickham, D. E. 1986. Epizootic infestations by nemertean brood parasites on commercially important crustaceans. *Canadian Journal of Fisheries and Aquatic Sciences*, 43:2295-2302.

Wickins, J. F. 1984. The effect of hypercapnic sea water on growth and mineralization in panaeid prawns. *Aquaculture*, 41:37-48.

Widdicombe, S. & J. I. Spicer. 2008. Predicting the impact of ocean acidification on benthic biodiversity: What can animal physiology tell us? *Journal of Experimental Marine Biology & Ecology*, 366:187–197. doi:10.1016/j.jembe.2008.07.024

Zheng, C.-q., J. Jeswin, K.-l. Shen, M. Lablche, K.-j. Wang, & H.-p. Liu. 2015. Detrimental effect of CO_2-

driven seawater acidification on a crustacean brine shrimp, *Artemia sinica*. *Fish and Shellfish Immunology*, 43:181-190. doi: 10.1016/j.fsi.2014.12.027

Zohar, Y., A. H. Hines, O. Zmora, E. G. Johnson, R. N. Lipcius, R. D. Seitz, D. B. Eggleston, A. R. Place, E. J. Schott, J. D. Stubblefield, & J. S. Chung. 2008. The Chesapeake Bay blue crab (*Callinectes sapidus*): A multidisciplinary approach to responsible stock replenishment. *Reviews in Fishery Science*, 16:25-35. doi: 10.1080/10641260701681623

Chapter Two:

The effect of increased acidity on blue crab (*Callinectes sapidus*) embryonic development

Abstract

The Chesapeake Bay is experiencing acidification derived from eutrophication and increasing carbon dioxide from anthropogenic sources. Currently there are no published studies evaluating the effects of these changes on a keystone species, the Atlantic blue crab (*Callinectes sapidus*), and its embryonic development. In this study, embryos obtained from the Eastern Shore of Virginia were placed in varying acidities (pH 6.8, 7.4, & 8.1) and observed over 16 days for changes in developmental progression, final yolk size, hatching day, hatching success, and embryo volume. At high acidity (pH 6.8), embryonic development was delayed and hatching day was delayed, with no changes in hatching success, final yolk size, or embryo volume compared with control (pH 8.1). At medium acidity (pH 7.4), hatching day was impaired, with no impact on hatching success, development, final yolk size, or embryo volume. These outcomes illustrate the negative effect of extremely high acidity on embryonic development and hatching, although the observed delays were minimal and unlikely to impact the species on a population level. Combining multiple stressors such as temperature and salinity will be important factors to consider in subsequent studies seeking to understand their response to multiple changes.

Introduction

As acidification continues to intensify globally (Doney *et al.*, 2014; Wallace *et al.*, 2014), the Chesapeake Bay estuarine system is expected to experience extreme localized acidity due to eutrophication from nitrogen and phosphorus as well as chronic depletion of carbonate (Cai *et al.*, 2017). These outcomes will likely negatively affect a majority of ecosystems in the Chesapeake (Najjar *et al.*, 2010), although new studies have observed potential buffering capability related to specific habitat restoration (Su *et al.*, 2020). The effects of increased acidity is broadly understood in commercially valuable mollusks such as juvenile eastern oysters (Beniash *et al.*, 2010; Waldbusser *et al.*, 2011; Branch *et al.*, 2013), whereas there are few studies exploring the impact on early life stages of the commercially and ecologically valuable crustacean, *Callinectes sapidus*, with no studies determining how increased acidity will affect its embryogenesis.

Responses to acidification are widely variable within crustaceans (Whiteley, 2011), although sensitivity broadly increases with exposure in the earliest life stages (Przeslawski *et al.*, 2015). In calanoids, adults are largely resilient to increased acidity, but exposure during early development reduced hatching success in some species to a minuscule 4% (Mayor *et al.*, 2007). However, some species of amphipods are relatively unaffected by acidity and are more strongly influenced by low salinity (Egilsdottir *et al.*, 2009). These varied outcomes, among many others (Kroeker *et al.*, 2013), are expected for such a diverse group of organisms and only serve to emphasize the continued value of individual studies, in addition to expanding research to include multi-stressor impacts, as well as long-term and mesocosm studies.

Embryonic and larval development slows in response to acidification in some barnacles (Findlay *et al.*, 2009), sand dollars (Gonzalez-Bernat *et al.*, 2013) and krill (Kawaguchi *et al.*, 2011). Additionally, morphological abnormalities were observed in a slipper limpet (Noisette *et al.*, 2014) and sea urchin (Lamare *et al.*, 2016) embryos, along with significantly reduced fertilization in sand dollars (Gonzalez-Bernat *et al.*, 2013). These crustaceans do exhibit adverse effects, whereas decapods display more varied responses. In the Norway lobster, *Nephrops norvegicus*, a long-term study found increased temperature to have a greater negative effect on yolk consumption, heart rate, and oxygen consumption than low pH, with only oxidative stress slightly increasing at low pH (Styf *et al.*, 2013). However, in the porcelain crab, *Petrolisthes cinctipes*, embryos experience delayed development and increased mortality with increased acidity (Carter *et al.*, 2013; Ceballos-Osuna *et al.*, 2013). In red king crabs, *Paralithodes camtschaticus*, developmental rates actually increased with acidification, alongside increased yolk consumption and eye size, with such changes expected to significantly reduce future population sizes (Long *et al.*, 2013).

Responses to increased acidity may vary widely, due to highly variable embryonic development within decapod brachyurans (Garcia-Guerrero & Hendrickx, 2004). In a marine brachyuran, the Tanner crab, a long-term study found little response to acidification during the first year of exposure. However, in the second year, hatching success was significantly reduced, embryonic development was significantly altered, and the number of viable larvae to hatch were significantly lower (Swiney *et al.*, 2015). The Florida stone crab exhibited highly variable hatching success between broods in acidic

conditions, although overall success was reduced by 28% and developmental rates were delayed by 24% (Gravinese, 2018). Although many estuarine brachyuran species, such as *Callinectes sapidus*, are strong iono-regulators as adults, this may not be true for embryos in chronic hypercapnic conditions (Whiteley, 2011). For instance, Dungeness crabs exposed to acidic conditions experience delayed hatching, despite exposure only starting near the end of embryonic development (Miller *et al.*, 2016). As such, there is a strong need for additional studies focused on estuarine brachyuran responses during embryonic development.

The effects of acidification on embryogenesis are currently unknown in Atlantic blue crabs. Here I explore how medium (pH 7.4) and high (pH 6.8) acidic conditions impact embryonic volume, yolk consumption, hatching day, hatching success, and developmental progression compared to control conditions (pH 8.1). It is expected that high and medium treatments will result in delayed development, increased yolk consumption, reduced hatching day and success, and unchanged embryonic volume.

Methods

Experimental Design: Nine 500 mL separatory funnels were connected to an air pump at the base to provide embryonic suspension (Cassels & Krebs, 1983; Helluy & Beltz, 1991). A CO_2 line from the top of the funnel bubbled CO_2 to maintain three funnels at pH 8.1 (control), three funnels at pH 7.4 (medium), and three final funnels at pH 6.8 (high; Figure 2.1). Carbon dioxide input was controlled by a microcontroller (Arduino) and solenoid system which input pure CO_2 every 10 minutes. Water changes were conducted every other day and salinity and temperature were maintained at 25‰

and 20° C (Table 2.1). To minimize infections, seawater was treated with an antibiotic (malachite green, 1 ppm) and an antifungal (nystatin 5 mg mL^{-1}; Bas & Spivak, 2000). Salinity, temperature, and pH were monitored daily.

Ovigerous female *C. sapidus* were obtained from the Anheuser-Busch Coastal Research Center (ABCRC) and transported to Charlottesville, Virginia. Broods were divided according to developmental stage, with only early-stage embryos used in experiments. Subsets of each early-stage brood were removed and scanned under a dissecting microscope for visible signs of infection. Embryos from the six healthiest broods were selected and removed from females with tweezers (Bas & Spivak, 2000; Gravinese, 2018) with initial developmental stage recorded and approximately 100,000 eggs placed into separate, randomly assigned separatory funnels. Each brood was considered one replicate (N=6), with each brood providing embryos for control and treatment funnels. Embryos were maintained for 16 days until hatching. Every other day approximately 100 embryos were removed, with a subset of ten healthy embryos removed for developmental, volume, and yolk analysis (Giovagnoli *et al.*, 2014). Any dead or rotting eggs were also removed from funnels (Giovagnoli *et al.*, 2014). Each egg was photographed, its developmental stage recorded (Gravinese, 2018), and stored in ethanol (95%). Embryo developmental stage was classified based on yolk content, the presence or absence of the eyespot, and primordia differentiation (Garcia-Guerrero & Hendrickx; Gravinese, 2018; Table 2.2). Hatching day was determined as the presence or absence of stage 1 zoea per day for each funnel. Hatching success was determined based on successful observed hatching by day 16 for each funnel and recorded as presence or

absence of stage I zoea.

Embryo Measurements: Embryo volume was determined using the following equation:

$$V=(\pi\times l\times h^2/6) \quad \text{(Eq. 1)}$$

where l is the longest axis and h is the shortest axis (Giovagnoli *et al.*, 2014). Final embryonic volume changes were also calculated:

$$V=((V_{16}-V_1)/V_1)\times 100 \quad \text{(Eq. 2)}$$

where V_{16} is the final day average embryonic volume and V_1 is the initial day average embryonic volume (Giovagnoli *et al.*, 2014).

Total yolk consumption was calculated for each funnel using the following equation:

$$(Y_{16}/S_{16})*100 \quad \text{(Eq. 3)}$$

where Y_{16} is average yolk surface and S_{16} is average embryo surface, with both measurements taken at day 16 (Giovagnoli *et al.*, 2014).

Statistical Analysis:

All data were tested for normality (Shapiro-Wilk) and heteroscedasticity (Bartlett Test or Fligner-Killeen, depending on normality distribution). If data failed the test for normality (embryo volume, developmental rate), they were log-transformed. Embryo volume and developmental rate log-transformed data were evaluated based on Q-Q plots and skewness and determined to fit normality assumptions enough to use parametric analysis (Webster & Oliver, 2007). Data (embryo volume, diameter, yolk consumption, developmental rate) were analyzed with 2-way factorial ANOVA, with brood and pH as

the factors (Witte & Witte, 2007). They were also analyzed with linear or generalized mixed effects models, with pH as the fixed effect and brood and day as the random effects (Qian, 2010). Significant results from ANOVA underwent Tukey HSD post-hoc testing (Gardener, 2012). Binomial data (hatching day) were analyzed with a logistic regression (Horton & Kleinman, 2011), with hatching success data analyzed with a Fisher's Exact Test (Horton & Kleinman, 2011). Power analysis was performed using R package 'pwr' v. 1.3.0 (Champely *et al.,* 2020). Water quality parameters were calculated using R package 'seacarb' v. 2.4.3 (Gattuso *et al.,* 2018), with total pH scale, K_f (Perez & Fraga, 1987), k_1, k_2 (Lueker *et al.,* 2000) and K_s (Dickson, 1990). Mean pH and standard error were determined after converting from pH to hydrogen ion concentration, which were then converted back to pH scale. All data were analyzed in R v. 3.5.2 (R Development Core Team, 2011) with the packages 'car' (Fox *et al.,* 2019) 'ggplot2' (Wickham, 2009), 'nlme' (Pinheiro *et al.,* 2020), and 'lme4' (Bates *et al.,* 2015). All data were considered statistically significant with an alpha of 0.05.

Results

Water quality: Temperature, salinity, and total alkalinity remained constant for each of the tanks (Table 2.1). In each tank, pH also remained fairly constant.

Embryo volume: In a linear mixed effects model, embryo volume did not change in response to high (Table 2.3; $p > 0.300$) or medium acidity ($p > 0.468$). Final embryo volume did not significantly differ between treatments (Figure 2.2; Table 2.3; $p > 0.674$), with brood source playing a stronger role in impacting embryo volume ($p > 0.084$). The change in initial and final volume did not significantly differ between treatments ($p >$

0.693) or brood ($p > 0.075$).

Embryo development: In a general linear mixed effects model, embryo developmental progression was significantly reduced (Table 2.3; $p \leq 0.006$) over time at high acidity only, with no observed effect ($p > 0.414$) at medium acidity (Figure 2.3). Final development significantly differed between treatments ($p \leq 0.021$), with high acidity significantly different from control ($p \leq 0.023$) and medium acidity not quite significantly different from control (Figure 2.4; Table 2.3; $p > 0.053$). Final development did not significantly differ between broods ($p > 0.821$).

Yolk consumption: Final yolk quantity did not significantly differ between treatments (Figure 2.5; $p > 0.768$) nor between broods ($p > 0.132$).

Hatching: Embryo hatching day significantly differed from the control at both high (Table 2.3; $p \leq 0.001$) and medium acidity ($p \leq 0.002$), whereas hatching success did not significantly differ between treatments ($p > 0.275$). Power analysis showed larger sample size was needed for nominal data (Table 2.3).

Discussion

As expected, blue crab final embryo volume did not significantly vary between treatment conditions, although comparing distribution curves reveals greater volume spread in control conditions, with narrower volume spreads at medium and high acidity. These reflect observed developmental delays, because with delayed development, final volume will differ between treatments. Brood origin played a greater role explaining differences than did treatments (Table 2.3), which is expected due to maternal condition and genetic differences (Carter *et al.*, 2013). The stone crab, *Menippe mercenaria*, also

revealed no significant difference in embryo volume when exposed to reduced seawater pH (Gravinese, 2018). Volume correlates with increasing developmental stage as well as lower salinities (Davis, 1965; Giménez & Anger, 2001; Jivoff *et al.,* 2007), indicating my observed differences between final and initial volume is likely natural developmental progression (Figure 2.6).

Red king crab embryos were significantly larger in acidic conditions and development increased in acidic conditions rather than decreased, which was attributed to embryos using more energy storage for growth in acidic conditions (Long *et al.,* 2013). Conversely, I expected and found development decreased significantly at high acidity. My findings of slowed development are also seen in other calcifying organisms, such as the amphipod *Echinogammarus marinus* (Egilsdottir *et al.,* 2009), the barnacle *Semibalanus balanoides* (Findlay *et al.,* 2009), and the sand dollar *Arachnoides placenta* (Gonzalez-Bernat *et al*., 2013). Additionally, at least two other species of crabs undergo delayed development (Swiney *et al.,* 2015; Miller *et al.,* 2016), with a third experiencing reduced dry mass and metabolism that may result in delays (Carter *et al.,* 2013). As a whole, blue crab developmental delays are relatively small (Figure 2.3). Similar to the Dungeness crab, *Cancer magister,* they are not likely to cause disruptions at the population scale (Miller *et al.,* 2016).

Final yolk quantity in blue crab embryos did not significantly differ with pH or between broods, contrary to my initial hypothesis. This also contrasts with red king crabs, which experienced yolk shrinkage in acidic conditions (Long *et al.,* 2013). These results can fit, however, with observed developmental delays. With more time required to reach

the final developmental stage, yolk usage rates will also likely diminish, resulting in greater final yolk quantity. Therefore, my final day comparison of yolk quantity may not be different in unhatched embryos.

In blue crabs, hatching day was delayed by changing acidity, as was expected. These results are similar to other crab species, such as the Florida stone crab (Gravinese, 2018), the Dungeness crab (Miller *et al.,* 2016), the Tanner crab (Swiney *et al.,* 2015), and the red king crab (Long *et al.,* 2013). These trends are further supported in other calcifying organisms (Kurihara, 2008; Mayor *et al.,* 2007; Yu *et al.,* 2013), although at least one crab species, *Petrolisthes cinctipes,* did not exhibit similar outcomes (Carter *et al.,* 2013) and serves to highlight the variability expected between species (Ries *et al.,* 2009). My observed delayed hatching day is a natural outcome of my observed developmental delays. However, I did not observe expected significant differences in hatching success between treatments, despite differences between tanks. All control tanks successfully hatched, whereas 50% of high and 67% of medium acidity tanks successfully hatched. Medium acidity tanks likely had some reduced hatching success due to fungal infections, despite continuous antifungal treatments. Such infections are common to decapod embryonic work and can be difficult to eliminate (Bas & Spivak, 2000). My findings suggest a trend towards declining hatching success, but future studies should utilize a larger sample size to confirm this trend (Table 2.3).

Across marine species, larvae have proven to be more sensitive to increasing acidification compared with embryos, with arthropod embryos being particularly robust (Przeslawski *et al.,* 2015), although high variability is still observed (Ries *et al.,* 2009).

Blue crab embryos illustrate similar robustness to changing acidity, although developmental and subsequent hatching delays did occur. However, these delays alone are not large enough to expect large repercussions in the population, although these impacts do not consider other factors such as adult female mortality during delayed development. Successful broods are distributed temporally across the summer months (Dittel & Epifanio, 1982), implying observed delays alone are unlikely to significantly impact recruitment dynamics into the Chesapeake Bay. Larvae are recruited well into late summer and early fall (McConaugha *et al.,* 1983), therefore delayed development and hatching alone are unlikely to prevent hatched larvae from entering the system at a slightly later time.

Blue crab embryos are already exposed to widely fluctuating environmental conditions because they develop in an estuarine environment. In other estuarine species, this may improve resilience to additional stressors such as increasing acidity from eutrophication and climate change (Whiteley, 2011; Cai *et al.,* 2017; Conradi *et al.,* 2019). However, other studies have exhibited increasing temperatures worsen embryonic outcomes (Brante *et al.,* 2003; Styf *et al.,* 2013). Future studies should include the effect of increased temperature and acidity in blue crab embryos to ascertain any interaction. Additionally, changing climate will bring about many more environmental disruptions to blue crab habitat, with increased rainfall and sea-level rise expected to greatly increase salinity variability in the Chesapeake Bay (Najjar *et al.,* 2010; Hong & Shen, 2012). Changes in salinity and temperature are already known to affect juvenile blue crabs (Cadman & Weinstein, 1988), thus it will be important to understand their impacts in

embryonic stages as well. Although my evaluation of acidification alone is a good introduction, future studies will benefit from combining multiple stressors such as temperature and salinity with acidification on blue crab embryonic development.

Figures and Tables

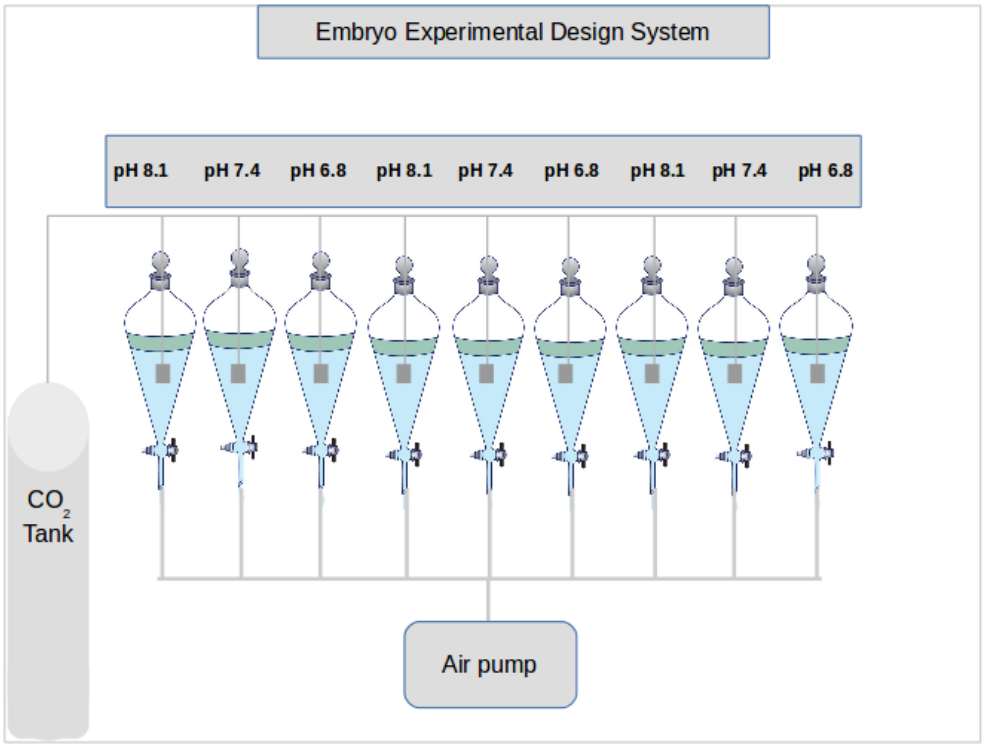

Figure 2.1. Experimental design. Embryos were placed in each separatory funnel and maintained in suspension through constant air bubbles from the base of the funnel. Carbon dioxide was added through a microcontroller and solenoid system to each funnel from the top every ten minutes. Water was changed in all funnels every other day and treated with an antibiotic (malachite green, 1 ppm) and antifungal (nystatin, 5 mg mL^{-1}).

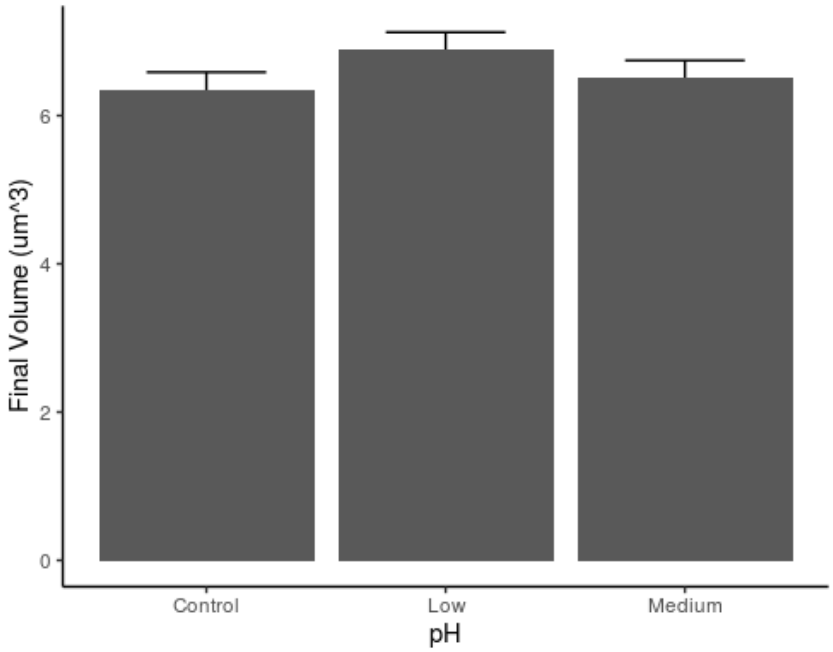

Figure 2.2. Effect of pH on final embryo volume in *C. sapidus*. Final volume (μm^3) varied little with increased acidity. Data are mean values ± standard error.

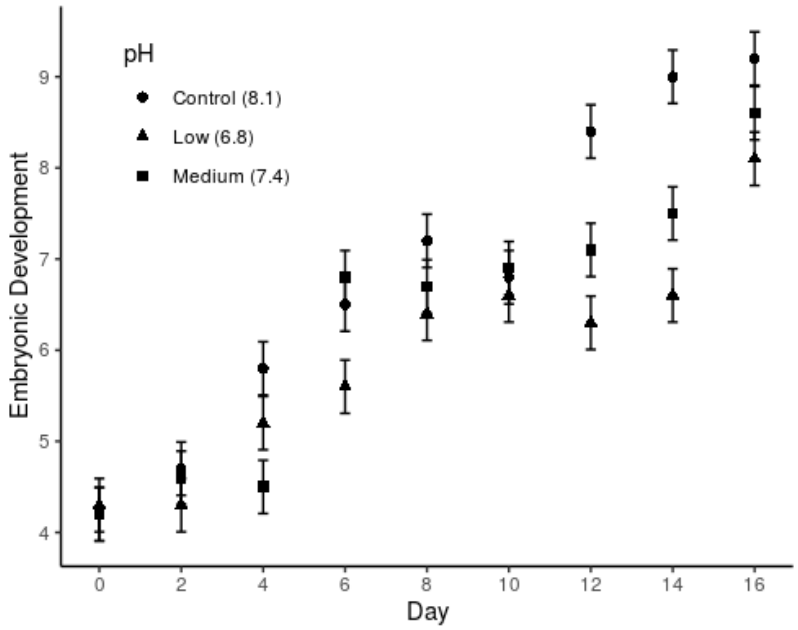

Figure 2.3. Effect of pH on embryo developmental stage in *C. sapidus*. Over a period of 16 days, embryonic development diverged with increasing acidity. Data are mean values ± standard error.

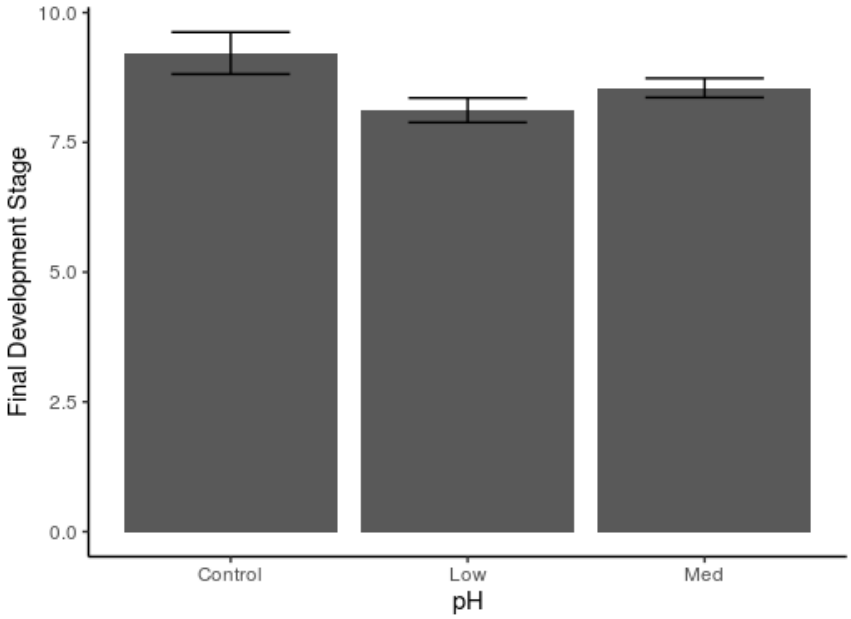

Figure 2.4. Effect of pH on final developmental stage in *C. sapidus*. Mean developmental stage was reduced with increased acidity. Data are mean values ± standard error.

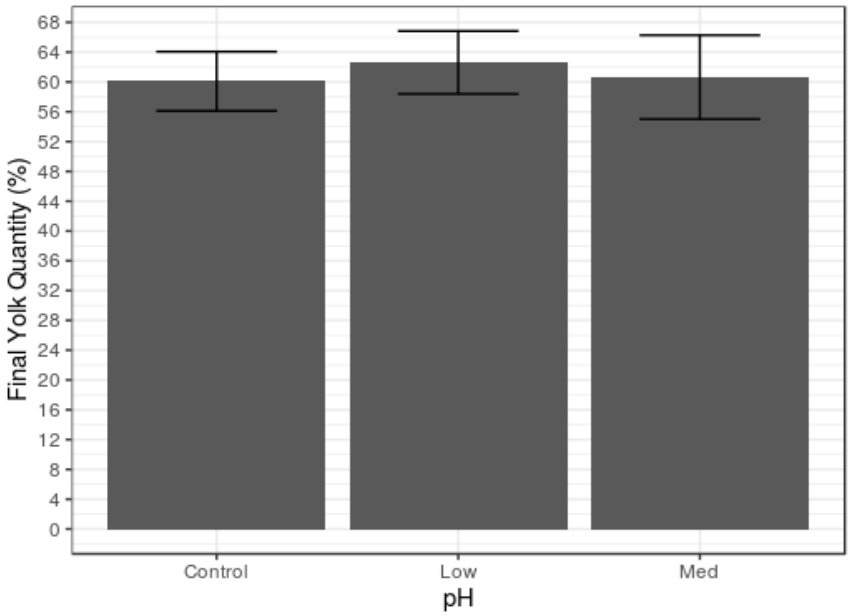

Figure 2.5. Effect of pH on final yolk quantity in *C. sapidus*. There was little difference in final yolk quantity (%) with increased acidity. Data are mean values ± standard error.

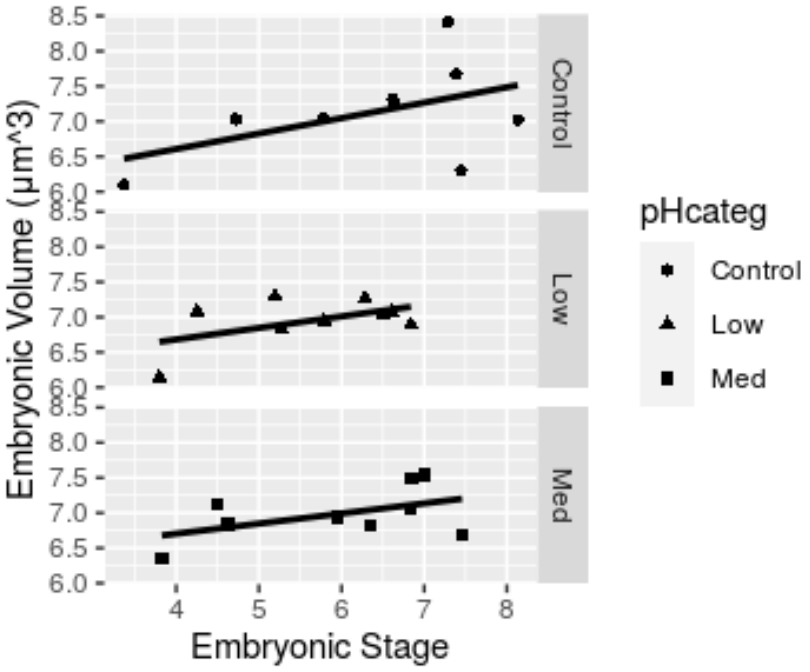

Figure 2.6. Relationship between embryonic volume and developmental stage. Embryonic volume (μm^3) increased with increasing developmental stages across all treatments.

Table 2.1. Water chemistry. Temperature, salinity, and pH were measured twice daily in experimental tanks. Total Alkalinity (A_T) was measured at the start of the experiment. Bicarbonate, carbonate, and dissolved inorganic carbon (DIC) were calculated at the end of the experiment. Data are mean values ± standard error.

Parameter	**Low**	**Medium**	**Control**
Temperature	20.66 ± 0.10	20.61± 0.04	20.69 ± 0.05
Salinity	25 ± 0.14	25 ± 0.12	26 ± 0.12
A_T (µmol kg^{-1})	1278 ± 1.48	1295 ± 0.97	1290 ± 1.22
pH	6.81 ± 0.01	7.36 ± 0.01	8.13 ± 0.01
$[HCO_3^-]$ (µmol kg^{-1})	1265.84 ± 1.59	1249.35 ± 2.19	1074.43 ± 2.24
$[CO_3^{2-}]$ (µmol kg^{-1})	6.32 ± 0.18	22.74 ± 0.75	107.72 ± 1.46
DIC	1440.81 ± 5.73	1319.01 ± 2.81	1188.93 ± 1.15

Table 2.2. Classification scheme used for embryonic development in *C. sapidus*. Stages were based on egg mass coloration, presence and absence of an eyespot, and yolk content. Descriptions were adapted from Garcia-Guerrero & Hendrickx (2004) and Gravinese (2018).

Stage	Description
1	90-100% yolk
2	Yolk composes 80–90% of egg, yolk divided into droplets
3	primordia of antenna-antenulle, maxilla-maxillule, and maxillipeds differentiated (3)
4	Primordia differentiation larger, abdomen in segmentation process, abdominal chromatophores appearing
5	<50% yolk, primordia differentiation more defined
6	Eyespot pigmented, ~10-20% yolk. abdominal segments defined along periphery of egg
7	eye differentiated; abdominal traces; heart formed
8	cephalic appendages larger; heartbeat; abdomen obvious
9	embryo ready to hatch, no traces of yolk, fully formed

Table 2.3. Results of linear models, ANOVA, and Tukey HSD. Significant results are in bold.

Embryo Volume	**AIC**	**BIC**			***P***
Mixed Effects Model	309.199	346.070			
Low pH					0.300
Medium pH					0.468
Final Volume: ANOVA	**d.f.**	**SS**	**MS**	***F***	***P***
pH	2	0.708	0.354	0.409	0.674
Brood	4	5.782	1.446	2.92	0.084
Volume Change: ANOVA	**d.f.**	**SS**	**MS**	***F***	***P***
pH	2	177.3	88.64	0.379	0.693
Brood	4	1587.0	396.8	3.062	0.075
Embryo Development	**AIC**	**BIC**			***P***
Mixed Effects Model	**405.8**	**469.1**			
Low pH					**0.006**
Medium pH					**0.048**
Final Development: ANOVA	**d.f.**	**SS**	**MS**	***F***	***P***
pH	**2**	**13.716**	**6.858**	**5.560**	**0.021**
Brood	4	3.899	0.975	0.375	0.821
Final Development: Tukey HSD	**Diff**	**Lower**	**Upper**		***P***
Low-Control	**-2.345**	**-4.357**	**-0.333**		**0.023**
Medium-Control	-1.985	-3.997	0.027		0.053
Medium-Low	0.360	-1.537	2.257		0.867
Final Yolk Quantity					
Final Yolk Quantity: ANOVA	**d.f.**	**SS**	**MS**	***F***	***P***
pH	2	125.370	62.687	0.271	0.768
Brood	4	1366.5	341.620	2.351	0.132
Hatching	**AIC**	**BIC**			***P***
Logistic Mixed Effects Model	**73.795**	**107.308**			
Low pH					**≤ 0.001**
Medium pH					**0.002**
Day					**≤ 0.001**
Fisher Exact Test					***P***
Hatching Success: pH					0.275
Power Analysis: Fisher	**d.f.**	**Effect Size**	**Alpha**	**Power**	**N**
	2	0.464	0.05	0.8	44

Literature Cited

Bas, C. C. & E. D. Spivak. 2000. Effect of salinity on embryos of two southwestern Atlantic estuarine grapsid crab species cultured *in vitro*. *Journal of Crustacean Biology*, 20:647-656. doi: 10.1651/0278-0372

Bates, D., M. Mächler, B. Bolker, & S. Walker. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67:1–48. doi:10.18637/jss.v067.i01

Beniash, E., A. Ivanina, N. Lieb, I. Kurochkin, & I. Sokolova. 2010. Elevated level of carbon dioxide affects metabolism and shell formation in oysters *Crassostrea virginica* (Gmelin). *Marine Ecology Progress Series*, 419:95-108. doi: 10.3354/meps08841

Branch, T. A., B. M. DeJoseph, L. J. Ray, & C. A. Wagner. 2013. Impacts of ocean acidification on marine seafood. *Trends in Ecology and Evolution*, 28:178-186. doi: 10.1016/j.tree.2012.10.001

Brante, A., M. Fernández, L. Eckerle, F. C. Mark, H. O. Pörtner, & W. Arntz. 2003. Reproductive investment in the crab *Cancer setosus* along a latitudinal cline: egg production, embryo losses and embryo ventilation. *Marine Ecology Progress Series*, 251:21–232.

Cadman, L. R., & M. P. Weinstein. 1988. Effects of temperature and salinity on the growth of laboratory-reared juvenile blue crabs *Callinectes sapidus* Rathbun. *Journal of Experimental Marine Biology and Ecology*, 121:193-207.

Cai, W.-J., W.-J. Huang, G. W. Luther III, D. Pierrot, M. Li, J. Testa, M. Xue, A. Joesoef, R. Mann, J. Brodeur, Y.-Y. Xu, B. Chen, N. Hussain, G. G. Waldbusser, J. Cornwall, & W. M. Kamp. 2017. Redox reactions and weak buffering capacity lead to acidification in the Chesapeake Bay. *Nature Communications*, 8:369. doi: 10.1038/s41467-017-00417-7

Cassels, F. & C. T. Krebs. 1983. A Comparison of Artificial Incubation Methods Using Ova of the Red Crab *Geryon quinquedens* Smith (Decapoda, Brachyura). *Journal of Crustacean Biology*, 3:565-574.

Carter, H. A., L. Ceballos-Osuna, N. A. Miller, & J. H. Stillman. 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. doi: 10.1242/jeb.078162

Ceballos-Osuna, L., H. A. Carter, N. A. Miller, & J. H. Stillman. 2013. Effects of ocean acidification on early life-history stages of the intertidal porcelain crab *Petrolisthes cinctipes*. *Journal of Experimental Biology*, 216:1405-1411. doi: 10.1242/jeb.078154

Champely, S., C. Ekstrom, P. Dalgaard, J. Gill, S. Weibelzahl, A. Anandkumar, C. Ford, R. Volcic, H. De Rosario. 2020. Basic Functions for Power Analysis. R package version 1.3.0. Available at: https://cran.r-project.org/web/packages/pwr/index.html

Conradi, M., J. E. Sanchez-Moyano, M. K. A. Bhuiyan, A. Rodriguez-Romero, A. Galotti, M. D. Basallote, A. DelValls, G. Parra, & I. Riba. 2019. Intraspecific variation in the response of the estuarine isopod *Cyathura carinata* (Krøyer, 1847) to ocean acidification. *Science of the Total Environment*, 134-145.

Davis, C. C. 1965. A study of the hatching process in aquatic invertebrates: XX. The blue crab, *Callinectes sapidus*, Rathbun, XXI. The nemertean, *Carcinonemertes carcinophila* (Kolliker). *Chesapeake Science*, 6:201-208.

Dickson, A. G. 1990. Standard potential of the reaction: AgCI(s) + 1/2H2(g) = Ag(s) + HCI(aq), and the standard acidity constant of the ion HSO_4 in synthetic sea water from 273.15 to 318.15 K. *Journal of*

Chemical Thermodynamics, 22:113-127.

Dittel, R., A. I. & C. E. Epifanio. 1982. Seasonal abundance and vertical distribution of crab larvae in Delaware Bay. *Estuaries*, 5:197-202.

Doney, S. C., L. Bopp, & M. C. Long. 2014. Historical and future trends in ocean climate and biogeochemistry. *Oceanography*, 27:108-119. doi: 10.5670/oceanog.2014.14

Egilsdottir, H., J. I. Spicer, & S. D. Rundle. 2009. The effect of CO_2 acidified seawater and reduced salinity on aspects of the embryonic development of the amphipod *Echinogammarus marinus* (Leach). *Marine Pollution Bulletin*, 58:1187–1191. doi: 10.1016/j.marpolbul.2009.03.017

Findlay, H. S., M. A. Kendall, J. I. Spicer, & S. Widdicombe. 2009. Future high CO_2 in the intertidal zone compromises development and hatching success of barnacle (*Semibalanus balanoides*) embryos, *Marine Ecology Progress Series*, 389:193-202. doi: 10.3354/meps08141

Fox, J., S. Weisburg, & B. Price. 2019. car: Companion to Applied Regression. R package version 3.0-5. Available at: https://cran.r-project.org/web/packages/car/index.html

Garcia-Guerrero, M. & M. E. Hendrickx. 2004. Embryology of decapod crustaceans I. Embryonic development of the mangrove crabs *Goniopsis pulchra* and *Aratus pisonii* (Decapoda: Brachyura). *Journal of Crustacean Biology*, 24:666–772. doi: 10.1651/C-2497

Gardener, M. 2012. *Statistics for Ecologists Using R and Excel*. Pelagic Publishing.

Gattuso, J.-P., J.-M. Epitalon, H. Lavigne, & J. Orr. 2018. seacarb: Seawater Carbonate Chemistry. R package version 3.2.12. Available at: https://cran.r-project.org/web/packages/seacarb/index.html

Giménez, L. & K. Anger. 2001. Relationships among salinity, egg size, embryonic development, and larval biomass in the estuarine crab *Chasmagnathus granulata* Dana, 1851. *Journal of Experimental Marine Biology & Ecology*, 260:241–257.

Giovagnoli, A., R. B. Ituarte, & E. D. Spivak. 2014. Effects of removal from the mother and salinity on embryonic development of *Palaemonetes argentinus* (Decapodae: Caridea: Palaemonidae). *Journal of Crustacean Biology*, 34:174-181. doi: 10.1163/1937240X-00002225

Gonzalez-Bernat, M. J., M. Lamare, S. Uthicke, & M. Byrne. 2013. Fertilisation, embryogenesis and larval development in the tropical intertidal sand dollar *Arachnoides placenta* in response to reduced seawater pH. *Marine Biology*, 160:1927–1941, doi: 10.1007/s00227-012-2034-2

Gravinese, P. M. 2018. Ocean acidification impacts the embryonic development and hatching success of the Florida stone crab, *Menippe mercenaria*. *Journal of Experimental Marine Biology and Ecology*, 500:140-146. doi: 10.1016/j.jembe.2017.09.001

Helluy, S. M. & B. S. Beltz. 1991. Embryonic development of the American lobster (*Homarus americanus*): quantitative staging and characterization of an embryonic molt cycle. *Biological Bulletin*, 80:355–371.

Hong, B. & J. Shen. 2012. Responses of estuarine salinity and transport processes to potential future sea-level rise in the Chesapeake Bay. *Estuarine, Coastal and Shelf Science*, 104-105:33-45. doi: 10.1016/j.ecss.2012.03.014

Horton, N. J., & K. Kleinman. 2011. *Using R for Data Management, Statistical Analysis, and Graphics.*

CRC Press, Taylor & Francis Group.

Jivoff, P., A. N. Hines & L. S. Quackenbush. 2007. Reproduction biology and embryonic development. In: Kennedy, V. S. & L. E. Cronin, editors. *The blue crab Callinectes sapidus*. College Park, MD: Maryland Sea Grant. pp. 255–298.

Kawaguchi, S., H. Kurihara, R. King, L. Hale, T. Berli, J. P. Robinson, A. Ishida, M. Wakita, P. Virtue, S. Nicol, & A. Ishimatsu. 2011. Will krill fare well under Southern Ocean acidification? *Biology Letters*, 7:288-291. doi: 10.1098/rsbl.2010.0777

Kroeker, K. J., R. L. Kordas, R. Crim, I. E. Hendriks, L. Ramajo, G. S. Singh, C. M. Duarte, & J.-P. Gattuso. 2013. Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. *Global Change Biology*, 19:1884-1896. doi: 10.1111/gcb.12179

Kurihara, H. 2008. Effects of CO_2-driven ocean acidification on the early developmental stages of invertebrates. *Marine Ecology Progress Series*, 373:275-284. doi:10.3354/meps07802

Lamare, M. D., M. Liddy, & S. Uthicke. 2016. *In situ* developmental responses of tropical sea urchin larvae to ocean acidification conditions at naturally elevated pCO_2 vent sites. *Proceedings of the Royal Society B*, 283:20161506. doi: 10.1098/rspb.2016.1506

Long, W. C., K. M. Swiney, & R. J. Foy. 2013. Effects of ocean acidification on the embryos and larvae of red king crab, *Paralithodes camtschaticus. Marine Pollution Bulletin*, 69:38-47. doi: 10.1016/j.marpolbul.2013.01.011

Lueker, T. J., A. G. Dickson & C. D. Keeling. 2000. Ocean pCO_2 calculated from dissolved inorganic carbon, alkalinity, and equations for K_1 and K_2: validation based on laboratory measurements of CO_2 in gas and seawater at equilibrium. *Marine Chemistry*, 70:105-119.

Mayor, D. J., C. Matthews, K. Cook, A. F. Zuur, & S. Hay. 2007. CO_2-induced acidification affects hatching success in *Calanus finmarchicus*. *Marine Ecology Progress Series*, 350:91–97. doi: 10.3354/meps07142

McConaugha, J. R., D. F. Johnson, A. J. Provenzano, & R. C. Maris. 1983. Seasonal distribution of larvae of *Callinectes sapidus* (crustacea: Decapoda) in the waters adjacent to Chesapeake Bay. *Journal of Crustacean Biology*, 3:582-591.

Miller, J. J., M. Maher, E. Bohaboy, C. S. Friedman, & P. McElhany. 2016. Exposure to low pH reduces survival and delays development in early life stages of Dungeness crab (*Cancer magister*). *Marine Biology*, 163:1–11. doi: 10.1007/s00227-016-2883-1

Najjar, R. G., C. R. Pyke, M. B. Adams, D. Breitburg, C. Hershner, M. Kemp, R. Howarth, M. R. Mulholland, M. Paolisso, D. Secor, K. Sellner, D. Wardrop, & R. Wood. 2010. Potential climate-change impacts on the Chesapeake Bay. *Estuarine, Coastal and Shelf Science*, 86:1-20. doi: 10.1016/j.ecss.2009.09.026

Noisette, F., T. Comtet, E. Legrand, F. Bordeyne, D. Davoult, & S. Martin. 2014. Does encapsulation protect embryos from the effects of ocean acidification? The example of *Crepidula fornicata*. *PLoS ONE*, 9:e93021. doi: 10.1371/journal.pone.0093021

Perez, F. F. & F. Fraga. 1987. Association constant of fluoride and hydrogen ions in seawater. *Marine Chemistry* 21:161-168.

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, & R Core Team. 2020. *nlme: Linear and Nonlinear Mixed Effects Models*. R package version 3.1-148. Available at: https://CRAN.R-project.org/package=nlme.

Przeslawski, R., M. Byrne, & C. Mellin. 2015. A review and meta-analysis of the effects of multiple abiotic stressors on marine embryos and larvae. *Global Change Biology*, 21:2122–2140. doi: 10.1111/gcb.12833

Qian, S.S. 2010. *Environmental and Ecological Statistics with R.* CRC Press, Taylor and Francis Group.

R Development Core Team. 2011. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: http://www.R-project.org

Ries, J. B., A. L. Cohen, & D. C. McCorkle. 2009. Marine calcifiers exhibit mixed responses to CO2-induced ocean acidification. *Geology*, 37:1131-1134. doi: 10.1130/G30210A.1

Styf, H. K., H. N. Skold, & S. P. Eriksson. 2013. Embryonic response to long-term exposure of the marine crustacean *Nephrops norvegicus* to ocean acidification and elevated temperature. *Ecology and Evolution*, 3:5055-5065. doi: 10.1002/ece3.860

Su, J., W.-J. Cai, J. Brodeur, B. Chen, N. Hussain, Y. Yao, C. Ni, J. M. Testa, M. Li, X. Xie, W. Ni, M. Scaboo, Y. Xu, J. Cornwell, C. Gurbisz, M. S. Owens, G. G. Waldbusser, M. Dai, & W. M. Kemp. 2020. Chesapeake Bay acidification buffered by spatially decoupled carbonate mineral cycling. *Nature Geoscience*, 13:441-447. doi: 10.1038/s41561-020-0584-3

Swiney, K. M., W. C. Long, & R. J. Foy. 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*, 73:825–835. doi: 10.1093/icesjms/fsv201

Waldbusser, G. G., E. P. Voigt, H. Bergschneider, M. A. Green, & R. I. E. Newell. 2011. Biocalcification in the Eastern oyster (Crassostrea virginica) in relation to long-term trends in Chesapeake Bay pH. *Estuaries and Coasts*, 34:221-231. doi: 10.1007/s12237-010-9307-0

Wallace, R. B., H. Baumann, J. S. Grear, R. C. Aller, & C. J. Gobler. 2014. Coastal ocean acidification: the other eutrophication problem. *Estuarine, Coastal and Shelf Science*, 148:1-13. doi: 10.1016/j.ecss.2014.05.027

Webster, R. & M. A. Oliver. 2007. *Geostatistics for environmental scientists*. John Wiley & Sons.

Witte, R. S. & J. S. Witte. 2010. *Statistics*. 9th ed. John Wiley & Sons.

Whiteley, N. M. 2011. Physiological and ecological responses of crustaceans to ocean acidification. *Marine Ecology Progress Series*, 430:257-271. doi: 10.3354/meps09185

Wickham, H. 2009. ggplot2: Elegant Graphics For Data Analysis. New York, NY:Springer.

Yu, P. C., M. A. Sewell, P. G. Matson, E. R. Rivest, L. Kapsenberg, & G. E. Hofmann. 2013. Growth attenuation with developmental schedule progression in embryos and early larvae of *Sterechinus neumayeri* raised under elevated CO_2. *PLOS One*, e52448. doi: 10.1371/journal.pone.0052448

Chapter Three:

Evaluating the effect of increased acidity on stage I zoea of the Atlantic blue crab, *Callinectes sapidus* Rathbun

Abstract

Declining ocean pH reduces survival in a wide variety of adult calcifying organisms, but less is known about the impact on their early life stages, particularly for decapod crustaceans. One such species, the Atlantic blue crab (*Callinectes sapidus*), is a commercially valuable fishery in the Chesapeake Bay that has not previously been evaluated. In this study, stage one zoea larvae were exposed to two treatment conditions (pH 7.4 and 6.8) as well as control conditions (pH 8.1) for seven days. Mortality rates significantly increased in both treatments compared with control. No major changes were observed in morphology, although total length and carapace length were nearly significantly reduced. Total protein and total lipid analysis revealed diminishing lipid reserves with increasing acidity, without similar protein changes. These responses reveal negative effects of increasing acidity on larval blue crabs and highlight the necessity of evaluating each stage of development, especially in species initially thought to be unaffected by ocean acidification as adults.

Introduction

The Chesapeake Bay experiences more rapid acidification from a combination of anthropogenic and biological sources of carbon dioxide (Cai *et al.,* 2017). Eutrophication and excess CO_2 from fossil fuel emissions are the primary contributors (Cai *et al.,* 2017; Le Quere *et al.,* 2009), with its large population of calcifying organisms regularly removing carbonate and further weakening its buffering capacity (Cai *et al.,* 2017). Juvenile eastern oysters (*Crassostrea virginica*) are already experiencing negative effects (Waldbusser *et al.,* 2011) and acidification is expected to only worsen with time (Feely *et al.,* 2004).

As a keystone species of the Chesapeake Bay (Epifanio, 2019), the blue crab (*Callinectes sapidus* Rathbun, 1896) holds a vital ecological and commercial position in the Chesapeake community, comprising the greatest volume of crab landings in the US (Miller *et al.,* 2005). Careful fishery management and habitat restoration over the past decade has created an overall stable population for commercial harvesting compared with earlier decades (Scheld *et al.,* 2016; Seitz, 2020). Nevertheless, increasing carbon dioxide is likely to affect future populations of blue crabs, with negative impacts observed across a range of adult and early life stages (Riebesell *et al.,* 2000; Orr *et al.,* 2005; Andersson *et al.,* 2005, 2007; Fabry *et al.,* 2008; Kroeker *et al.,* 2013).

Of these early life stages in crustaceans, species at higher latitudes in cold water are the most sensitive to increased acidification (Whiteley, 2011). Survival is greatly diminished in larval stages of red king crabs (Long *et al.,* 2013a), blue king crabs (Long *et al.,* 2017), spider crabs (Schiffer *et al.,* 2013), porcelain crabs (Ceballos-Osuna *et al.,*

2013), and Dungeness crabs (Miller *et al.,* 2016). Such strong declines in survival are likely due to increased energy expenditure on acid-base regulation, changes in molting frequency, or metabolized substrates (Carter *et al.*, 2013; Long *et al.*, 2017, Giltz & Taylor, 2017; Tomasetti *et al.*, 2018). Morphological responses are more varied, with some species unaffected (Miller *et al.,* 2016; Long *et al.*, 2017; Gravinese, 2018), one species slightly declining in total length (Giltz & Taylor, 2017), and another species slightly increasing in total length (Long *et al.*, 2013a). These findings are expected to be found across species (Whiteley, 2011) and will exert significant influence on distribution, abundance, ecosystem function, and food resources (Macko *et al.,* 2017; Macko & Fantasia, 2018).

As a representative of a midlatitude estuarine crustacean, the blue crab is found in a habitat different from most other studies, which have primarily focused on coastal cold water species, as described above. When larval blue crabs in a Gulf of Mexico population were exposed to increased acidity, survival and size were reduced by 23% and 10%, respectively (Giltz & Taylor, 2017). In New York, blue crab larval mortality rose to 49% when combined with low dissolved oxygen (Tomasetti *et al.,* 2018). My study complements these findings by determining whether larval blue crabs from the Chesapeake Bay respond differently. Within broods and populations, responses to acidification can be greatly varied. In the porcelain crab (*Petrolisthes cinctipes*), certain broods evidenced greater metabolic stress (Carter *et al.,* 2013). In two separate spider crab (*Hyas araneus*) populations, one revealed greater sensitivity to acidity through reduced growth and fewer successful molts (Walther *et al.*, 2010). There are no similar

studies determining such responses for larval blue crabs in the Chesapeake Bay, despite its economic importance to the region (National Marine Fisheries Service, 2016). Additionally, these earlier studies only evaluated survival and morphology in larval blue crabs. I expand on these and determine potential sublethal outcomes that have been observed in other larval crustaceans (Arnold *et al.*, 2009) by evaluating lipid and protein content.

In my study, I expect larval blue crabs will increase in mortality, reduce lipids and protein, and experience no morphological changes after exposure to acidification for seven days. These hypothesized outcomes are likely to produce a bottleneck effect and reduce larval survival to adulthood, either through direct mortality or through reduced lipid and protein storage and subsequent successful molting.

Methods

Five blue crab females from the Institute of Marine and Environmental Technology (Baltimore, Maryland, USA) were the source for approximately 12,500 larvae, with larvae obtained one day after hatching and transported to the University of Virginia (Charlottesville, Virginia, USA). A total of five trips were made, with each round of experimentation containing larvae from only one female.

Experimental Design: The experimental system (Figure 3.1) was composed of nine different tanks each containing 11 L of artificial sea water (Instant Ocean, Blacksburg, Virginia, USA), with all tanks connected to a reservoir where water quality was cleaned and maintained by particulate and biological filters, UV light sterilization, and aeration to restore water to pH 8.1. Water was re-circulated four times daily for 45

minutes through all nine tanks. Three tanks were maintained at pH 8.1 (control pH), three tanks at treatment pH 7.4 (medium pH), and three final tanks at treatment pH 6.8 (low pH; Table 3.1). Each tank contained a pump at the bottom of the tank to create moderate flow and were separated from larvae by a mesh insert (100 μm). Each tank also contained its own air stone to provide aeration above the mesh and a line for CO_2 distribution below the mesh. A microcontroller (Arduino Uno, Somerset, Massachusetts, USA) regulated the length of time each solenoid was open through a relay board to control pH. When open, individual solenoids released CO_2 into each tank. Solenoids released CO_2 every half hour, with each tank receiving individualized lengths of open solenoid time to maintain the required pH, with monitoring and updates performed twice daily. Salinity was monitored and maintained at 30‰ through freshwater inputs with temperature maintained at 23° C (Costlow & Bookhout, 1959; Sulkin & Epifanio, 1975).

Five batches of larvae were collected and evenly distributed between the nine tanks. The zoea were daily fed a mixture of *Rotifera plicatilus* S-type (Reed Mariculture, Campbell, California, USA) and *Nannochloropsis* algae (Instant Algae, Reed Mariculture, Campbell, California, USA) at a density of 50 individuals per milliliter (Sulkin, 1978; Zmora *et al.,* 2005). Over an experimental period of seven days, larvae were daily removed from the base and separated into live and dead larvae, with the number of deceased larvae counted, placed in 95% ethanol, and preserved for later analysis (Prendini *et al.,* 2002). Live larvae counted and returned to their original tank. On the final day, all live larvae were also collected and placed in vials. Mortality was evaluated for each tank daily and total mortality was calculated at the end of the

experiments.

Total Protein: Live larvae were collected on days 0, 4, and 7 from all nine tanks. They were rinsed in Milli-Q water (MilliporeSigma, Massachusetts, USA) and placed in -80° C freezer. For analysis, vials were thawed on ice and homogenized with 500 µL of phosphate buffer (0.1 M phosphate, 0.15 M NaCl, pH 7.2) and protease inhibitors (1 M PMSF (phenylmethylsulfonyl fluoride), 10 mg mL^{-1} leupeptin, 1 mg mL^{-1} aprotinin). A Micro BCA Protein Assay Kit (ThermoScientific, Waltham, Massachusetts, USA) was used with the homogenate, which was then incubated at 37° C for 2 hours and read in a plate reader (SpectraMax M3, San Jose, California, USA) at 562 nm.

Total Lipid: Following the modified spectrophotometric sulfophosphovanillin method (Carter *et al.*, 2013), live larvae were collected on days 0, 4, and 7 from all nine tanks. Larvae were rinsed in Milli-Q water and placed in -80° C freezer. For analysis, vials were thawed on ice and samples homogenized with 500 µL methanol:chloroform (1:1) and placed on a dry block heater (95° C) until the solvent was completely evaporated. Concentrated sulfuric acid (300 µL) was then added to each sample and mixed thoroughly. Samples (200 µL) were mixed with 1 mL of colored reagent (85% phosphoric acid and vanillin). A serial dilution (0-200 µg) using triglyceride and 95% sulfuric acid was used for quantification. The samples and standards were read at 540 nm in a plate reader (SpectraMax M3, San Jose, California, USA).

Morphology: Total length, carapace length, rostrum spine length, and dorsum spine length were determined with ToupView software (ToupTek Photonics, Zhejiang, P.R. China). Data were obtained from larvae in all vials, which were randomized to

prevent knowledge from which tank they were derived.

Statistical Methods: Mortality, morphology, total protein, and lipid results were checked for normality (Shapiro-Wilk test) and heteroscedasticity (Levene's Test). When the data did not pass these assumptions for an ANOVA (total protein, rostral length, total length), they were log-transformed (total protein, rostrum length, total length). After transformation, total protein and rostrum length passed these assumptions. Total length was then Box-Cox transformed and found to pass these assumptions. For mortality, a Kaplan-Meier survival curve and a Cox proportional hazards regression was performed to determine the effect of pH and brood (Horton & Kleinman 2011). For total protein, lipid, and morphology, a two-way factorial ANOVA was performed to test for the effect of factors pH and treatment length (Qian, 2010). When the interaction between factors was significant, a Tukey HSD post-hoc test was performed to detect the differences between treatments (Qian, 2010). Total lipid and total protein were also evaluated with a linear mixed effects model, with pH as a fixed effect and treatment length as a random effect (Horton & Kleinman, 2011). Survival curves were created using the Kaplan-Meier log-rank test in R 'survival' package (Thernau 2011). Water quality parameters were calculated using R package 'seacarb' v. 2.4.3 (Lavigne & Gattuso, 2018) with total pH scale, K_f (Perez & Fraga, 1987), k_1, k_2 (Lueker *et al.*, 2000) and K_s (Dickson, 1990). Mean pH and standard error were determined after converting from pH to hydrogen ion concentration, which were then converted back to the pH scale. All data were analyzed in R v. 3.5.2 (R Development Core Team, 2011) with the packages 'car' (Fox *et al.* 2019), 'nlme' (Pinheiro *et al.*, 2020) and 'ggplot2' (Wickham 2020). All data were considered

statistically significant with an alpha of 0.05.

Results

Water Quality: Each tank was maintained at low (pH 6.8), medium (pH 7.4), or control (pH 8.1) conditions, with salinity and temperature also kept constant (Table 3.1). Each tank's acidity was variable throughout the day, with maximum variation observed in tanks at medium pH of 7.4.

Mortality: Survival significantly decreased over a period of 7 days when larvae were exposed to low and medium pH (Figure 3.2). When brood effects are included, mortality significantly differs between broods as well (Figure 3.3). On day 7, average total mortality was greatest at low pH (71%) and least at control pH (15.8%; Figure 3.4).

Total Protein: There was little difference in final total protein between control and treatment pH conditions (Figure 3.5). Between day 0 and day 7, total protein was highest at control pH on day 4 and subsided to the original concentration by day 7. At medium pH total protein varied little over 7 days, and at low pH total protein increased (Figure 3.6), with these trends significantly different from control pH (Table 3.2). There was no significant difference in final total protein between control pH and low pH, nor between control pH and medium pH (Table 3.2).

Total Lipid: There were significant differences in total lipid between control pH and low pH and between medium pH and low pH, with no significant difference between control pH and medium pH (Figure 3.7; Table 3.3). On day 0, total lipid was greatest at control pH, with slightly reduced total lipid at medium pH and greatly reduced total lipid at low pH. After 7 days, total lipid increased at control pH, remained nearly constant at

medium pH, and greatly decreased at low pH (Figure 3.8).

Morphology: When total length of larvae was contrasted between control, medium, and low pH, there were no significant differences. There were significant differences in total length between days 4 & 6 when compared to day 2 (Table 3.4). There were no significant differences in carapace or rostral spine length at either medium or low pH. Dorsal spine length was significantly different when contrasting control and low pH and when contrasting medium and low pH, with no significant difference between control and medium pH.

Discussion

Increased acidity was found to primarily affect survival, with the greatest effects observed at low pH, moderate effects at medium pH, and the best survival at control pH. In morphology, changes were minimal at low and medium pH, although dorsal spine length at low pH was significantly shorter than at control pH. Total protein revealed no significant differences between low, medium, and control pH. In contrast, total lipid content significantly decreased at low pH. These results are partially consistent with my initial hypotheses. I did not expect to find any significant differences in dorsal spine length and I had expected total protein to decline.

My observed declines in survival are corroborated in larval blue crabs from the Gulf of Mexico (Giltz & Taylor, 2017), while later juvenile stages are unaffected (Ries *et al.,* 2009). Such divergent responses at different life stages emphasizes the importance of evaluating every molt stage in complex life histories (Kurihara, 2008), in order to identify bottleneck effects upstream or downstream of the life stage studied (Whiteley, 2011). My

observed reduced survival may impact population size and therefore affect larval dispersal patterns (Duarte, 2007), fishery management plans (O'Connor *et al.,* 2007), and other ecosystem-wide impacts due to the ecological importance of crustaceans (Gutierrez *et al.,* 2003; Baum & Worm, 2009; Boudreau & Worm, 2012).

Alternatively, I found that brood sources significantly affect survival. Such differing responses between broods likely indicates high genetic variability, which would reduce or eliminate population level effects as corroborated in blue king crabs (Long *et al.,* 2017). The Atlantic population as a whole contains high genetic diversity (Feng *et al.,* 2017) and acclimation is therefore worth considering in future studies.

I observed dorsal spine length was reduced in low pH, which has not been observed in other studies. This is likely due to noticeably broken off dorsal tips with decreasing pH observed in live larvae. Although dorsal spines' thickness was not measured, increasing acidity may cause dorsal spine degradation and result in easier breakage, thus changing the length measured. Alternatively, spinal structural integrity may be affected, as has been found in carapaces in other crustaceans. In red king and blue king crabs, carapace strength was compromised in acidic conditions (Coffey *et al.,* 2017). Further research into this finding is merited. The majority of larvae did not have the opportunity to molt and therefore my other morphological measurements did not statistically differ between treatments, although larval total length and carapace length were nearly significant (Table 3.4). After Tanner crabs were exposed to acidic conditions through the 5^{th} molt, crab size diminished compared with the control (Long *et al.,* 2013b), and blue crabs are likely to follow this trend, based on my nearly significant changes.

My results reveal significant loss of total lipids at low pH. As a source for rapid energy mobilization in stressful environments (Anger, 2001) and as an extremely important energy source for regular growth and development, this decline conveys larvae were utilizing their energy stores more rapidly in low pH, as found in other larval crustaceans (Long *et al.,* 2013a).

Other larval crustaceans show diminished protein content with increasing acidity, which may be from suppressing protein synthesis or switching to protein catabolism (Anger & Harms, 1990; Pörtner *et al.,* 1998; Carter *et al.,* 2013; Schiffer *et al.,* 2013). While I found significant variability between treatments over the course of seven days, I did not find significant differences in final protein content. Red king crabs also decreased total lipid content without diminishing final protein content (Long *et al.,* 2013a). My observed differences over seven days may indicate delayed protein production (Figure 3.6), with healthier larvae in control conditions producing protein earlier in order to aid transition to the next molt.

Reduced survival and lipid content may be alternatively due to diminished food consumption in acidic conditions. I could not evaluate food consumption rates, nor have similar studies included this consideration (Carter *et al.,* 2013; Long *et al.,* 2017; Giltz & Taylor, 2017). Future studies should determine whether feeding rates diminish with declining pH in order to accurately assess the impact of pH alone. Additionally, larvae are known to require narrower temperature ranges compared with adults (Costlow and Bookhout, 1959; Epifanio, 2019). Although I do not address this constraint, future studies should determine whether there are interactive effects of increased temperature and

acidity. In other crustaceans, negative responses to increased acidity were amplified when combined with increasing temperature (Walther *et al.,* 2010; Whiteley, 2011; Walther *et al.,* 2011).

Overall, larval blue crabs respond poorly to acidification, with significantly increased mortality at medium and low pH and reduced total lipids at low pH. I utilized pH values that are not expected in surface waters where stage I larvae are found in order to elucidate whether larvae are negatively affected by any change in pH. Even so, some models predict an average surface ocean pH of 7.4 to be reached by the year 2300 (IPCC, 2013), which will likely be exacerbated in coastal waters (Cai *et al.,* 2017). It is possible blue crabs may adapt by the year 2300, however it remains unknown at what rate they can adapt in order to optimize survival.

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Figures and Tables

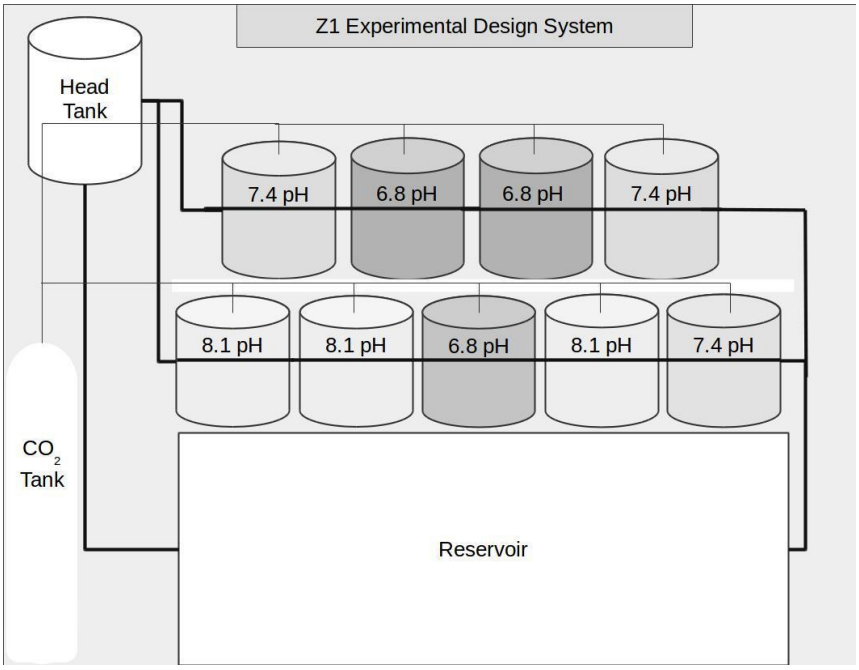

Figure 3.1. Experimental design. Carbon dioxide flow was regulated by a microcontroller that individualized flow and opened individual solenoids every half hour. Salt water flow was regulated to circulate four times/day to remove waste products through a life support system in the reservoir. Air stones were placed in each tank and moderate currents were produced in each tank through a small pump at the base of each tank. A mesh insert separated pumps from approximately 250 zoea per tank.

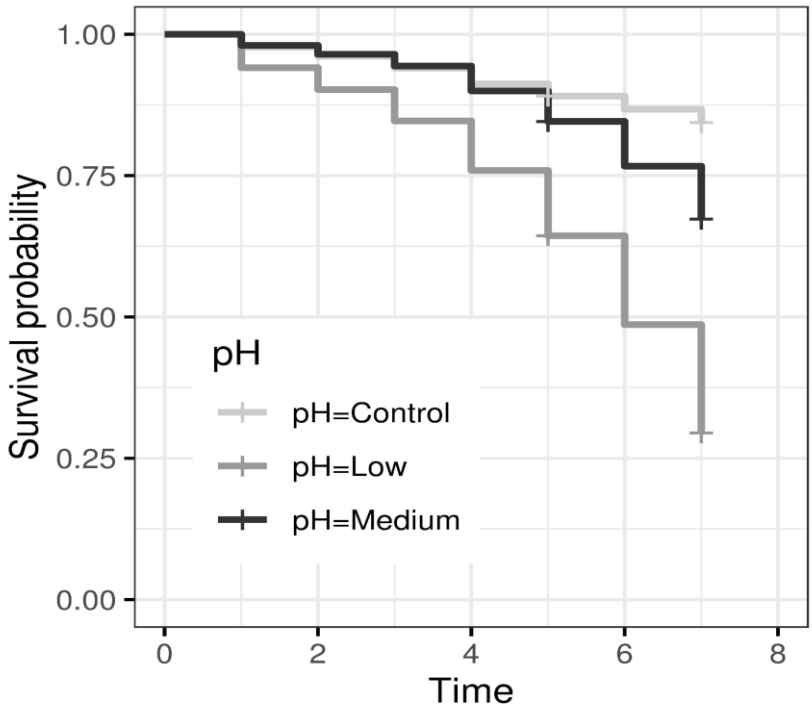

Figure 3.2. Effect of pH on larvae survival. Over a period of 7 days, survival diminished with increasing acidity.

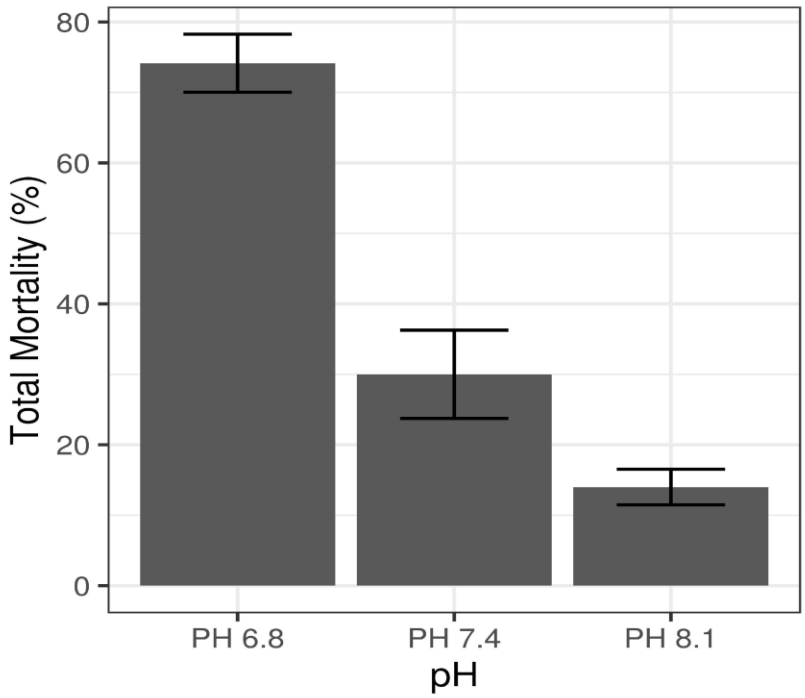

Figure 3.3. Effect of pH on total mortality. Average final mortality (%) significantly increased with increasing acidity. Data are mean values ± standard error.

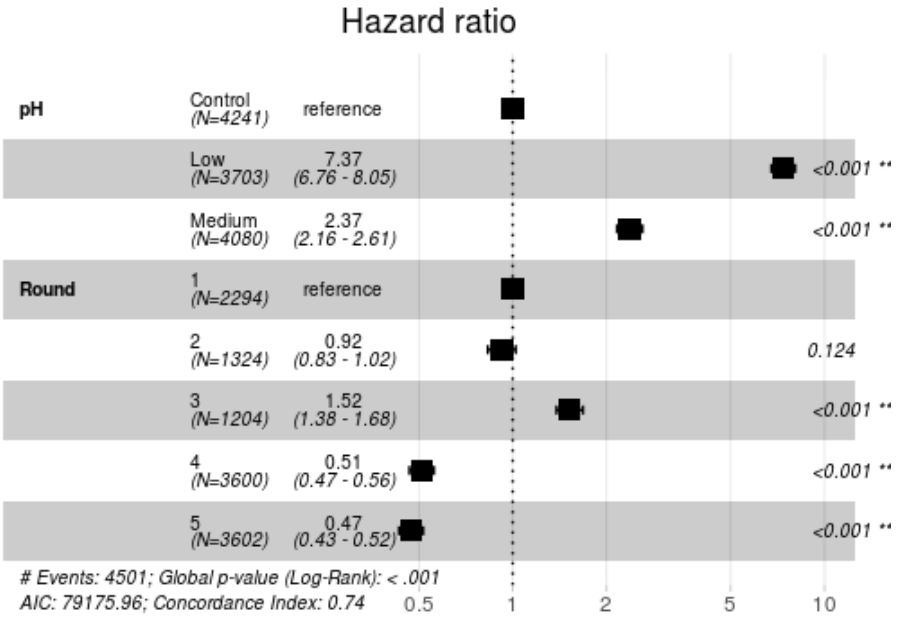

Figure 3.4. Effect of pH and brood on mortality. Mortality increased with increasing acidity, with brood (Round) source also affecting mortality.

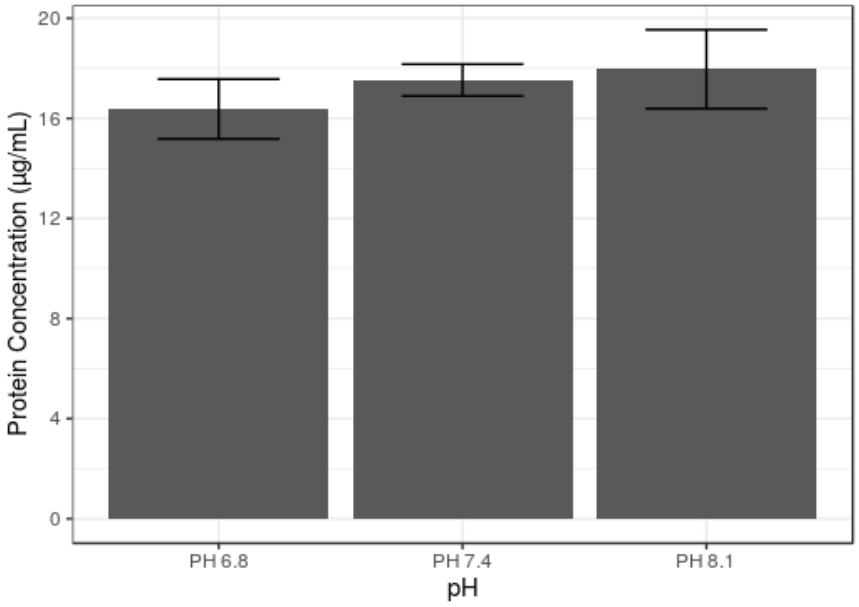

Figure 3.5. Effect of pH on larvae protein concentration. On the final day, total protein (µg/mL) did not differ widely between treatments. Data are mean values ± standard error.

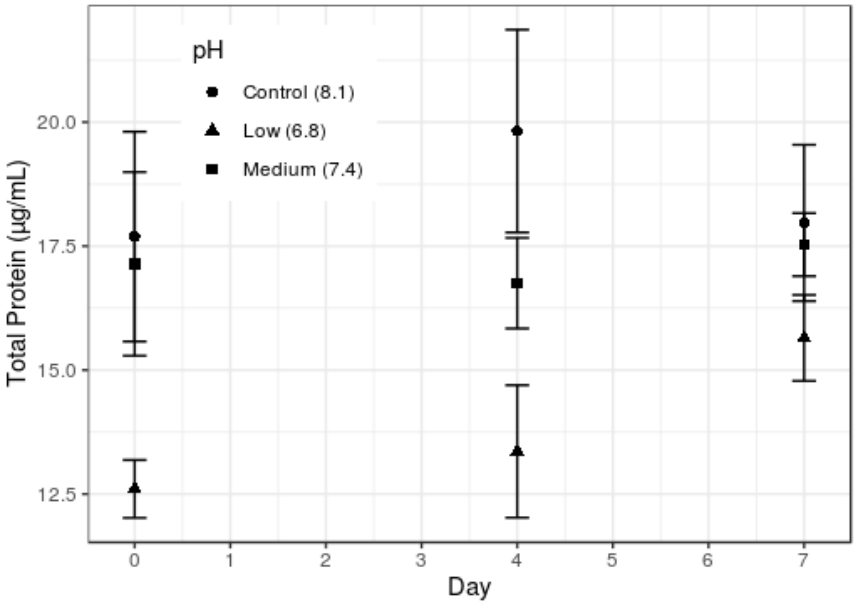

Figure 3.6. Change in average total protein. With increasing acidity, protein (µg/mL) increases were delayed in comparison with control. Data are mean values ± standard error.

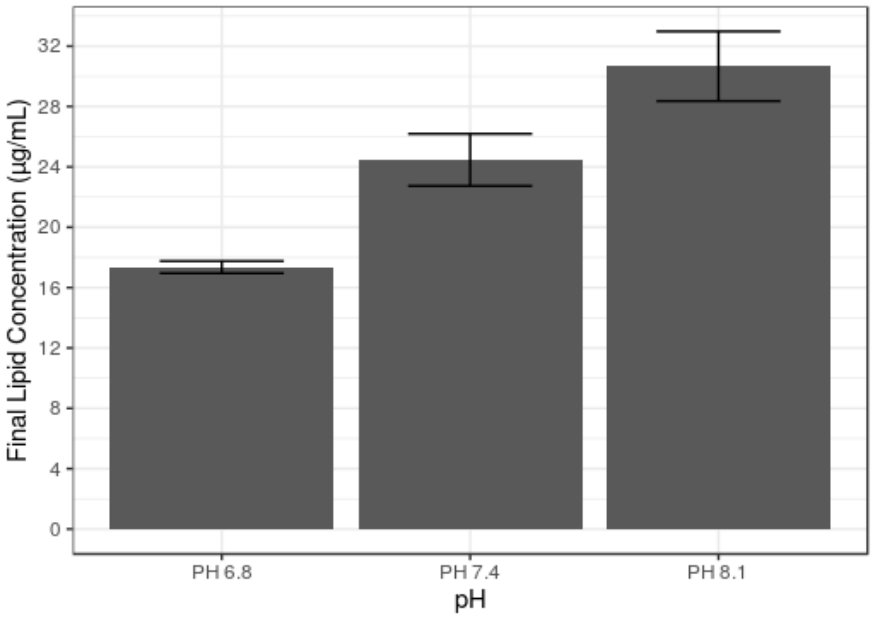

Figure 3.7. Effect of pH on larvae lipid concentration. With increasing acidity, total lipids (µg/mL) decreased. Data are mean values ± standard error.

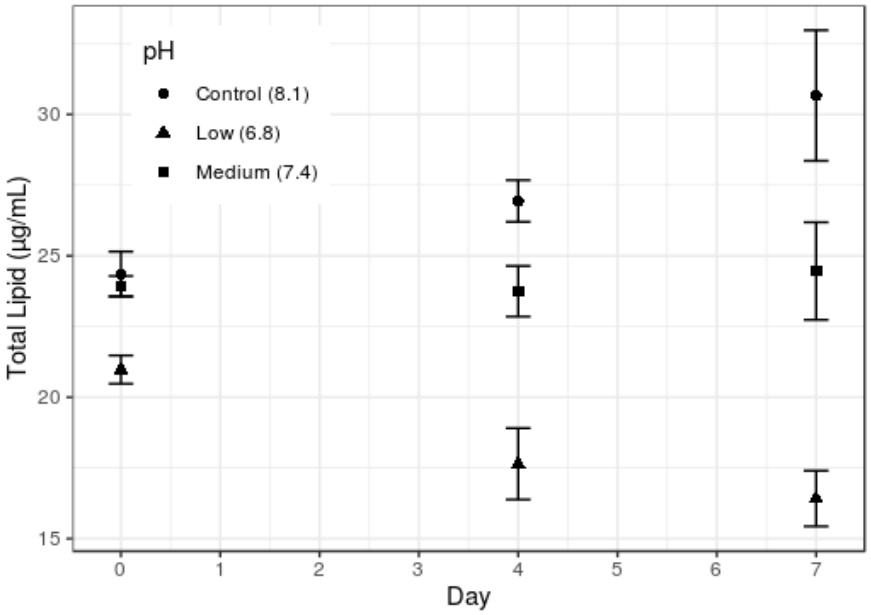

Figure 3.8. Change in average total lipid. Over a period of seven days, total lipids (µg/mL) diverged, with increasing acidity reducing lipid content. Data are mean values ± standard error.

Table 3.1. Water chemistry. Temperature, salinity, and pH were measured twice daily in experimental tanks. Bicarbonate, carbonate, and dissolved inorganic carbon (DIC) were calculated after the experiment. Total Alkalinity (A_T) was measured at the start of the experiment. Data are mean values ± standard error.

Parameter	**Low**	**Medium**	**Control**
Temperature	23.51 ±0.10	23.29 ±0.11	23.17 ±0.14
Salinity	29.83 ±0.15	29.83 ±0.15	29.83 ±0.15
A_T (µmol kg^{-1})	1683 ±1.3	1680 ±1.5	1693 ±3.4
pH	6.73 ±0.01	7.38 ±0.02	8.13 ±0.01
[HCO_3^-] (µmol kg^{-1})	1666 ±0.94	1600 ±4.47	1360 ±3.82
[CO_3^{2-}] (µmol kg^{-1})	9 ±0.26	40 ±2.15	167 ±2.88
DIC	1896 ±4.94	1688 ±4.51	1534 ±2.23

Table 3.2. Total protein ANOVA and linear regression results for *C. sapidus.* Significant results in bold.

Total Protein					
Two-Way ANOVA	**d.f.**	**SS**	**MS**	***F***	***P***
Day	2	0.0127	0.0064	0.438	0.653
pH	2	0.0586	0.0293	2.016	0.164
Day:pH	4	0.0125	0.0031	0.214	0.927
Residuals	17	0.2470	0.0145		
	AIC	**BIC**			***P***
Mixed Effects Model	**452.9**	**464.5**			
Low pH					**≤ 0.001**
Medium pH					**0.008**

Table 3.3. Total lipid ANOVA, Tukey HSD, and linear regression results for *C. sapidus.* Only statistically significant Tukey HSD results are reported. Significant results in bold.

Total Lipid					
Two-Way ANOVA	**d.f.**	**SS**	**MS**	***F***	***P***
Day	2	19.3	9.65	0.685	0.530
pH	**2**	**300.21**	**150.11**	**10.231**	**0.001**
Day:pH	4	58.87	14.72	1.003	0.432
Residuals	18	264.09	14.67		
Tukey HSD	**Diff**	**Lower**	**Upper**		***P***
Med pH: Low pH	**4.843**	**0.235**	**9.452**		**0.039**
Control pH: Low pH	**8.117**	**3.509**	**12.726**		**0.001**
	AIC	**BIC**			***P***
Mixed Effects Model	**421.7**	**433.0**			
Low pH					**≤ 0.001**
Medium pH					**≤ 0.001**

Table 3.4. Morphology ANOVA and Tukey HSD results for *C. sapidus.* Only statistically significant Tukey HSD results are reported. Significant results in bold.

Morphology					
Total Length					
Two-Way ANOVA	**d.f.**	**SS**	**MS**	***F***	***P***
Day	**6**	**0.024**	**0.004**	**2.775**	**0.013**
pH	2	0.009	0.004	3.041	0.0503
Day:pH	12	0.019	0.002	1.125	0.342
Residuals	181	0.258	0.001		
Tukey HSD	**Diff**	**Lower**	**Upper**		***P***
Day 4: Day 2	**-0.036**	**-0.066**	**-0.007**		**0.005**
Day 6: Day 2	**-0.031**	**-0.059**	**-0.002**		**0.023**
Carapace Length					
Two-Way ANOVA	**d.f.**	**SS**	**MS**	***F***	***P***
Day	6	0.051	0.009	0.643	0.696
pH	2	0.002	0.001	0.089	0.915
Day:pH	12	0.288	0.024	1.804	0.0502
Residuals	182	2.421	0.013		
Rostrum Length					
Two-Way ANOVA	**d.f.**	**SS**	**MS**	***F***	***P***
Day	6	0.165	0.027	1.662	0.133
pH	2	0.007	0.004	0.225	0.798
Day:pH	12	0.045	0.004	0.224	0.997
Residuals	176	2.905	0.017		
Dorsal Length					
Two-Way ANOVA	**d.f.**	**SS**	**MS**	***F***	***P***
Day	6	0.023	0.004	0.680	0.666
pH	**2**	**0.109**	**0.055**	**9.501**	**≤ 0.001**
Day:pH	12	0.094	0.008	1.365	0.187
Residuals	174	0.999	0.006		
Tukey HSD	**Diff**	**Lower**	**Upper**		***P***
Med pH: Low pH	**-0.052**	**-0.084**	**-0.020**		**≤ 0.001**
Control pH: Low pH	**-0.046**	**-0.076**	**-0.015**		**0.002**

Literature Cited

Andersson, A. J., F. T. Mackenzie, & A. Lerman. 2005. Coastal ocean and carbonate systems in the high CO_2 world of the anthropocene. *American Journal of Science,* 305:875–918. doi: 10.2475/ajs.305.9.875

Andersson, A. J., N. R. Bates, & F. T. Mackenzie. 2007. Dissolution of carbonate sediments under rising pCO_2 and ocean acidification: observations from Devil's Hole, Bermuda. *Aquatic Geochemistry,* 13:237–264. doi: 10.1007/s10498-007-9018-8

Anger, K., & J. Harms. 1990. Elemental (CHN) and proximate biochemical composition of decapod crustacean larvae, *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry,* 97:9-80.

Anger, K. 2001. *The Biology of Decapod Crustacean Larvae. Crustacean Issues, Vol. 14.* CRC Press, Boca Raton, FL.

Arnold, K. E., H. S. Findlay, J. I. Spicer, C. L. Daniels,& D. Boothroyd. 2009. Effect of CO_2-related acidification on aspects of the larval development of the European lobster, *Homarus gammarus* (L.). *Biogeosciences,* 6:1747-1754. doi: 10.5194/bg-6-1747-2009

Baum, J. K., & B. Worm. 2009. Cascading top-down effects of changing oceanic predator abundances. *Journal of Animal Ecology*, 78:699-714. doi: 10.1111/j.1365-2656.2009.01531.x

Blue Crab Winter Dredge Survey. 2020. Maryland Department of Natural Resources. [Online]. Available: http://dnr.maryland.gov/fisheries/Pages/blue-crab/dredge.aspx [2020, June 15].

Boudreau, S. A., & B. Worm. 2012. Ecological role of large benthic decapods in marine ecosystems: a review. *Marine Ecology Progress Series*, 469:195-212. doi: 10.3354/meps09862

Cai, W-J., W.-J. Huang, G. W. Luther III, D. Pierrot, M. Li, J. Testa, M. Xue, A. Joesoef, R. Mann, J. Brodeur, Y.-Y. Xu, B. Chen, N. Hussain, G. G. Waldbusser, J. Cornwell, & W. M. Kemp. 2017. Redox reactions and weak buffering capacity lead to acidification in the Chesapeake Bay. *Nature Communications,* 8:369. doi: 10.1038/s41467-017-00417-7

Carter, H. A., L. Ceballos-Osuna, N. A. Miller, & J. H. Stillman. 2013. Impact of ocean acidification on metabolism and energetics during early life stages of the intertidal porcelain crab *Petrolisthes cinctipes. Journal of Experimental Biology,* 216:1412-1422. doi: 10.1242/jeb.078162

Ceballos-Osuna, L., H. A. Carter, N. A. Miller, & J. H. Stillman. 2013. Effects of ocean acidification on early life-history stages of the intertidal porcelain crab *Petrolisthes cinctipes. Journal of Experimental Biology,* 216:1405-1411. doi: 10.1242/jeb.078154

Coffey, W. D., J. A. Nardone, A. Yarram, W. C. Long, K. M. Swiney, R. J. Foy, & G. H. Dickinson. 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. doi: 10.1016/j.jembe.2017.05.011

Costlow, J. D. & C. G. Bookhout. 1959. The larval development of *Callinectes sapidus* rathbun reared in the laboratory. *Biological Bulletin,* 116:373-396.

Dickson, A. G. 1990. Standard potential of the reaction: AgCI(s) + 1/2H2(g) = Ag(s) + HCI(aq), and the standard acidity constant of the ion HSO_4 in synthetic sea water from 273.15 to 318.15 K. *Journal of Chemical Thermodynamics,* 22:113-127.

Duarte, C. M. C. 2007. Marine ecology warms up to theory. *Trends in Ecology and Evolution*, 22:331–333. doi: 10.1016/j.tree.2007.04.001

Epifanio, C. E. 2019. Early life history of the blue crab *Callinectes sapidus*: A review. *Journal of Shellfish Research,* 38:1-22. doi: 10.2983/035.038.0101

Fabry, V. J., B. A. Seibel, R. A. Feely, & J. C. Orr. 2008. Impacts of ocean acidification on marine fauna and ecosystem processes. *ICES Journal of Marine Science,* 65:414-432. doi: 10.1093/icesjms/fsn048

Feely, R. A., C. L. Sabine, K. Lee, W. L. Berelson, J. Kleypas, V. J. Fabry, & F. J. Millero. 2004. Impact of anthropogenic CO_2 on the $CaCO_3$ system in the oceans, *Science,* 305:362-366. doi: 10.1126/science.1097329

Feng, X., E. P. Williams, & A. R. Place. 2017. High genetic diversity and implications for determining population structure in the blue crab, *Callinectes sapidus*. *Journal of Shellfish Research*, 36:231-242. doi: 10.2983/035.036.0126

Fox, J., S. Weisburg, & B. Price. 2019. car: Companion to Applied Regression. R package version 3.0-5. Available at: https://cran.r-project.org/web/packages/car/index.html

Gattuso, J.-P., J.-M. Epitalon, H. Lavigne, & J. Orr. 2018. seacarb: Seawater Carbonate Chemistry. R package version 3.2.12. Available at: https://cran.r-project.org/web/packages/seacarb/index.html

Giltz, S. M. & C. M. Taylor. 2017. Reduced growth and survival in the larval blue crab *Callinectes sapidus* under predicted ocean acidification. *Journal of Shellfish Research,* 36:481-485. doi: 10.2983/035.036.0219

Gravinese, P. M. 2018. Ocean acidification impacts the embryonic development and hatching success of the Florida stone crab, *Menippe mercenaria. Journal of Experimental Marine Biology and Ecology,* 500:140-146. doi: 10.1016/j.jembe.2017.09.001

Gutierrez, J. L., C. G. Jones, D. L. Strayer, & O. O. Iribarne. 2003. Mollusks as ecosystem engineers: the role of shell production in aquatic habitats. *Oikos,* 101:79-90.

Horton, N. J., & K. Kleinman. 2011. *Using R for Data Management, Statistical Analysis, and Graphics.* CRC Press, Taylor & Francis Group.

Intergovernmental Panel on Climate Change. 2013. Fifth Assessment Report: Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Kroeker, K. J., R. L. Kordas, R. Crim, I. E. Hendriks, L. Ramajos, G. S. Singh, C. M. Duartes, & J.-P. Gattuso. 2013. Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. *Global Change Biology* 19:1884-1896. doi: 10.1111/gcb.12179

Kurihara, H. 2008. Effects of CO_2-driven ocean acidification on the early developmental stages of invertebrates. *Marine Ecology Progress Series,* 373:275-284. doi: 10.3354/meps07802

Lavigne, H. & J.-P. Gattuso. 2018. seacarb: Seawater Carbonate Chemistry. R package version 2.4.3. Available at: https://CRAN.R-project.org/package=seacarb.

Lueker, T. J., A. G. Dickson & C. D. Keeling. 2000. Ocean pCO_2 calculated from dissolved inorganic carbon, alkalinity, and equations for K1 and K2: validation based on laboratory measurements of CO_2 in gas and seawater at equilibrium. *Marine Chemistry,* 70:105-119.

Le Quere, C., R. J. Andres, T. Boden, T. Conway, R. A. Houghton, J. I. House, G. Marland, G. P. Peters, G. van der Werf, A. Ahlstrom, R. M. Andrew, L. Bopp, J. G. Canadell, P. Ciais, S. C. Doney, C. Enright, P. Friedlingstein, C. Huntingford, A. K. Jain, C. Jourdain, E. Kato, R. F. Keeling, K. Klein Goldewijk, S. Levis, P. Levy, M. Lomas, B. Poulter, M. R. Raupach, J. Schwinger, S. Sitch, B. D. Stocker, N. Viovy, S. Zaehle, & N. Zeng. 2012. The global carbon budget, 1959-2011. *Earth System Science Data Discussion,* 5:1107-1157. doi: 10.5194/essdd-5-1107-2012

Long, W. C., K. M. Swiney, & R. J. Foy. 2013a. Effects of ocean acidification on the embryos and larvae of red king crab, *Paralithodes camtschaticus*. *Marine Pollution Bulletin,* 69:38-47. doi: 10.1016/j.marpolbul.2013.01.011

Long, W. C., K. M. Swiney, C. Harris, H. N. Page, & R. J. Foy. 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:e6095. doi: 10.1371/journal.pone.0060959

Long, W. C., S. B. Van Sant, K. M. Swiney, & R. J. Foy. 2017. Survival, growth, and morphology of blue king crabs: effect of ocean acidification decreases with exposure time. *ICES Journal of Marine Science,* 74:1033-1041. doi: 10.1093/icesjms/fsw197

Macko, S. A., C. M. Fantasia, & G. Xue. 2017. Potential global economic impacts of ocean acidification. *Center for Oceans Law & Policy: International Marine Economy*. Eds. M.H. Nordquist, J.N. Moore, & R. Long. 20:79-97. doi: 10.1163/9789004323445_003

Macko, S. A., & C. M. Fantasia. 2018. An overview of ocean acidification: relationships. *Center for Oceans Law & Policy:The Marine Environment and United Nations Sustainable Development Goal 14, Life Below Water.* Eds. M.H. Nordquist, J.N. Moore, & R. Long. 21:391-417. doi: 10.1163/9789004366619_025

Miller, T. J., S. J. D. Martell, D. B. Bunnell, G. Davis, L. Fegley, A. Sharov, C. Bonzek, D. Hewitt, J. Hoenig, & R. N. Lipcius. 2005. Stock assessment of the blue crab in Chesapeake Bay. University of Maryland Center for Environmental Science, Technical Report Series TS-487-05, Solomons. Available: hjort.cbl.umces.edu/crabs/Assessment05.html.

Miller, J. J., M. Maher, E. Bohaboy, C. S. Friedman, & P. McElhany. 2016. Exposure to low pH reduces survival and delays development in early life stages of Dungeness crab (*Cancer magister*). *Marine Biology*, 163:118. doi: 10.1007/s00227-016-2883-1

O'Connor, M. I., J. F. Bruno, S. D. Gaines, B. S. Halpern, S. E. Lester, B. P. Kinlan & J. M. Weiss. 2007. Temperature control of larval dispersal and the implications for marine ecology, evolution, and conservation. *PNAS*, 104:1266–1271. doi: 10.1073/pnas.0603422104

Orr, J. C., V. J. Fabry, O. Aumont, L. Bopp, S. C. Doney, R. A. Feely, A. Gnanadesikan, N. Gruber, A. Ishida, F. Joos, R. M. Key, K. Lindsay, E. Maier-Reimer, R. Matear, P. Monfray, A. Mouchet, R. G. Najjar, G-K. Plattner, K. B. Rodgers, C. L. Sabine, J. L. Sarmiento, R. Schlitzer, R. D. Slater, I. J. Totterdell, M-F. Weirig, Y. Yamanaka, & A. Yool, 2005. Anthropogenic ocean acidification over the 21st century and its impact on calcifying organisms. *Nature,* 437:681-686. doi: 10.1038/nature04095

Perez, F. F. & F. Fraga. 1987. Association constant of fluoride and hydrogen ions in seawater. *Marine Chemistry*, 21:161-168.

Pinheiro J., D. Bates, S. DebRoy, D. Sarkar, & R Core Team. 2020. *nlme: Linear and Nonlinear Mixed Effects Models*. R package version 3.1-148. Available at: https://CRAN.R-project.org/package=nlme.

Pörtner, H. O., A. Reipschlager, & N. Heisler. 1998. Acid-base regulation, metabolism, and energetics in S*ipunculus nudus* as a function of ambient carbon dioxide level. *Journal of Experimental Biology,* 201:43-55.

Prendini, L., R. Hanner, R. Desalle, & P. Fitzpatrick. 2002. Obtaining, Storing and Archiving Specimens and Tissue Samples for Use in Molecular Studies. Pp. 176-248 in *Techniques in Molecular Evolution and Systematics*, R. DeSalle, G. Giribet, and W. Wheeler, eds. Birkhäuser, Basel.

Qian, S.S. 2010. *Environmental and Ecological Statistics with R.* CRC Press, Taylor and Francis Group.

R Development Core Team. 2011. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: http://www.R-project.org

Ries, J. B., A. L. Cohen, & D. C. McCorkle. 2009. Marine calcifiers exhibit mixed responses to CO_2-induced ocean acidification. *Geology,* 37:1057-1152. doi: 10.1130/G30210A.1

Riebesell, U., I. Zondervan, B. Rost, P. D. Tortell, R. E. Zeebe, & F. M. M. Morel. 2000. Reduced calcification of marine plankton in response to increased atmospheric CO_2. *Nature,* 407:364–67. doi: 10.1038/35030078

Scheld, A. M., D. M. Bilkovic, & K. J. Havens. 2016. The dilemma of derelict gear. *Nature Scientific Reports,* 6:19671. doi: 10.1038/srep19671

Schiffer, M., L. Harms, H. O. Pörtner, M. Lucassen, F. C. Mark, & D. Storch. 2013. Tolerance of *Hyas araneus* zoea I larvae to elevated pCO_2 despite elevated metabolic costs. *Marine Biology,* 160:1943-1953. doi: 10.1007/s00227-012-2036-0

Seitz, R. D. 2020. "Blue Crabs: Beautiful Savory Swimmers of the Chesapeake Bay." In: Cuker B. (ed) Diet for a Sustainable Ecosystem. Estuaries of the World. Springer, Cham. doi: 10.1007/978-3-030-45481-4_7

Sulkin, S. D. & C. E. Epifanio. 1975. Comparison of rotifers and other diets for rearing early larvae of the blue crab, *Callinectes sapidus* Rathbun. *Estuarine and Coastal Marine Science,* 3:109-113.

Sulkin, S. D. 1978. Nutritional requirements during larval development of the portunid crab, *Callinectes sapidus* Rathbun. *Journal of Experimental Marine Biology and Ecology,* 34:29-41.

Therneau, T. 2019. Survival: survival analysis, including penalized likelihood. R package version 2.44-1.0. Available at: http://CRAN.R-project.org/package=survival.

Tomasetti, S. J., B. K. Morrell, L. R. Merlo, & C. J. Gobler. 2018. Individual and combined effects of low dissolved oxygen and low pH on survival of early life stage blue crabs, *Callinectes sapidus. PLoS ONE,* 13:e0208629. doi: 10.1371/journal.pone.0208629

Waldbusser, G. G., E. P. Voigt, H. Bergschneider, M. A. Green, & R. I. E. Newell. 2011. Biocalcification in the Eastern oyster (*Crassostrea virginica*) in relation to long-term trends in Chesapeake Bay pH. *Estuaries and Coasts,* 34:221-231. doi: 10.1007/s12237-010-9307-0

Walther, K., K. Anger, & H. O. Pörtner. 2010. Effects of ocean acidification and warming on the larval development of the spider crab *Hyas araneus* from different latitudes (54° vs. 79° N). *Marine Ecology Progress Series,* 417:159-170. doi: 10.3354/meps08807

Walther, K., F. J. Sartoris, & H. O. Pörtner. 2011. Impacts of temperature and acidification on larval

calcium incorporation of the spider crab *Hyas araneus* from different latitudes (54° vs. 79° N). *Marine Biology,* 158:2043-2053. doi: 10.1007/s00227-011-1711-x

Whiteley, N. M. 2011. Physiological and ecological responses of crustaceans to ocean acidification. *Marine Ecology Progress Series,* 430:257-271. doi: 10.3354/meps09185

Wickham, H. 2020. ggplot2: Create Elegant Data Visualisations Using the Grammar of Graphics. R package version 3.3.2. Available at: https://cran.r-project.org/web/packages/ggplot2/index.html.

Wood, H. L., H. N. Skold, & S. P. Eriksson. 2014. Health and population-dependent effects of ocean acidification on the marine isopod *Idotea balthica*. *Marine Biology,* 161:2423–2431. doi: 10.1007/s00227-014-2518-3

Zmora, O., A. Findiesen, J. Stubblefield, V. Frenkel, and Y. Zohar. 2005. Large-scale juvenile production of the blue crab *Callinectes sapidus*. *Aquaculture,* 244:129-139. doi: 10.1016/j.aquaculture.2004.11.012

Chapter Four:

The effect of increased acidity and temperature on stage 1 zoea in the blue crab (*Callinectes sapidus*)

Abstract

Increasing ocean acidification from increased fossil fuel usage profoundly affects early life stage crustaceans, including the blue crab (*Callinectes sapidus*). However, little is known about the combined effects of increased temperature with increased acidification on early stages. This study focuses on these combined stressors and their impact on stage one larvae in terms of survival, total lipid, total protein, morphology, swimming activity, and carapace calcium content. Larvae were placed in nine tanks at a combination of temperatures (23º C, 26º C, 28º C) and acidities (pH 8.1, pH 7.8, pH 7.4). Survival, total lipid, total protein, and swimming activity were negatively affected most strongly when these stressors were combined, whereas morphology and carapace calcium content were not significantly affected by the combination. When temperature alone was increased, survival, total lipid, and total protein were significantly reduced. Swimming activity, morphology, carapace calcium content were unaffected by increased temperatures alone. With increased acidity alone, total lipid, total protein, and swimming activity were diminished, although survival, morphology, and carapace calcium content were not significantly affected. Taken together, these results indicate general sensitivity to acidification and temperature alone, with negative interactions when combined. My results suggest they experience reduced thermal tolerance, which may be detrimental to their population and stability in future ocean conditions.

Introduction

With continued reliance on carbon-based fuels, carbon dioxide concentrations are expected to continue rising (IPCC, 2013) and accumulating in one of the largest carbon sinks, the ocean (Sabine *et al.*, 2004). A natural consequence will be further calcium carbonate dissolution (Feely *et al.*, 2004). This significant change in ocean chemistry is expected to negatively impact a variety of marine organisms and systems (Doney *et al.*, 2014; Wallace *et al.*, 2014; Macko & Fantasia, 2018) and will ultimately affect the economic status of many nations, especially those heavily dependent on fisheries and marine based tourism (Macko *et al.*, 2017). In the Chesapeake Bay, diminishing carbonate concentrations in the water column will be exacerbated by high populations of calcifying organisms and eutrophication (Najjar *et al.*, 2010; Cai *et al.*, 2017). Although most of these negative effects will occur over the next several decades, juvenile eastern oysters (*Crassostrea virginica*) have already begun to experience calcification loss from limited carbonate availability (Waldbusser *et al.*, 2011).

Alongside changes in ocean chemistry, temperatures are increasing in the ocean's mixed layer and will continue to increase (IPCC, 2013). Surface water temperatures in the Chesapeake Bay have been warming at least since 1957 (Wood *et al.*, 2002; Preston, 2004) and will continue to increase (Najjar *et al.*, 2010; Du *et al.*, 2018). These warming temperatures will alter species distribution and reduce winter intensity and duration, which is likely to enhance over-winter survival in juvenile and adult blue crabs (Hines *et al.*, 2010). Although winter mortality is expected to decrease (Glandon *et al.*, 2019), increasing temperatures are also expected to increase pathogen susceptibility, disease

outbreaks (Najjar *et al.*, 2010; Du *et al.*, 2018; Huchin-Mian *et al.*, 2018; Shields, 2019), predation, and cannibalism (Hines *et al.*, 2010). Additionally, the habitat of blue crabs is likely to become more sensitive to eutrophication, thus expanding current anoxic and hypoxic zones (Du *et al.*, 2018).

Warming temperatures and their effects on the Chesapeake Bay have been supported (Huchin-Mian *et al.*, 2018; Shields, 2019), however there are no published studies ascertaining the combined effect of increased temperature and acidity on potentially vulnerable early life stage blue crabs. With both increased temperatures and acidity predicted for the habitat of stage one larvae, I sought to understand the interaction of these two stressors on survival and physiological responses.

The effects of increased acidity in crustaceans are wide ranging and include parasite transmission (Harland *et al.*, 2015), altered metabolism (Long *et al.*, 2017; Tomasetti *et al.*, 2018), reduced survival (Long *et al.*, 2013a; Miller *et al.*, 2016), and taxonomic repercussions (Whiteley, 2011; Branch *et al.*, 2013). These physiological changes are most likely from acidosis across a range of internal systems (Pörtner *et al.*, 2004; Pörtner, 2008). Some crab species (*Necora puber*) can mitigate this acidosis through actively pumping bicarbonate, although this was achieved through exoskeletal dissolution (Spicer *et al.*, 2007). Even so, most invertebrates are weak or poor iono-regulators (Pörtner, 2004), although adult blue crabs are strong iono-regulators due to their estuarine habitat (Whiteley, 2011). However, it is unknown whether their coastal ocean-dwelling early life stages are also strong regulators. It is likely iono-regulation in acidic conditions will require greater energy allocation in developing larvae and reduce

overall survival. Including thermal pressures will likely exacerbate these expenditures (Pörtner *et al.,* 2005) and further reduce survival, along with compounding sublethal effects.

Increasing temperature alone has already been proven to affect organisms through reducing thermal tolerance and limits (Pörtner, 2008). Oxygen supply is reduced in adult spider crabs when exposed to extreme temperatures and demonstrates the outer limits of crustacean circulatory systems (Frederich & Pörtner, 2000). Higher temperatures cause reduced growth, reproduction, and changed metabolism across a range of invertebrates (Pörtner *et al.,* 2014). In particular, larval development is affected by increasing temperatures (Gillooly *et al.,* 2002; Byrne, 2011) and thermal windows have been well defined in invertebrates, with the outcomes explaining ecosystem effects (Pörtner, 2001, 2002; Pörtner & Knust, 2007), including regime shifts and food web structural changes (Pörtner & Farrell, 2008; Pörtner *et al.,* 2014). Such shifts could result in lost ecosystems (Pörtner *et al.,* 2014).

Combining temperature and acidity exacerbated negative effects in coral reefs (Reynaud *et al.,* 2003; Hoegh-Guldberg *et al.,* 2007), reduced bivalve shell hardness (Ivanina *et al.,* 2013), elevated bivalve metabolic rates (Matoo *et al.,* 2013), and altered parasite and bacteria abundance in a commercial mussel species, *Mytilus edulis* (Mackenzie *et al.,* 2014). Stress indicators in an adult lobster (*Nephrops norvegicus*) became more pronounced, extracellular pH increased, and their immune response was reduced when exposed to increased acidity and temperature (Hernroth *et al.,* 2012), with cardiac performance affected in another lobster species (*Homarus americanus;* Qadri *et*

al., 2007). This combination narrowed thermal windows in adult crabs, *Cancer pagurus* and *Hyas araneus* (Metzger *et al.,* 2007; Walther *et al.,* 2009), and reduced survival and protein synthesis across species (Pörtner *et al.,* 2005). With reduced oxygen consumption from increased temperatures and acidosis, metabolic rates are ultimately impacted and these outcomes are only expected to increase with time (Pörtner, 2008).

These multi-stressor studies have focused on adult life stages, with minimal information known about their impacts on early life stages. In embryonic and larval cuttlefish (*Sepia officinalis*), growth, survival, and hatching success were relatively unaffected, although hypercalcification was observed (Dorey *et al.,* 2013). Norway lobster (*Nephrops norvegicus*) embryos also revealed no interactions (Styf *et al.,* 2013). However, spider crab larvae (*Hyas araneus*) do evidence narrowing thermal windows when exposed to both stressors (Walther *et al.,* 2009, 2010). Currently there are no published studies evaluating both stressors on blue crab stage 1 larvae. My study aims to address this and expects the combination of temperature and pH to negatively impact larval survival, carapace calcium content, total lipid and protein content, and swimming activity. Morphology is expected to be unaffected. These hypothesized results are expected to produce a bottleneck effect and limit successful larval development to adulthood, with these implications negatively affecting populations.

Methods

Approximately 13,500 larvae were obtained from the Institute of Marine and Environmental Technology (Baltimore, Maryland, USA) from six female blue crabs. Each batch was collected one day after hatching and transported to the University of

Virginia (Charlottesville, Virginia, USA). In total, six trips were made, with each round of experimentation containing larvae from one female.

Experimental Design: Nine tanks (Figure 4.1) contained 11 L of artificial sea water (Instant Ocean, Blacksburg, Virginia, USA) and each were connected to a main reservoir where water quality was maintained through use of particulate and biological filters, UV light sterilization, and aeration to restore water to pH 8.1. Over a period of 24 hours, water was re-circulated four times through all nine tanks, with three tanks maintained at pH 8.1 (control acidity), three tanks at treatment pH 7.8 (medium acidity), and three final tanks at treatment pH 7.4 (high acidity; Table 4.1). Within these control and treatment tanks were three differing temperatures: control temperature of 23° C, medium temperature of 26° C, and high temperature of 28° C. Each tank contained a small pump to maintain circulation, a mesh insert (100 μm) to separate larvae from the pump, an air stone bubbling in ambient air to maintain oxygen, and a CO_2 line and small water heater below the mesh. The CO_2 lines were connected to solenoids controlled with an Arduino microcontroller (Arduino Uno, Somerset, Massachusetts, USA), which determined how many milliseconds of CO_2 each tank received. When open, individual solenoids released CO_2 every half hour, with each tank receiving individualized bursts of CO_2. Monitoring and small changes were performed twice daily. Temperature and salinity were also monitored twice daily, with salinity maintained at 30‰ (Costlow & Bookhout, 1959; Sulkin & Epifanio, 1975) and temperature maintained at the treatment conditions described above.

Each brood of larvae was evenly distributed between all nine tanks for seven days

and daily fed a mixture of *Rotifera plicatilus* S-type (Reed Mariculture, Campbell, California, USA) and enriched *Nannochloropsis* algae (Instant Algae, Reed Mariculture, Campbell, California, USA) at a density of 50 individuals per milliliter (Sulkin, 1978; Zmora *et al.*, 2005). Larvae were removed daily from the bottom of each tank and separated into live and dead larvae, with the number of deceased larvae counted, placed in ethanol (95%), and preserved for later analysis (Prendini *et al.*, 2002). Live larvae were counted and returned to their original tank. On the final day, all live larvae were also collected and placed in vials of ethanol (95%). Daily mortality was determined in each tank and total mortality was calculated at the end of the experiments.

Total Protein: Live larvae were collected on days 0, 4, and 7 from all nine tanks. They were rinsed in Milli-Q water (MilliporeSigma, Massachusetts, USA) and placed in -80° C freezer. For analysis, vials were thawed on ice and homogenized with 500 µL of phosphate buffer (0.1 M phosphate, 0.15 M NaCl, pH 7.2) and protease inhibitors (1 M PMSF (phenylmethylsulfonyl fluoride), 10 mg mL^{-1} leupeptin, 1 mg mL^{-1} aprotinin). A Micro BCA Protein Assay Kit (ThermoScientific, Waltham, Massachusetts, USA) was used with the homogenate, which was then incubated at 37° C for 2 hours and read in a plate reader (SpectraMax M3, San Jose, California, USA) at 562 nm.

Total Lipid: Following the modified spectrophotometric sulfophosphovanillin method (Carter *et al.*, 2013), live larvae were collected on days 0, 4, and 7 from all nine tanks. Larvae were rinsed in Milli-Q water and placed in -80° C freezer. For analysis, vials were thawed on ice and samples homogenized with 500 µL methanol:chloroform (1:1) and placed on a dry block heater (95° C) until the solvent was completely

evaporated. Concentrated sulfuric acid (300 μL) was then added to each sample and mixed thoroughly. Samples (200 μL) were mixed with 1 mL of colored reagent (85% phosphoric acid and vanillin). A serial dilution (0-200 μg) using triglyceride and 95% sulfuric acid was used for quantification. The samples and standards were read at 540 nm in a plate reader (SpectraMax M3, San Jose, California, USA).

Swimming Activity: All zoea were collected on days 0 and 7 from each tank and observed under a dissecting microscope. Individual larval movements were placed into six categories from highly active (6) to dead (0). An average of swimming activity per tank was then determined.

Carapace Calcium Content: Live larvae were collected on days 0 and 6, rinsed in Milli-Q water, and placed in ethanol (95%) for later analysis. Larvae were then removed from ethanol, dried, placed on carbon tape, and mounted on a metal disc for placement in a scanning x-ray photoelectron spectrometer (XPS; PHI Versaprobe III, Chanhassen, Minnesota, USA) for surface carapace composition analysis. Once samples were placed in the XPS and sealed in a vacuum, a sub-10 μm x-ray beam produced an image of the sample to visualize location of analysis, with each sample analyzed on the main carapace body. After surface compositional analysis was complete, a depth profile was conducted after sputtering with an Argon Gas Cluster Ion Beam (GCIB) removed the first few surface nanometers. Each analysis produced a profile of the surface and sub-surface molecular components, with each sample providing three data points from which to create an average composition. From these, ratios and composition percentage of carbon, nitrogen, calcium, carbonate, and magnesium were determined.

Morphology: Telson, abdomen, carapace, rostrum, dorsum, and total length, as well as carapace height and width, were determined with ToupView software (ToupTek Photonics, Zhejiang, P.R. China). Data were obtained from larvae in all vials, which were randomized to prevent knowledge from which tank they were derived.

Statistical Methods: Mortality, morphology, total protein, total lipid, swimming activity, and carapace calcium content were checked for normality (Shapiro-Wilk test) and heteroscedasticity (Bartlett or Levene's Test). When the data did not pass these assumptions for a two-way ANOVA or MANOVA on final day results (total lipid, mortality, swimming activity, carapace calcium content), they were evaluated based on Q-Q plots and skewness, with all but swimming activity fitting normality assumptions enough to use parametric analysis (Webster & Oliver 2007). Swimming activity was log-transformed. A Kaplan-Meier survival curve was created for mortality for each pH treatment, with a Cox Proportional Hazards regression evaluating the impact of both stressors (Horton & Kleinman, 2011). Morphology data (total length, rostrum length, dorsum length, carapace length, telson length, abdomen length, carapace height, carapace width) were evaluated with a two-way multivariate ANOVA (MANOVA) with a Pillai-Bartlett trace statistic (Hand & Taylor, 1987) to determine the effect of factors pH and temperature. All other metrics were tested with a two-way factorial ANOVA (total protein, total lipid, swimming activity, carapace calcium content) to determine the effect of pH and temperature (Qian, 2010). When the interaction between factors was significant, a Tukey HSD post-hoc test was performed to detect the differences between treatments (Qian, 2010).

Survival curves were created using the Kaplan-Meier log-rank test in R 'survival' package (Thernau, 2011). Water quality parameters were calculated using R package 'seacarb' v. 2.4.3 (Gattuso *et al.,* 2018) with total pH scale, K_f (Perez & Fraga, 1987), k_1, k_2 (Lueker *et al.,* 2000) and K_s (Dickson, 1990). Mean pH and standard error were determined after converting from pH to hydrogen ion concentration, which were then converted back to the pH scale. All data were analyzed in R v. 3.5.2 (R Development Core Team, 2011) with the packages 'car' (Fox *et al.,* 2019), 'ggplot2' (Wickham, 2020), 'lme4' (Bates *et al.,* 2020), and 'Rmisc' (Hope, 2013). All tests were considered statistically significant with an alpha of 0.05.

Results

Mortality: Increased temperature alone significantly decreased survival across all treatments, with acidity alone also significantly decreasing survival (Figures 4.2-4.5). Combining both stressors significantly reduced survival in most conditions (Figure 4.6). At control acidity (pH 8.1) and medium temperature (26º C), there was higher mortality than expected due to large deviation in one brood's response. When this brood is removed, mortality is within 1% of mortality rates at control and high temperatures.

Total lipid: In control, medium, and high acidity, total lipid content declined with increasing temperature (Figure 4.7). On the final day of the experiment, total lipid content significantly differed with pH and with temperature, although final lipid content did not differ significantly with the combination of pH and temperature (Figure 4.8; Table 4.2).

Total protein: In control and medium acidity, total protein content declined at control and medium temperature (Figure 4.9). At high temperature, total protein declined

at medium acidity, but slightly increased at control acidity. In high acidity, total protein increased slightly at control temperature and fell more steeply with increasing temperature. On day 7 (Figure 4.10), there were significant effects of pH as well as temperature, with no effects observed with the combination of both treatments (Table 4.3).

Swimming activity: During the length of the experiment, swimming activity steeply diminished in all treatments, except for tanks at control acidity and high temperature (Figure 4.11). When larvae were exposed to higher temperatures alone, there was no significant difference in their final swimming activity (Figure 4.12). However, when larvae were exposed to pH alone, activity was significantly reduced, with the combination of pH and temperature also significantly reducing activity (Table 4.4).

Morphology: On the final day of the experiment, there were no significant differences observed between the metrics and pH, temperature, or the combination of the two (Table 4.4).

Carapace calcium content: Over the period of 7 days, calcium content increased throughout all tanks, except for the tanks at medium acidity and high temperature and control acidity and high temperature (Figure 4.13). However, by the final day there was no significant difference between the treatments (Figure 4.14; Table 4.4).

Discussion

Overall, the effects of combined increased temperature and acidity were greater than either factor alone in larval blue crab outcomes and fits with my initial hypothesis. The narrow mortality ranges in control acidity at varied temperatures evidence that larvae

are able to compensate for higher temperatures when acidity is within normal ocean ranges. However, as acidity increases, this tolerance with respect to temperature declines. In open circulatory systems such as decapods, large volumes of extracellular fluid must be modulated through acid-base regulation (Pörtner, 2008). Compensating for increasing acidity becomes a more arduous task and leads to increased temperature sensitivity. When increased thermal stress is included with acidification stress, oxygen supply through tissues is reduced (Pörtner, *et al.*, 2005; Metzger *et al.*, 2007) and shifts extracellular pH, reducing the function of various internal systems and ultimately negatively affecting the whole organism (Pörtner, 2008). Failing functional capacity results in reduced pO_2 in body fluids, with thermal stress pushing the organism to reach their limits more quickly (Pörtner, 2008). In blue crabs, my results illustrate how such declining function is likely occurring. Although I was unable to include oxygen consumption in this study, it should be evaluated in subsequent studies.

In all treatment tanks, mortality was greater than control tanks. These likely affected my total lipid and protein sampling, with all larvae collected on day 7 necessarily the most fit larvae and therefore containing higher concentrations of lipids and proteins. All larvae most sensitive to both stressors would be deceased by day 7 and thus would obscure negative interactions. Collecting larvae on each day of the experiment for analysis may improve detection of these outcomes. Future studies should incorporate these suggestions in order to detect these subtleties. Even with this obscuring effect, the trends in my results support the hypothesis that combined stressors reduce metabolic output (Pörtner, 2008). Such findings should be confirmed in future work

determining the effects on respiration (Carter *et al.*, 2013). Additionally, the marked decline in total lipid and protein in higher temperatures when also in higher acidity suggests reduced thermal tolerance (Pörtner, 2002; Walther *et al.*, 2009, 2010) when compared with lower temperatures at higher acidity.

Swimming activity was not significantly affected by increased temperature, although increased acidity did reduce activity. These findings are similar to results in adult blue crabs which found locomotory fatigue increased with hypercapnic conditions (Stover *et al.*, 2013). Swimming activity was likely unaffected by temperature due to efforts to achieve thermoregulation, as is observed in adult decapods (Lewis & Ayers, 2014). The combination of increased temperature and acidity significantly reduced swimming activity, which would be expected if increased acidity narrows overall thermal tolerance in larval blue crabs.

Morphology was unaffected by either treatment, which is not surprising because the experimental time did not allow for larvae to molt into the next stage. In other studies where early life stage crustaceans were exposed to longer term acidity alone, larvae increasingly diminished in size through successive molts (Walther *et al.*, 2010; Long *et al.*, 2013b), whereas combining increased temperature and acidity in larval American lobsters (*Homarus americanus*) revealed an interactive effect on carapace length in one population (Niemisto, 2019). Future long-term studies in blue crabs should continue to evaluate their morphological response after several molting periods. When evaluating carapace calcium content specifically, I observed continuous increases across temperature and pH, except at the highest acidity. This suggests blue crabs are able to maintain

calcium carbonate mineralization in their carapace overall, but once temperature and acidity reach a tipping point, the process of mineralization is negatively affected and calcium content declines. Initial calcium measurements were from larvae unexposed to any changes in acidity or temperature, which at high temperature and acidity indicates natural variability producing low initial calcium content. Larvae on the final day had been exposed to increased acidity and temperature for six days. At high temperature and high acidity, this produced the lowest calcium content out of all tanks, suggesting there is a trend towards reduced calcium content at high temperature and acidity. Although these results were not statistically significant, they are worth further examination in future studies.

I found that future populations of blue crabs will likely experience diminished survival and increased sublethal effects. Although adults are relatively resistant to changes in acidification, their larval stages have proven to be more sensitive, with increased temperatures further reducing their adaptation potential. These negative consequences due to increased carbon dioxide are strong enough to reduce the Chesapeake Bay's population of blue crabs, unless continued adaptation occurs. If blue crabs are unable to adapt rapidly, it is likely to produce ecological and economic repercussions throughout the Chesapeake (Miller *et al.,* 2005; Johnson *et al.,* 2014). Efforts to support sensitive larval stages continue to be explored (Zohar *et al.,* 2008) in order to ensure blue crabs will continue to provide economic support to the region, as well as remain an iconic and ecological linchpin for the Chesapeake Bay.

Figures and Tables

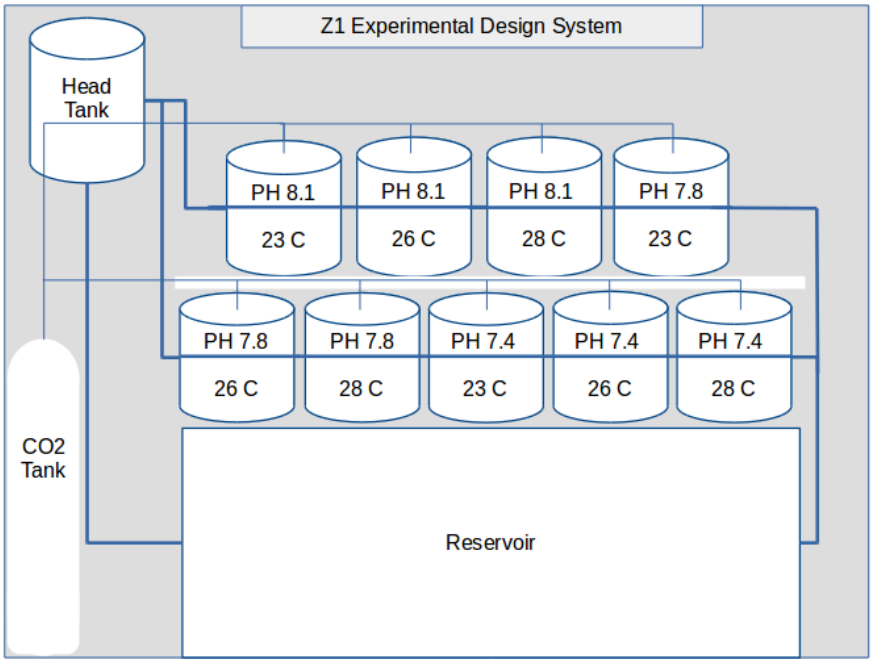

Figure 4.1. Experimental design. Approximately 250 zoea were placed in each of the nine tanks at different temperatures and pH. Water was circulated through the system every 4 hours, temperature was individually controlled in each tank, and pH was controlled through a microcontroller and solenoid system that let carbon dioxide flow into each tank every half hour. Temperature and pH were monitored twice daily.

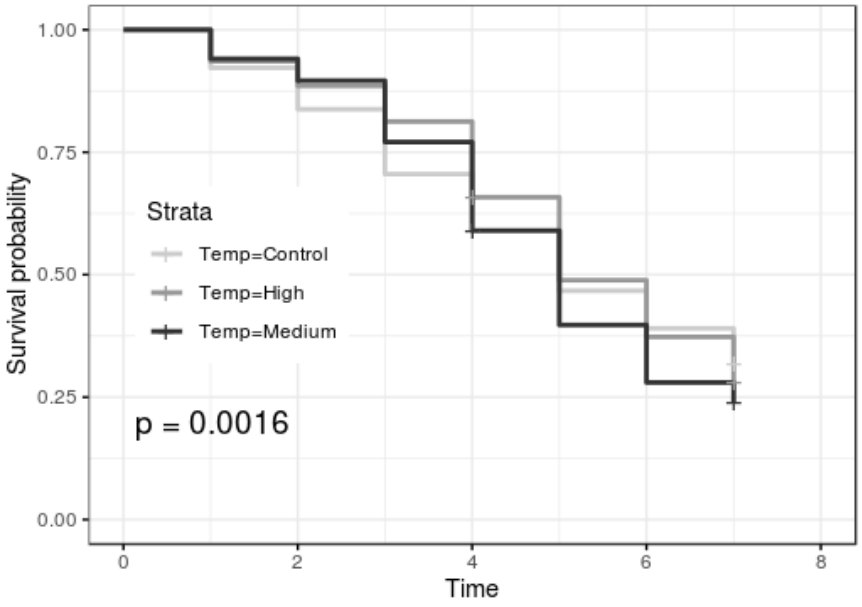

Figure 4.2. Effect of temperature on larvae survival at 8.1 pH. Over 7 days, survival was reduced across all temperatures, with medium temperature of 26º C most strongly affecting survival.

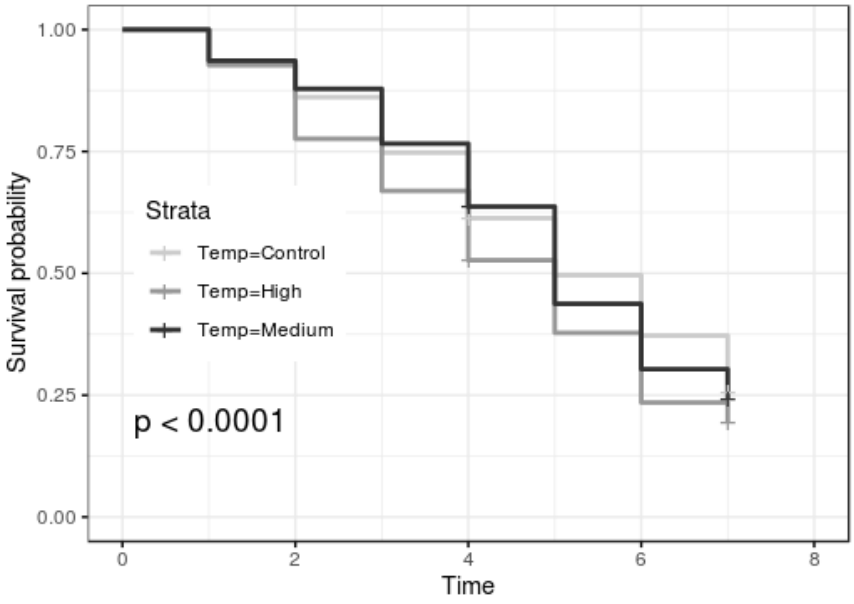

Figure 4.3. Effect of temperature on larvae survival at 7.8 pH. Over 7 days, survival was reduced across all temperatures, with high temperature of 28º C most strongly affecting survival.

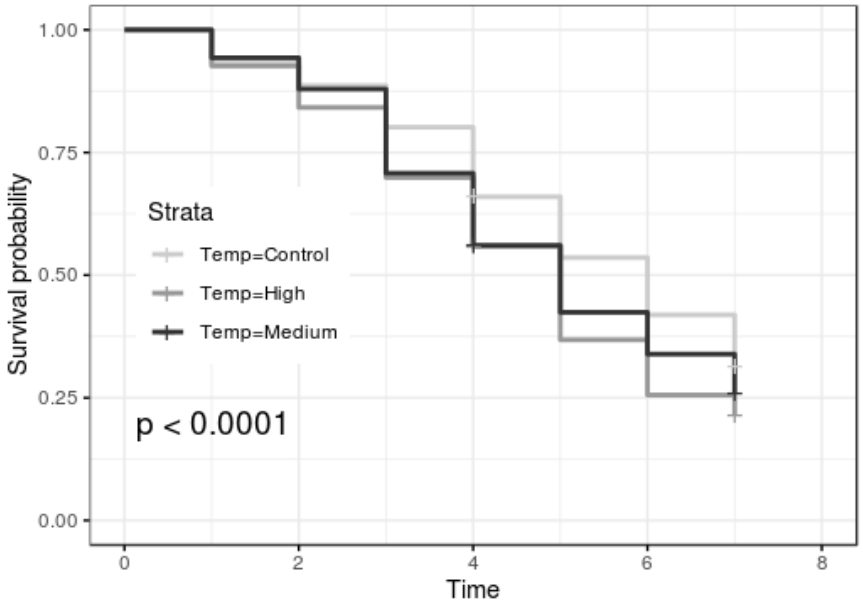

Figure 4.4. Effect of temperature on larvae survival at 7.4 pH. Over 7 days, survival was reduced across all temperatures, with high temperature of 28º C most strongly affecting survival.

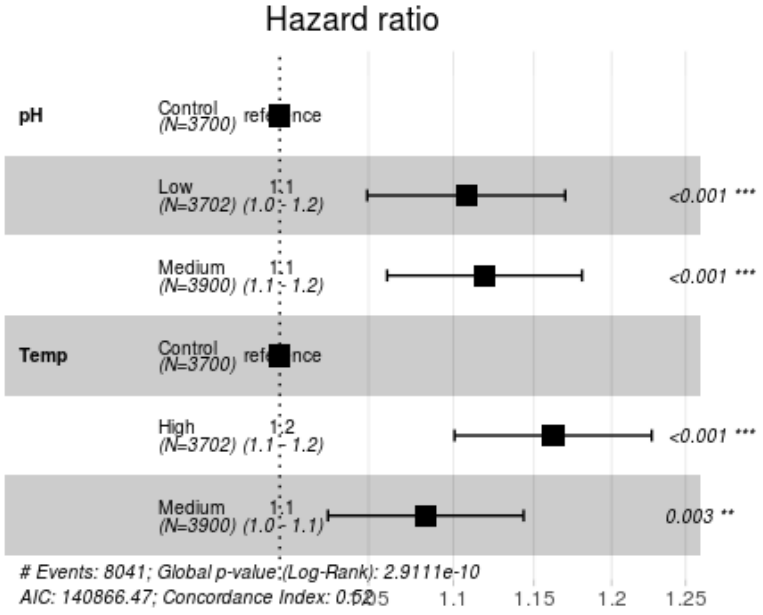

Figure 4.5. Effect of pH and temperature on larvae survival. Both temperature and pH significantly affected survival. Data are mean values ± standard error.

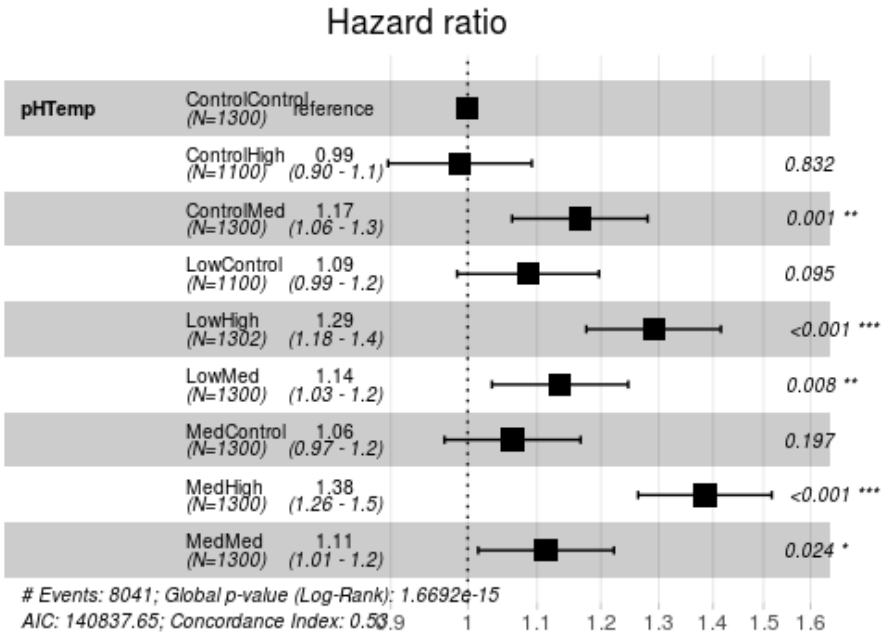

Figure 4.6. Effect of combined pH and temperature on larvae survival. Mortality rates significantly differed from control pH and temperature in the following treatments: ControlMed, LowHigh, LowMed, MedHigh, and MedMed. Data are mean values ± standard error.

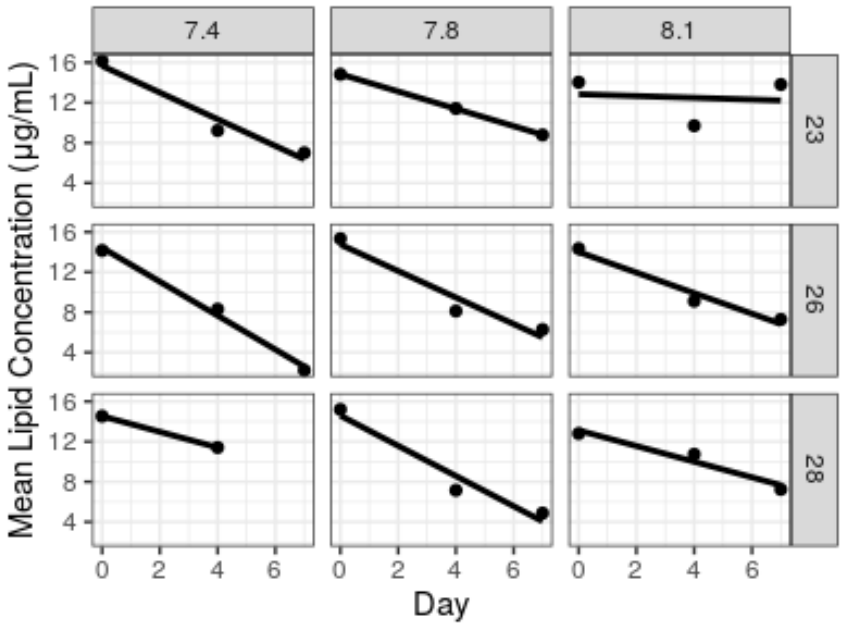

Figure 4.7. Effect of pH and temperature on total lipids. Mean concentration (µg/mL) remained steady or declined over a period of seven days.

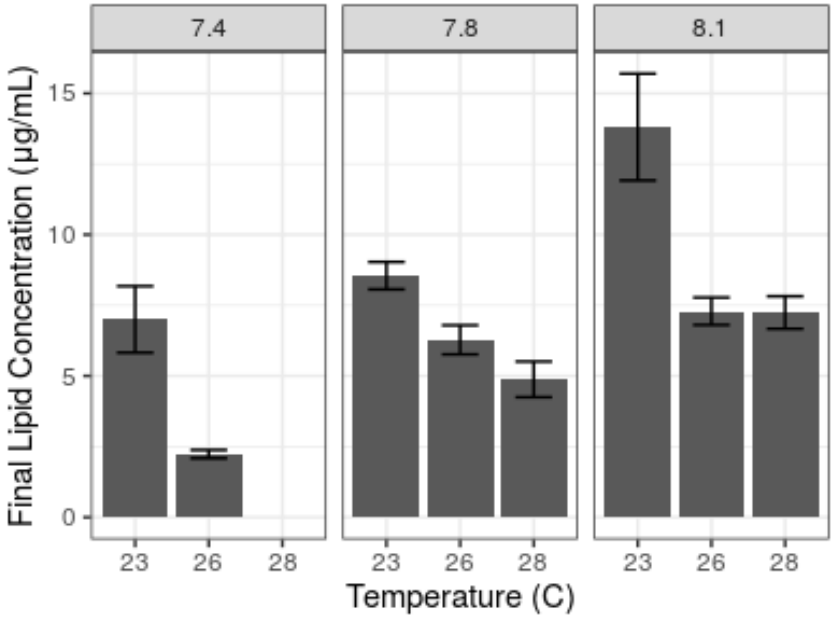

Figure 4.8. Effect of pH and temperature on final total lipids. Increasing acidity and temperature reduced mean lipid content (µg/mL). Data are mean values ± standard error.

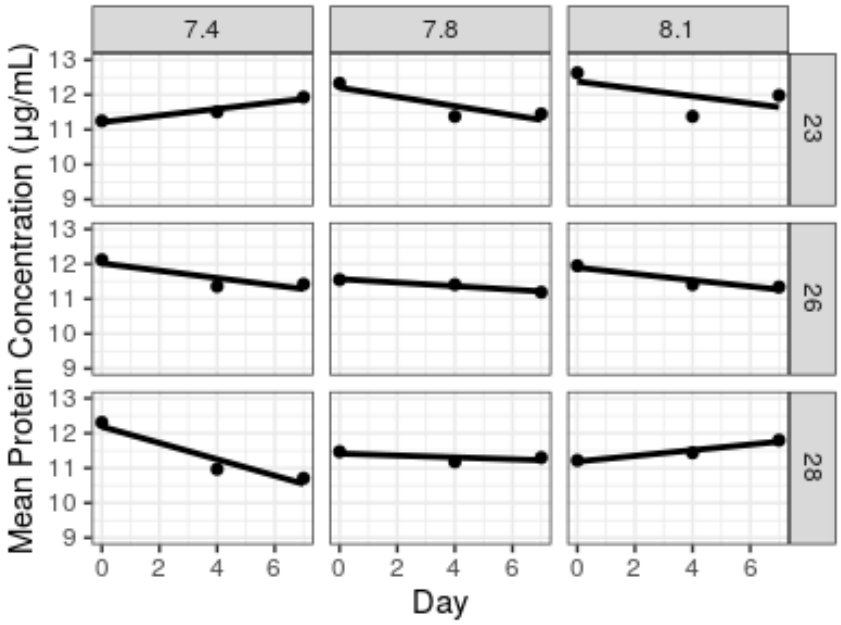

Figure 4.9. Effect of pH and temperature on total protein. Protein content (µg/mL) remained fairly constant or slightly diminished over seven days. At highest acidity and temperature, protein steeply declined.

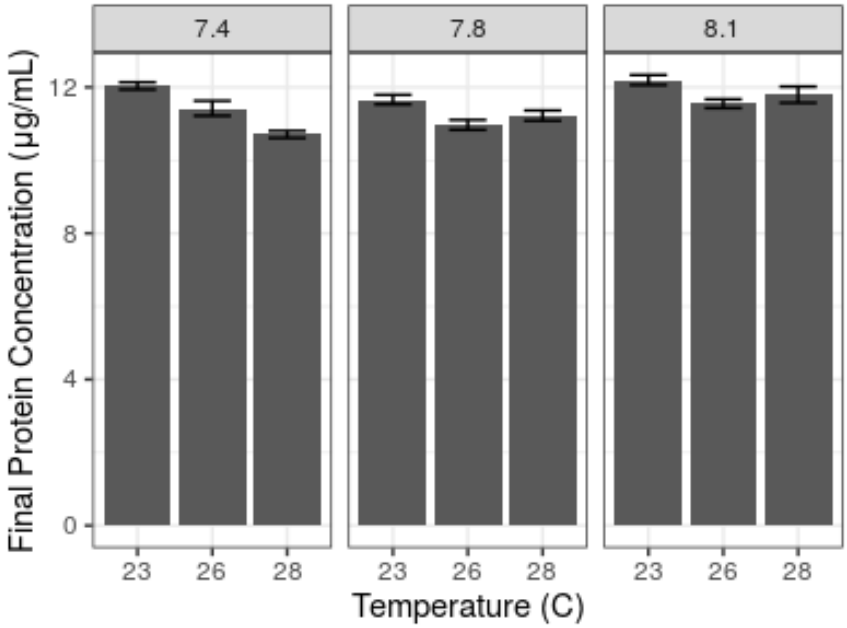

Figure 4.10. Effect of pH and temperature on final total protein. With increased acidity, protein content (µg/mL) declined. With increased temperature, protein was diminished, with protein at medium temperature the most reduced at medium and control pH. Data are mean values ± standard error.

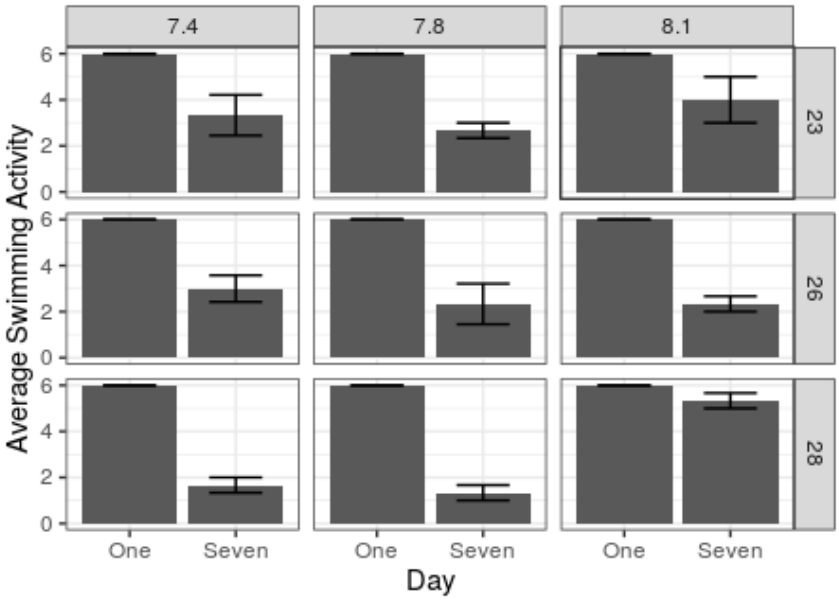

Figure 4.11. Effect of pH and temperature on swimming activity. Over a period of 7 days, swimming activity diminished across all tanks. Data are mean values ± standard error.

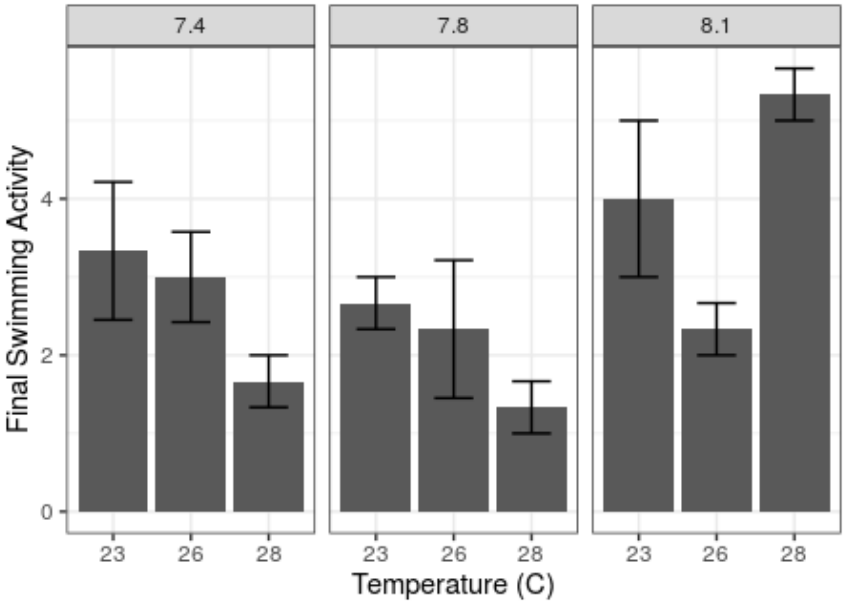

Figure 4.12. Effect of pH and temperature on final swimming activity. Mean swimming activity was reduced with temperature and acidity, except at control pH and high temperature. Data are mean values ± standard error.

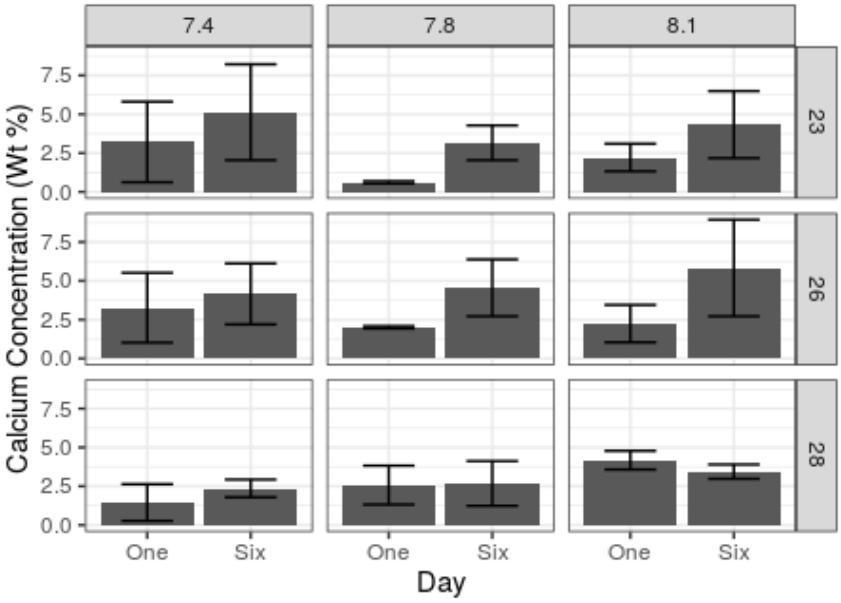

Figure 4.13. Effect of pH and temperature on carapace calcium content. Over a period of six days, calcium concentration (wt %) increases with increasing acidity and temperature. Calcium concentration only diminished at the highest temperature. Data are mean values ± standard error.

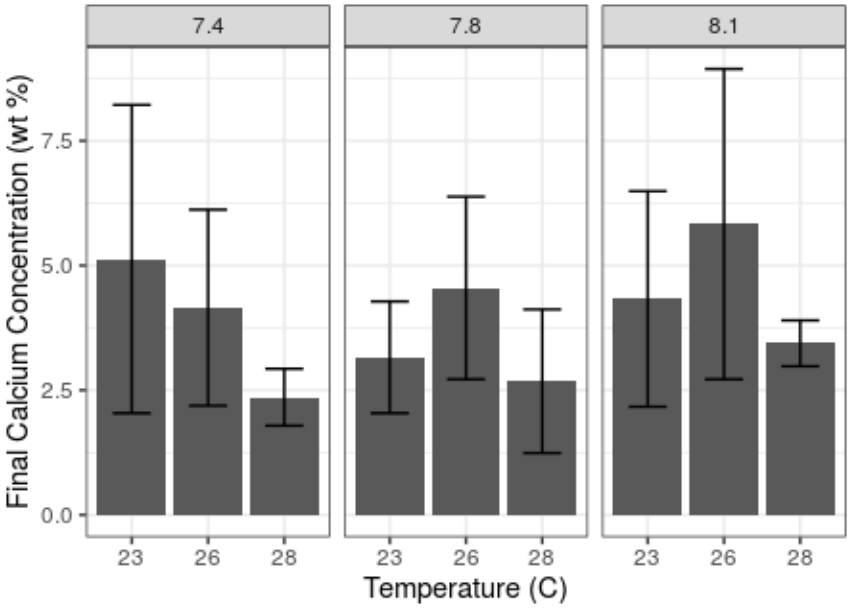

Figure 4.14. Effect of pH and temperature on final carapace calcium content. Temperature alone may have impacted calcium content (wt %) more than pH alone. Data are mean values ± standard error.

Table 4.1. Water chemistry. Temperature, salinity, and pH were measured twice daily in experimental tanks. Total Alkalinity (A_T) was measured at the start of the experiment. Bicarbonate, carbonate, and dissolved inorganic carbon (DIC) were calculated. Data are mean values ± standard error.

Parameter (Acidity-Temp)	**High-Control**	**High-Medium**	**High-High**	**Med-Control**	**Med-Medium**	**Med-High**	**Control-Control**	**Control-Medium**	**Control-High**
Temperature	23.3 ± 0.10	26.1 ± 0.06	27.7 ± 0.16	23.8 ± 0.13	26.4 ± 0.09	28.9 ± 0.19	23.7 ± 0.13	26.5 ± 0.18	27.9 ± 0.22
Salinity	31 ± 0.36	31 ± 0.36	31 ± 0.36	31 ± 0.36	31 ± 0.36	31 ± 0.36	31 ± 0.36	31 ± 0.36	31 ± 0.36
A_T (µmol kg^{-1})	1665 ± 1.69	1666 ± 0.99	1667 ± 1.38	1662 ± 1.50	1662 ± 1.47	1662 ± 1.69	1669 ± 2.22	1662 ± 0.39	1665 ± 1.07
pH	7.44 ± 0.02	7.36 ± 0.05	7.41 ± 0.02	7.81 ± 0.01	7.80 ± 0.02	7.74 ± 0.02	8.06 ± 0.01	8.05 ± 0.01	8.01 ± 0.01
$[HCO_3^-]$ (µmol kg^{-1})	1580 ± 3.97	1583 ± 3.23	1574 ± 3.84	1478 ± 4.83	1466 ± 5.48	1472 ± 5.68	1370 ± 6.18	1350 ± 4.98	1358 ± 5.78
$[CO_3^{2-}]$ (µmol kg^{-1})	43 ± 1.73	42 ± 1.60	47 ± 1.85	92 ± 2.64	98 ± 3.06	95 ± 2.97	149 ± 3.54	156 ± 2.34	154 ± 3.04
DIC	1666 ± 3.96	1675 ± 7.39	1663 ± 3.70	1588 ± 2.83	1581 ± 3.15	1586 ± 3.63	1529 ± 3.26	1515 ± 2.82	1521 ± 3.05

Table 4.2. Total lipid ANOVA and Tukey HSD results for *C. sapidus.* Significant results are in bold, and only significant Tukey HSD output has been included. C=Control, L=Low, M=Medium.

Total Lipid					
2-Way ANOVA	**d.f.**	**SS**	**MS**	***F***	***P***
Temperature	**2**	**0.53**	**0.26**	**17.896**	**≤ 0.005**
pH	**2**	**0.69**	**0.34**	**23.359**	**≤ 0.005**
Temperature:pH	3	0.11	0.04	2.456	0.088
Tukey HSD					
Temp:pH	**Diff**	**Lwr**	**Upper**		***P***
C:L-C:C	**-0.307**	**-0.544**	**-0.069**		**0.005**
M:L-C:C	**-0.770**	**-1.061**	**-0.479**		**≤ 0.001**
H:M-C:C	**-0.440**	**-0.731**	**-0.149**		**≤ 0.001**
M:M-C:C	**-0.327**	**-0.618**	**-0.036**		**0.020**
M:L-H:C	**-0.503**	**-0.840**	**-0.167**		**≤ 0.001**
M:L-M:C	**-0.510**	**-0.846**	**-0.174**		**≤ 0.001**
M:L-C:L	**-0.463**	**-0.754**	**-0.172**		**≤ 0.001**
C:M-M:L	**0.578**	**0.277**	**0.879**		**≤ 0.001**
M:M-M:L	**0.443**	**0.107**	**0.780**		**0.004**

Table 4.3. Total protein ANOVA and Tukey HSD results for *C. sapidus.* Significant results are in bold, and only significant Tukey HSD output has been included. C=Control, L=Low, M=Medium.

Total Protein					
2-Way ANOVA	**d.f.**	**SS**	**MS**	***F***	***P***
Temperature	**2**	**11.41**	**5.71**	**15.099**	**≤ 0.005**
pH	**2**	**8.30**	**4.15**	**10.978**	**≤ 0.005**
Temperature:pH	**4**	2.10	0.52	1.388	0.242
Tukey HSD					
Temp:pH	**Diff**	**Lwr**	**Upper**		***P***
H:L-C:C	**-1.491**	**-2.689**	**-0.293**		**0.004**
M:L-C:C	**-0.774**	**-1.548**	**-0.0005**		**0.050**
H:M-C:C	**-0.973**	**-1.630**	**-0.317**		**≤ 0.001**
M:M-C:C	**-1.228**	**-1.885**	**-0.572**		**≤ 0.001**
M:M-H:C	**-0.826**	**-1.505**	**-0.148**		**0.006**
H:L-C:L	**-1.330**	**-2.557**	**-0.102**		**0.023**
H:M-C:L	**-0.812**	**-1.520**	**-0.103**		**0.013**
M:M-C:L	**-1.067**	**-1.776**	**-0.358**		**≤ 0.001**
M:M-C:M	**-0.693**	**-1.350**	**-0.037**		**0.030**

Table 4.4. ANOVA, MANOVA and Tukey HSD results for swimming activity, morphology, and calcium content in *C. sapidus.* Significant results are in bold, and only significant Tukey HSD output has been included.

Swimming Activity					
2-Way ANOVA	**d.f.**	**SS**	**MS**	***F***	***P***
Temperature	2	0.12	0.06	2.010	0.163
pH	**2**	**0.36**	**0.18**	**6.081**	**0.010**
Temperature:pH	**4**	**0.39**	**0.10**	**3.312**	**0.034**
Tukey HSD: pH	**Diff**	**Lwr**	**Upper**		***P***
Medium-Control	**-0.290**	**-0.539**	**-0.041**		**0.02**
Morphology					
2-Way MANOVA	**d.f.**	**Pillai**		***Approx. F***	***P***
Temperature	2	0.02		1.038	0.443
pH	2	0.08		1.104	0.387
Temperature:pH	4	0.12		1.216	0.239
Calcium Content					
2-Way ANOVA	**d.f.**	**SS**	**MS**	***F***	***P***
Temperature	2	0.14	0.07	0.771	0.491
pH	2	0.05	0.23	0.250	0.784
Temperature:pH	4	0.03	0.01	0.091	0.983

Literature Cited

Bates, D., M. Maechler, B. Bolker, S. Walker, R. H. B. Christensen, H. Singmann, B. Dai, F. Scheipl, G. Grothendieck, P. Green and J. Fox. 2020. lme4: Linear Mixed-Effects Models using 'Eigen' and S4. R package version 1.1-23. Available at: https://cran.r-project.org/web/packages/lme4/index.html

Branch, T. A., B. M. DeJoseph, L. J. Ray, & C. A. Wagner. 2013. Impacts of ocean acidification on marine seafood. *Trends in Ecology and Evolution,* 28:178-186. doi: 10.1016/j.tree.2012.10.001

Byrne, M. 2011. Impact of ocean warming and ocean acidification on marine invertebrate life history stages: vulnerabilities and potential persistence in a changing ocean. In: Gibson, R. N., R. J. A. Atkinson, & J. D. M. Gordon, editors. *Oceanog. Marine Biol.: Ann. Rev. (SAUS) Vol. 49*, Boca Raton, FL: CRC Press, pp. 1-42.

Cai, W.-J., W.-J. Huang, G. W. Luther III, D. Pierrot, M. Li, J. Testa, M. Xue, A. Joesoef, R. Mann, J. Brodeur, Y.-Y. Xu, B. Chen, N. Hussain, G. G. Waldbusser, J. Cornwall, & W. M. Kamp. 2017. Redox reactions and weak buffering capacity lead to acidification in the Chesapeake Bay. *Nature Communications*, 8:369. doi: 10.1038/s41467-017-00417-7

Carter, H. A., L. Ceballos-Osuna, N. A. Miller, and J. H. Stillman. 2013. Impact of ocean acidification on metabolism and energetics during early life stages of the intertidal porcelain crab *Petrolisthes cinctipes. Journal of Experimental Biology,* 216:1412-1422. doi: 10.1242/jeb.078162

Costlow, J. D. & C. G. Bookhout. 1959. The larval development of *Callinectes sapidus* rathbun reared in the laboratory. *Biological Bulletin,* 116:373-396.

Dickson, A. G. 1990. Standard potential of the reaction: AgCI(s) + 1/2H2(g) = Ag(s) + HCI(aq), and the standard acidity constant of the ion HSO4 in synthetic sea water from 273.15 to 318.15 K. *Journal of Chemical Thermodynamics,* 22:113-127.

Doney, S. C., L. Bopp, & M. C. Long. 2014. Historical and future trends in ocean climate and biogeochemistry. *Oceanography,* 27:108-119. doi: 10.5670/oceanog.2014.14

Dorey, N., F. Melzner, S. Martin, F. Oberhänsli, J. L. Teyssié, P. Bustamante, J.-P. Gattuso, & T. Lacoue-Labarthe. 2013. Ocean acidification and temperature rise: effects on calcification during early development of the cuttlefish *Sepia officinalis. Marine Biology,* 160:2007–2022. doi: 10.1007/s00227-012-2059-6

Du, J., J. Shen, K. Park, Y. P. Wang, & X. Yu. 2018. Worsened physical condition due to climate change contributes to the increasing hypoxia in Chesapeake Bay. *Science of the Total Environment,* 630:707-717. doi: 10.1016/j.scitotenv.2018.02.265

Feely, R. A., C. L. Sabine, K. Lee, W. L. Berelson, J. Kleypas, V. J. Fabry, & F. J. Millero. 2004. Impact of anthropogenic CO_2 on the $CaCO_3$ system in the oceans, *Science*, 305:362-366. doi: 10.1126/science.1097329

Fox, J., S. Weisburg, and B. Price. 2019. car: Companion to Applied Regression. R package version 3.0-5. Available at: https://cran.r-project.org/web/packages/car/index.html

Frederich, M. & H. O. Pörtner. 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.

Gattuso, J.-P., J.-M. Epitalon, H. Lavigne, and J. Orr. 2018. seacarb: Seawater Carbonate Chemistry. R

package version 3.2.12. Available at: https://cran.r-project.org/web/packages/seacarb/index.html

Gillooly, J. F., E. L. Charnov, G. B. West, V. M. Savage, & J. H. Brown. 2002. Effects of size and temperature on development time. *Nature*, 417:70–73. doi: 10.1126/science.1061967

Glandon, H. L., K. H. Kilbourne & T. J. Miller. 2019. Winter is (not) coming: Warming temperatures will affect the overwinter behavior and survival of blue crab. *PLoS ONE*, 14:e0219555. doi: 10.1371/journal.pone.0219555

Hand, D. J. & C. C. Taylor. 1987. *Multivariate Analysis of Variance and Repeated Measures.* Chapman and Hall, London, UK.

Harland, H., C. D. MacLeod, & R. Poulin. 2015. Non-linear effects of ocean acidification on the transmission of a marine intertidal parasite. *Marine Ecology Progress Series*, 536:55–64. doi: 10.3354/meps11416

Hernroth, B., H. N. Sköld, K. Wiklander, F. Jutfelt, & S. Baden. 2012. Simulated climate change causes immune suppression and protein damage in the crustacean *Nephrops norvegicus. Fish and Shellfish Immunology*, 33:1095–1101. doi: 10.1016/j.fsi.2012.08.011

Hines, A. H., E. G. Johnson, M. Z. Darnell, D. Rittschof, T. J. Miller, L. J. Bauer, P. Rodgers, and R. Aguilar. 2010. Predicting Effects of Climate Change on Blue Crabs in Chesapeake Bay. In: G. H. Kruse, G. L. Eckert, R. J. Foy, R. N. Lipcius, B. Sainte-Marie, D. L. Stram, and D. Woodby (eds.), Biology and Management of Exploited Crab Populations under Climate Change. Alaska Sea Grant, University of Alaska Fairbanks. doi:10.4027/bmecpcc.2010.22

Hoegh-Guldberg, O., P. J. Mumby, A. J. Hooten, R. S. Steneck, P. Greenfield, E. Gomez, C. D. Harvell, P. F. Sale, A. J. Edwards, K. Caldeira, N. Knowlton, C. M. Eakin, R. Iglesias-Prieto, N. Muthiga, R. H. Bradbury, A. Dubi, & M. E. Hatziolos. 2007. Coral Reefs under rapid climate change and ocean acidification. *Science*, 318:1737–1742. doi: 10.1126/science.1152509

Hope, R. M. 2013. Rmisc: Rmisc: Ryan Miscellaneous. R package version 1.5. Available at: https://cran.r-project.org/web/packages/Rmisc/index.html.

Horton, N. J., & K. Kleinman. 2011. *Using R for Data Management, Statistical Analysis, and Graphics.* CRC Press, Taylor & Francis Group.

Huchin-Mian, J. P., H. J. Small, & J. D. Shields. 2018. The influence of temperature and salinity on mortality of recently recruited blue crabs, Callinectes sapidus, naturally infected with *Hematodinium perezi* (Dinoflagellata). *Journal of Invertebrate Pathology*, 152:8–16. doi: 10.1016/j.jip.2018.01.003

Intergovernmental Panel on Climate Change. 2013. Fifth Assessment Report: Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Ivanina, A. V., G. H. Dickinson, O. B. Matoo, R. Bagwe, A. Dickinson, E. Beniash, & I. M. Sokolova. 2013. Interactive effects of elevated temperature and CO_2 levels on energy metabolism and biomineralization of marine bivalves *Crassostrea virginica* and *Mercenaria mercenaria. Comparative Biochemistry and Physiology Part A*, 166:101-111. doi: 10.1016/j.cbpa.2013.05.016

Johnson, K. D., J. H. Grabowski, & D. L. Smee. 2014. Omnivory dampens trophic cascades in estuarine communities. *Marine Ecology Progress Series*, 507:197-206. doi: 10.3354/meps10815

Lavigne, H. & J.-P. Gattuso. 2018. seacarb: Seawater Carbonate Chemistry. R package version 2.4.3.

Available at: https://CRAN.R-project.org/package=seacarb.

Lewis, L. & J. Ayers. 2014. Temperature preference and acclimation in the Jonah crab, *Cancer borealis*. *Journal of Experimental Marine Biology and Ecology*, 455:7-13. doi: 10.1016/j.jembe.2014.02.013

Long, W. C., K. M. Swiney, & R. J. Foy. 2013a. Effects of ocean acidification on the embryos and larvae of red king crab, *Paralithodes camtschaticus*. *Marine Pollution Bulletin,* 69:38-47. doi: 10.1016/j.marpolbul.2013.01.011

Long, W. C., K. M. Swiney, C. Harris, H. N. Page, & R. J. Foy. 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:e6095. doi: 10.1371/journal.pone.0060959

Long, W. C., S. B. Van Sant, K. M. Swiney, & R. J. Foy. 2017. Survival, growth, and morphology of blue king crabs: effect of ocean acidification decreases with exposure time. *ICES Journal of Marine Science,* 74:1033-1041. doi: 10.1093/icesjms/fsw197

Lueker, T. J., A. G. Dickson & C. D. Keeling. 2000. Ocean pCO_2 calculated from dissolved inorganic carbon, alkalinity, and equations for K1 and K2: validation based on laboratory measurements of CO_2 in gas and seawater at equilibrium. *Marine Chemistry,* 70:105-119.

Mackenzie, C. L., S. A. Lynch, S. C. Culloty, & S. K. Malham. 2014. Future oceanic warming and acidification alter immune response and disease status in a commercial shellfish species, *Mytilus edulis L. PLoS ONE*, 9:e99712. doi: 10.1371/journal.pone.0099712

Macko, S. A., C. M. Fantasia, & G. Xue. 2017. Potential global economic impacts of ocean acidification. Center for Oceans Law & Policy: International Marine Economy. Eds. M.H. Nordquist, J.N. Moore, & R. Long. 20:79-97. doi: 10.1163/9789004323445_003

Macko, S. A., & C. M. Fantasia. 2018. An overview of ocean acidification: relationships. Center for Oceans Law & Policy: The Marine Environment and United Nations Sustainable Development Goal 14, Life Below Water. Eds. M.H. Nordquist, J.N. Moore, & R. Long. 21:391-417. doi: 10.1163/9789004366619_025

Matoo, O. B., A. V. Ivanina, C. Ullstad, E. Beniash, & I. M. Soklova. 2013. Interactive effects of elevated temperature and CO_2 levels on metabolism and oxidative stress in two common marine bivalves (*Crassostrea virginica* and *Mercenaria mercenaria*). *Comparative Biochemistry and Physiology- Part A: Molecular and Integrative Physiology,* 164:545–553. doi: 10.1016/j.cbpa.2012.12.025

Metzger, R., F. J. Sartoris, M. Langenbuch, & H. O. Pörtner. 2007. Influence of elevated CO_2 concentrations on thermal tolerance of the edible crab *Cancer pagurus. Journal of Thermal Biology,* 32:144–151. doi: 10.1016/j.jtherbio.2007.01.010

Miller, T. J., S. J. D. Martell, D. B. Bunnell, G. Davis, L. Fegley, A. Sharov, C. Bonzek, D. Hewitt, J. Hoenig, & R. N. Lipcius. 2005. Stock assessment of the blue crab in Chesapeake Bay. University of Maryland Center for Environmental Science, Technical Report Series TS-487-05, Solomons. Available: hjort.cbl.umces.edu/crabs/Assessment05.html.

Miller, J. J., M. Maher, E. Bohaboy, C. S. Friedman, & P. McElhany. 2016. Exposure to low pH reduces survival and delays development in early life stages of Dungeness crab (*Cancer magister*). *Marine Biol*ogy, 163:118. doi: 10.1007/s00227-016-2883-1

Najjar, R. G., C. R. Pyke, M. B. Adams, D. Breitburg, C. Hershner, M. Kemp, R. Howarth, M. R. Mulholland, M. Paolisso, D. Secor, K. Sellner, D. Wardrop, & R. Wood. 2010. Potential climate-change

impacts on the Chesapeake Bay. *Estuarine, Coastal and Shelf Science*, 86:1-20. doi: 10.1016/j.ecss.2009.09.026

Niemisto, Maura K. 2019. "Response of Early Life Stage Homarus americanus to Ocean Warming and Acidification: an Interpopulation Comparison". *Electronic Theses and Dissertations*. 3076. https://digitalcommons.library.umaine.edu/etd/3076

Perez, F. F. & F. Fraga. 1987. Association constant of fluoride and hydrogen ions in seawater. *Marine Chemistry,* 21:161-168.

Pörtner, H. O. 2001. Climate change and temperature dependent biogeography: oxygen limitation of thermal tolerance in animals. *Naturwissenschaften,* 88:137–146. doi: 10.1007/s001140100216

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 and Integrative Physiology*, 132:739–761. doi: 10.1016/S1095-6433(02)00045-4

Pörtner, H. O. 2004. Climate variability and the energetic pathways of evolution: the origin of endothermy in mammals and birds. *Physiological and Biochemical Zoology*, 77:959–981. doi: 10.1086/423742

Pörtner, H. O. 2008. Ecosystem effects of ocean acidification in times of ocean warming: a physiologist's view. *Marine Ecology Progress Series*, 373:203-217. doi: 10.3354/meps07768

Pörtner, H. O., & R. Knust. 2007. Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *Science*, 315:95–97. doi: 10.1126/science.1135471

Pörtner, H. O., & A. P. Farrell. 2008. Physiology and climate change. *Science*, 322:690–692. doi: 10.1126/science.ll63156

Pörtner, H. O., M. Langenbuch, & A. Reipschläger. 2005. Biological impact of elevated ocean CO_2 concentrations: lessons from animal physiology and earth history? *Journal of Oceanography*, 60:705–718. doi: 10.1007/s10872-004-5763-0

Pörtner, H.-O., D. M. Karl, P. W. Boyd, W. W. L. Cheung, S. E. Lluch-Cota, Y. Nojiri, D. N. Schmidt, and P. O. Zavialov, 2014: Ocean systems. In: Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Field, C. B., V. R. Barros, D. J.
Prendini, L., R. Hanner, R. Desalle, and P. Fitzpatrick. 2002. Obtaining, Storing and Archiving Specimens and Tissue Samples for Use in Molecular Studies. Pp. 176-248 in *Techniques in Molecular Evolution and Systematics,* R. DeSalle, G. Giribet, and W. Wheeler, eds. Birkhäuser, Basel.

Preston, B. L. 2004. Observed winter warming of the Chesapeake Bay estuary (1949-2002): implications for ecosystem management. *Environmental Management,* 34:125-139. doi: 10.1007/s00267-004-0159-x

Qadri, S. A., J. Camacho, H. Wang, J. R. Taylor, M. Grosell, & M. K. Worden. 2007. Temperature and acid–base balance in the American lobster *Homarus americanus*. *Journal of Experimental Biology*, 210:1245–1254. doi: 10.1242/jeb.02709

Qian, S.S. 2010. *Environmental and Ecological Statistics with R.* CRC Press, Taylor and Francis Group.

R Development Core Team. 2011. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: http://www.R-project.org.

Reynaud, S., N. Leclercq, S. Romaine-Lioud, C. Ferrier-Pagès, J. Jaubert, & J. P. Gattuso. 2003. Interacting effects of CO_2 partial pressure and temperature on photosynthesis and calcification in a scleractinian coral. *Global Change Biology,* 9:1660–1668. doi: 10.1046/j.1529-8817.2003.00678.x

Sabine, C. L., R. A. Feely, N. Gruber, R. M. Key, K. Lee, J. L. Bullister, R. Wanninkhof, C. S. Wong, D. W. R. Wallace, B. Tilbrook, F. J. Millero, T.-H. Peng, A. Kozyr, T. Ono, & A. F. Rios. 2004. The oceanic sink for anthropogenic CO_2. *Science,* 305:367-371. doi: 10.1126/science.1097403

Shields, J. D. 2019. Climate change enhances disease processes in crustaceans: case studies in lobsters, crabs, and shrimps. *Journal of Crustacean Biology*, 39:673-683. doi: 10.1093/jcbiol/ruz072

Spicer, J. I., A. Raffo, & S. Widdicombe. 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. doi: 10.1007/s00227-006-0551-6

Stover, K. K., K. G. Burnett, E. J. McElroy, & L. E. Burnett. 2013. Locomotory fatigue during moderate and severe hypoxia and hypercapnia in the Atlantic blue crab, *Callinectes sapidus*. *Biological Bulletin,* 224:68-78. doi: 10.1086/BBLv224n2p63

Styf, H. K., H. N. Skold, & S. P. Eriksson. 2013. Embryonic response to long-term exposure of the marine crustacean *Nephrops norvegicus* to ocean acidification and elevated temperature. *Ecology and Evolution,* 3:5055-5065. doi: 10.1002/ece3.860

Sulkin, S. D. & C. E. Epifanio. 1975. Comparison of rotifers and other diets for rearing early larvae of the blue crab, *Callinectes sapidus Rathbun. Estuarine and Coastal Marine Science*, 3:109-113.

Sulkin, S. D. 1978. Nutritional requirements during larval development of the portunid crab, *Callinectes sapidus Rathbun. Journal of Experimental Marine Biology and Ecology,* 34:29-41.

Therneau, T. 2019. Survival: survival analysis, including penalized likelihood. R package version 2.44-1.0. Available at: http://CRAN.R-project.org/package=survival.

Tomasetti, S. J., B. K. Morrell, L. R. Merlo, and C. J. Gobler. 2018. Individual and combined effects of low dissolved oxygen and low pH on survival of early life stage blue crabs, *Callinectes sapidus. PLoS ONE,* 13:e0208629. doi: 10.1371/journal.pone.0208629

Waldbusser, G. G., E. P. Voigt, H. Bergschneider, M. A. Green, & R. I. E. Newell. 2011. Biocalcification in the Eastern oyster (*Crassostrea virginica*) in relation to long-term trends in Chesapeake Bay pH. *Estuaries and Coasts,* 34:221-231. doi: 10.1007/s12237-010-9307-0

Wallace, R. B., H. Baumann, J. S. Grear, R. C. Aller, & C. J. Gobler. 2014. Coastal ocean acidification: The other eutrophication problem. *Estuarine, Coastal and Shelf Science,* 148:1-13. doi: 10.1016/j.ecss.2014.05.027

Walther, K., F. J. Sartoris, C. Bock, & H. O. Pörtner. 2009. Impact of anthropogenic ocean acidification on thermal tolerance of the spider crab *Hyas araneus. Biogeosciences,* 6:2837–2861. doi: 10.5194/bg-6-2207-2009

Walther, K., K. Anger, & H.O. Pörtner. 2010. Effects of ocean acidification and warming on the larval development of the spider crab *Hyas araneus* from different latitudes (54° vs. 79° N). *Marine Ecology Progress Series*, 417:159-170. doi: 10.3354/meps08807

Whiteley, N. M. 2011. Physiological and ecological responses of crustaceans to ocean acidification. *Marine Ecology Progress Series*, 430:257-271. doi: 10.3354/meps09185

Wickham, H. 2020. ggplot2: Create Elegant Data Visualisations Using the Grammar of Graphics. R package version 3.3.2. Available at: https://cran.r-project.org/web/packages/ggplot2/index.html.

Webster, R. & M. A. Oliver. 2007. Geostatistics for Environmental Scientists. John Wiley & Sons, Hoboken, New Jersey, USA. https://doi.org/10.1002/9780470517277

Wood, R. J., D. F. Boesch, & V. S. Kennedy. 2002. Future consequences of climate change for the Chesapeake Bay ecosystem and fisheries. *American Fisheries Society Symposium*, 32:171-184.

Zmora, O., A. Findeisen, J. Stubblefield, V. Frenkel, & Y. Zohar. 2005. Large-scale juvenile production of the blue crab *Callinectes sapidus*. *Aquaculture*, **244**:129-139. doi: 10.1016/j.aquaculture.2004.11.012

Zohar, Y., A. H. Hines, O. Zmora, E. G. Johnson, R. N. Lipcius, R. D. Seitz, D. B. Eggleston, A. R. Place, E. J. Schott, J. D. Stubblefield, & J. S. Chung. 2008. The Chesapeake Bay blue crab (*Callinectes sapidus*): a multidisciplinary approach to responsible stock replenishment. *Reviews in Fisheries Science*, 16:24-34. doi: 10.1080/10641260701681623

Chapter Five:

Conclusion

The primary effects of increasing acidity on blue crabs were slightly delayed hatching times in embryos, with significantly diminished survival and loss of lipids in stage 1 larvae. Combining increased temperatures with acidification in larvae exacerbated these effects, with further decreases in survival, lipids, protein, and swimming activity. These outcomes were observed within the first seven days and, if similar to other crab species, are likely to worsen with molt progression (Long *et al.,* 2013; Miller *et al.,* 2016). Two of the acidic conditions larvae were exposed to (pH 7.8, 7.4) represented worst case scenarios predicted in the open ocean by years 2100 and 2300, respectively (IPCC, 2013), although coastal systems are expected to worsen at a faster rate (Cai *et al.,* 2017). My final acidic condition (pH 6.8) was not representative of any expected surface ocean conditions, and rather served as a baseline for survival and physiological responses. However, long-term acidity trends in the Chesapeake evidence female crabs are currently exposed to hypercapnic events under pH 7.5 in bottom waters which continue to increase in duration (Shen *et al.,* 2020). Further studies should therefore expose embryos to these bursts of extreme acidity, as well as determine how oocytes are affected in females.

As larvae are progressively exposed to increasingly acidic coastal waters, it is possible adaptation will occur. I observed varied brood responses that may result in better adapted blue crabs by the year 2100. Similar observations have been reported in other decapods (Long *et al.,* 2017), whereas certain geographically distinct populations within

a species have varied responses (Walther *et al.,* 2010; Niemisto, 2019). Blue crabs in the Atlantic are known to contain high genetic diversity (Feng *et al.,* 2017) and are thus well situated to adapt to changing conditions.

Although this potentially offers good news, we must better understand how adaptation could occur. Alternatively, if further research suggests adaptation is not occurring at a rapid enough rate, reduced blue crab populations could have broad reaching effects across the Chesapeake Bay. As omnivorous predators, blue crabs currently control populations of several species at differing trophic levels, including small bivalves (Arnold, 1984), smaller crustaceans (Hill & Weissburg, 2013), and small fishes (Kneib, 1982). Isotope analysis suggests great flexibility in prey consumption that shifts with habitat constraints and prey availability (Mancinelli *et al.*, 2017). This omnivory helps to reduce potential trophic cascades in estuaries, provides some buffering effect to environmental shocks (Johnson *et al.,* 2014), and may control populations of invasive crustaceans (DeRivera *et al.,* 2005). However, smaller populations of blue crabs would reduce this service and accentuate trophic cascades, which are currently observed in the Chesapeake as a result of eutrophication, invasive species, habitat loss, and climate change effects (Kemp *et al.,* 2005). Additionally, various life stages of blue crabs are prey for fishes, birds, marine mammals, and cephalopods (Boudreau & Worm, 2012). Blue crabs have been identified as a keystone species specifically in salt marshes, where they exert control over marsh grass cover through periwinkle predation. Overfishing of blue crabs has been postulated as the mechanism for major salt marsh die-offs (Silliman & Bertness, 2002). In experiments with other benthic decapod crustaceans, their removal

consistently resulted in increased infaunal and epifaunal densities, changes in species composition, and sometimes cascading effects (Boudreau & Worm, 2012).

With the loss of ground fisheries, invertebrate fisheries have gained commercial importance (Choi & Zisserson, 2008). In the Chesapeake Bay, blue crab harvests are the largest single source in the US (Miller *et al.*, 2005). The turn of the century brought with it an overburdened fishery in degrading habitat with record low production and an 80% drop in spawning stock (Lipcius & Stockhausen, 2002). These drastic declines precipitated recovery efforts that have successfully and remarkably brought it to current healthy population levels (Seitz, 2020). Currently there are no published assessments determining the economic impact of this previously observed decline, although it would be an excellent case study for determining how future shocks could affect the region. Economic considerations have been called for inclusion in management strategies, given the socio-economic importance of this species (Chesapeake Bay Commission, 2001), and newer models have begun to incorporate these factors (Bunnell *et al.*, 2010). As the Chesapeake experiences continued acidification and we consistently expand our understanding of its complex effects across the Chesapeake's systems (Su *et al.*, 2020), it is important for further research to explore the interconnection between blue crab populations and human society.

When considering the effects of future acidification to the blue crab population, it cannot be considered in a vacuum. Climate change is expected to intensify storms (IPCC, 2013), which may have profound effects on recruitment dynamics into the Chesapeake Bay (Miller *et al.*, 2019). Therefore, in addition to my observed effects of acidification

and temperature on larval survival and fitness, populations could further decline from weather related events. Identifying strategies to adapt to a more hostile natural system could include aquaculture for early life stages. After reaching the juvenile stage, where they can more successfully acclimatize to acidic conditions, crabs could be released into the Chesapeake. Previous studies have promoted the feasibility of such efforts and evidenced local population surges between 50-250% after release of cultured juveniles (Zohar *et al.*, 2008). Although this stock replenishment is technically feasible, such an endeavor would require cooperation between multiple stakeholders due to the scale and expense of the effort, and is best conducted in focused efforts for local populations throughout Chesapeake Bay (Secor *et al.*, 2004). It is thus more likely to be one aspect of an approach that would include further management strategies, such as scientifically based fisheries management and habitat restoration (Secor *et al.*, 2004).

The primary findings of this thesis suggest blue crabs will decrease in response to changing environmental conditions. Although there is potential for adaptation, it is wise to explore ways to mitigate these effects to the ecosystem and to regional human populations. Such pathways may include direct intervention, such as investing in early life stage aquaculture, or in less direct means, such as protecting nursery habitat. In habitat such as submerged aquatic vegetation, this may have a two-fold effect. Already important for juveniles seeking protection from predators (Mizerek *et al.*, 2011), new research has discovered its importance for buffering acidification within the Chesapeake (Su *et al.*, 2020), which may improve outlooks for the entire estuary. By finding sustainable ways to protect sensitive larval stages, we can enable blue crabs to

successfully reach this haven and continue to thrive in the Chesapeake Bay for future generations.

Literature Cited:

Arnold, W. S. 1984. The effects of prey size, predator size, and sediment composition on the rate of predation of the blue crab, *Callinectes sapidus* Rathbun, on the hard clam, *Mercenaria mercenaria* (Linne). *Journal of Experimental Marine Biology and Ecology*, 80:207-219.

Boudreau, S. A., & B. Worm. 2012. Ecological role of large benthic decapods in marine ecosystems: a review. *Marine Ecology Progress Series*, 469:195-212. doi: 10.3354/meps09862

Bunnell, D. B., D. W. Lipton, & T. J. Miller. 2010. The bioeconomic impact of different management regulations on the Chesapeake Bay blue crab fishery. *North American Journal of Fisheries Management*, 30:1505-1521. doi: 10.1577/M09-182.1

Chesapeake Bay Commission. 2001. Taking action for the blue crab: managing and protecting the stock and its fisheries. Chesapeake Bay Commission, Bi-State Blue Crab Advisory Committee, Annapolis, Maryland.

Choi, J. S., & B. M. Zisserson. 2008. An assessment of the snow crab resident on the Scotian Shelf in 2006, focusing upon CFA 4X. *Canadian Science Advisory Secretariat,* Research Doc: 2008/003

DeRivera, C. E., G. M. Ruiz, A. H. Hines, & P. Jivoff. 2005. Biotic resistance to invasion: native predator limits abundance and distribution of an introduced crab. *Ecology*, 86:3364–3376. doi: 10.1890/05–0479

Feng, X., E. P. Williams, & A. R. Place. 2017. High genetic diversity and implications for determining population structure in the blue crab, *Callinectes sapidus*. *Journal of Shellfish Research,* 36:231-242. doi: 10.2983/035.036.0126

Hill, J. M., & M. J. Weissburg. 2013. Habitat complexity and predator size mediate interactions between intraguild blue crab predators and mud crab prey in oyster reefs. *Marine Ecology Progress Series,* 488:209-219. doi: 10.3354/meps10386

Intergovernmental Panel on Climate Change. 2013. Fifth Assessment Report: Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Johnson, K. D., J. H. Grabowski, & D. L. Smee. 2014. Omnivory dampens trophic cascades in estuarine communities. *Marine Ecology Progress Series*, 507:197-206. doi: 10.3354/meps10815

Kemp, W. M., W. R. Boynton, J. E. Adolf, D. F. Boesch, W. C. Boicourt, G. Brush, J. C. Cornwell, T. R. Fisher, P. M. Gilbert, J. D. Hagy, L. W. Harding, E. D. Houde, D. G. Kimmel, W. D. Miller, R. I. E. Newell, M. R. Roman, E. M. Smith, & J. C. Stevenson. 2005. Eutrophication of Chesapeake Bay: historical trends and ecological interactions. *Marine Ecology Progress Series*, 303:1-29. doi:10.3354/meps303001

Kneib, R. T. 1982. The effects of predation by wading birds (Ardeidae) and blue crabs (*Callinectes sapidus*) on the population size structure of the common mummichog, *Fundulus heterclitus*. *Estuarine, Coastal and Shelf Science,* 14:159-165.

Lipcius, R., & W. Stockhausen. 2002. Concurrent decline of the spawning stock, recruitment, larval abundance, and size of the blue crab *Callinectes sapidus* in Chesapeake Bay. *Marine Ecology Progress Series*, 226:45–61. doi: 10.3354/meps226045

Long, W. C., Swiney, K. M., Harris, C., Page, H. N., & Foy, R. J. 2013. Effects of ocean acidification on juvenile red king crab (*Paralithodes camtschaticus*) and Tanner crab (*Chionoecetes bairdi*) growth,

condition, calcification, and survival. *PLoS ONE,* 8:e6095. doi: 10.1371/journal.pone.0060959

Long, W. C., S. B. Van Sant, K. M. Swiney, & R. J. Foy. 2017. Survival, growth, and morphology of blue king crabs: effect of ocean acidification decreases with exposure time. *ICES J. Mar. Sci.* 74:1033-1041. doi: 10.1093/icesjms/fsw197

Mancinelli, G., M. T. Guerra, K. Alujevic, D. Raho, M. Zotti, & S. Vizzini. 2017. Trophic flexibility of the Atlantic blue crab *Callinectes sapidus* in invaded coastal systems of the Apulia region (SE Italy): A stable isotope analysis. *Estuarine, Shelf and Coastal Science,* 198:421-431. doi: 10.12681/mms.13886

Miller T. J., S. J. D. Martell, D. B. Bunnell, G. Davis, L. Fegley, A. Sharov, C. Bonzek, D. Hewitt, J. Hoenig, & R. N. Lipcius. 2005. Stock assessment of the blue crab in Chesapeake Bay. University of Maryland Center for Environmental Science, Technical Report Series TS-487-05, Solomons. Available: hjort.cbl.umces.edu/crabs/Assessment05.html.

Miller, J. J., M. Maher, E. Bohaboy, C. S. Friedman, and P. McElhany. 2016. Exposure to low pH reduces survival and delays development in early life stages of Dungeness crab (*Cancer magister*). *Marine Biology,* 163:118. doi: 10.1007/s00227-016-2883-1

Miller, T. J., D. Liang, G. M. Nesslage, & M. J. Wilberg. 2019. Ranking ecosystem impacts on Chesapeake Bay blue crab (*Callinectes sapidus*) using empirical Gaussian graphical models. University of Maryland Center for Environmental Science, Technical Report Series TS-737-19, Solomons.

Mizerek, T., H. M. Regan, & K. A. Hovel. 2011. Seagrass habitat loss and fragmentation influence management strategies for a blue crab *Callinectes sapidus* fishery. *Marine Ecology Progress Series,* 427:247-257. doi: 10.3354/meps09021

Niemisto, Maura K. 2019. "Response of Early Life Stage Homarus americanus to Ocean Warming and Acidification: an Interpopulation Comparison". *Electronic Theses and Dissertations*. 3076. https://digitalcommons.library.umaine.edu/etd/3076

Secor, D. H., A. H. Hines, & A. R. Place. 2004. Japanese hatchery-based stock enhancement: Lessons for the Chesapeake Bay blue crab. Maryland Sea Grant, College Park, Maryland, 44 p. UM-SG-TS-2002-02.

Seitz, R. D. 2020. “Blue Crabs: Beautiful Savory Swimmers of the Chesapeake Bay.” In: Cuker B. (ed) Diet for a Sustainable Ecosystem. Estuaries of the World. Springer, Cham. doi: 10.1007/978-3-030-45481-4_7

Shen, C., J. M. Testa, M. Li, & W.-J. Cai. 2020. Understanding anthropogenic impacts on pH and aragonite saturation state in Chesapeake Bay: insights from a 30-year model study. *Journal of Geophysical Research: Biogeoscience*s, 125:e2019JG005620. doi: 10.1029/2019JG005620

Silliman B. R., & M. D. Bertness. 2002. A trophic cascade regulates salt marsh primary production. *Proc Natl Acad Sci USA*, 99:10500–10505. doi: 10.1073/pnas.162366599

Su, J., W.-J. Cai, J. Brodeur, B. Chen, N. Hussain, Y. Yao, C. Ni, J. M. Testa, M. Li, X. Xie, W. Ni, M. Scaboo, Y. Xu, J. Cornwell, C. Gurbisz, M. S. Owens, G. G. Waldbusser, M. Dai, & W. M. Kemp. 2020. Chesapeake Bay acidification buffered by spatially decoupled carbonate mineral cycling. *Nature Geoscience,* 13:441-447. doi: 10.1038/s41561-020-0584-3

Walther, K., K. Anger, & H.O. Pörtner. 2010. Effects of ocean acidification and warming on the larval development of the spider crab *Hyas araneus* from different latitudes (54° vs. 79° N). *Marine Ecology Progress Series,* 417:159-170. doi: 10.3354/meps08807

Zohar, Y., A. H. Hines, O. Zmora, E. G. Johnson, R. N. Lipcius, R. D. Seitz, D. B. Eggleston, A. R. Place, E. J. Schott, J. D. Stubblefield, & J. S. Chung. 2008. The Chesapeake Bay blue crab (*Callinectes sapidus*): a multidisciplinary approach to responsible stock replenishment. *Reviews in Fisheries Science*, 16:24-34. doi: 10.1080/10641260701681623

Appendix I:

Potential global economic impacts of ocean acidification*

S.A. Macko, C. Fantasia, & G. Xue

Abstract

The addition of massive amounts of carbon dioxide to the atmosphere and oceans as a result of anthropogenic emissions from fossil fuel use, is changing the ocean chemistry by increasing acidity through lowering ocean pH. Acidification influence on calcareous organisms at primary production levels could lead to catastrophic effects on higher organisms of food chains. The ocean influences all activities on Earth, being a source of nutrition and energy while simultaneously buffering climate. Fisheries, the source of 16% of human nutrition, are already in a state of near collapse for some species owing to overfishing and mismanagement of a sustainable infrastructure. The modification of the pH of this ecosystem will add further stress to the life cycles of marine organisms and will likely impact declining fisheries harvests even further. Impacts on larval stages of shellfish, and the reef-building corals add even more to the complexity of the effects of "the other carbon dioxide problem" of acidification. We are only in the initial stages in the evaluation of the potential economic losses resulting from the lower pH, which are likely to exceed a significant portion of the multi-billion dollar U.S. fishery harvest. Projections for the global economic impacts of ocean acidification are being attempted by combining economic models with ecosystem ones, and including the observations derived from laboratory experiments, and suggest that the global impact may exceed billions of dollars.

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Introduction

Ocean Acidification Arising from Increased Carbon Dioxide Emissions: The "Other Carbon Dioxide Problem"

Carbon dioxide levels in the atmosphere have now reached over 400ppm, and did not seasonally adjust to lower than 400 ppm in 2016 for the first time in the recent history of the planet. These levels are more than 40% above the pre-industrial levels of carbon dioxide and are a direct consequence of carbon dioxide emissions by human activities including the burning of fossil fuels (National Academy of Sciences 2008a; 2008b). With the increased atmospheric concentration of carbon dioxide have come increased levels of dissolved carbon dioxide in the ocean as marine waters scavenge the gas out of the atmosphere thus increasing the amount dissolved in the ocean (Caldeira and Wickett, 2003). Over the past 100 years, the oceans have absorbed about one third of the carbon dioxide emitted by anthropogenic sources. This scavenging of the gaseous carbon dioxide can have great and predictable responses in the ocean water chemistry, affecting carbonate ion concentrations, calcite and aragonite mineral saturation levels and eventually influencing the pH of the ocean water (Figure 1). The ocean carbon cycle involves two forms of carbon: organic carbon and the inorganic carbon. The inorganic carbon cycle is particularly relevant when discussing ocean acidification for it includes the many forms of dissolved CO_2. When CO_2 dissolves, it reacts with water to form ions from the dissolved carbon dioxide: carbonic acid (H_2CO_3), bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}). The relative abundance of these species depends on factors such as seawater temperature and alkalinity (Tyrrell, 2008a, 2008b).

Although the natural absorption of CO_2 by the world's oceans has helped mitigate the atmospheric climatic effects of anthropogenic emissions of CO_2, it is believed that increased levels in the ocean have caused a decrease in pH of approximately 0.15 units on the pH scale (Doney, 2006), or a 30% increase in acidity since this scale is logarithmic. This increase will likely have negative consequences, primarily for oceanic calcifying organisms. These span the food chain from autotrophs to heterotrophs and include organisms such as coccolithophores, corals, foraminifera, echinoderms, crustaceans and mollusks. The "skeletons" of these organisms are composed of calcite and aragonite (mineral forms of calcium carbonate) and are stable in surface waters since the carbonate ion is at supersaturating concentrations. However, as ocean pH falls further, so does the concentration of this ion, and when carbonate becomes undersaturated, structures made of calcium carbonate are vulnerable to dissolution (Feely *et al.*, 2004). The ocean is approaching pH levels not seen in millions of years. Increasing ocean acidity (OA) will affect a vast majority of marine life (either directly or indirectly), but some of the first to feel the effects are shellfish, such as oysters. The "slight" decrease of 0.1 units of pH has adverse repercussions on calcifiers. Acidification results in the water becoming unstable for calcium carbonate minerals that shellfish produce to make their shells. Without their protective shells, oysters are vulnerable and simply cannot live. Although the corrosion of their defensive shell seems enough to rapidly increase their death rate, the devastation of oysters from acidification goes even further (Waldbusser *et al.*, 2011).

An important aspect of this "other carbon dioxide problem" is that, unlike models of climatic warming which are based on complex models of many forcings and

feedbacks, heightened acidity, or lower pH of the ocean, is fairly predictable. The mechanisms for increasing acidity are well-established, physical chemical processes: increasing carbon dioxide in the atmosphere will increase the amount dissolved in the ocean (Figure 2). The pH of the ocean is dependent on the amount of the dissolved CO_2. The "unknowns" are simply the levels that atmospheric carbon dioxide will reach, and the rate at which the surface ocean attains equilibrium with that level. As fossil fuels continue to contribute carbon dioxide to the atmosphere, the pH of the ocean will continue to decline.

Most studies have found that coccolithophores, a type of planktonic algae, coralline algae, corals, shellfish, foraminifera, and pteropods all experience reduced calcification or increased dissolution under lower pH or elevated CO_2 (Raven *et al*., 2005). However, a few studies have suggested that with ocean acidification, the direction of the response, enhanced or declining, varies between species. Although the full ecological consequences of these changes in calcification are still uncertain, it appears likely that many calcifying species will be adversely affected. Lower pH also appears to negatively impact non-calcifying larvae during planktonic stages, affecting hardening of chitin and resulting in increased mortality.

Aside from calcification stress, organisms may suffer other adverse effects, either directly as reproductive or physiological effects, including CO_2-induced acidification of body fluids, or indirectly through negative impacts on food resources. With diminished calcifying planktonic organisms, the entire food resource may be disrupted, with a cascading effect up the food chain, should no other primary food source be readily

available (Kleypas *et al*., 2006). A change in any part of the food web may have consequences on the rest of the food web, ocean biogeochemistry and the whole ecosystem. Such a modification has already been observed in the Antarctic: in the Southern Ocean GLOBEC study with diminishing krill, predators of krill have turned to alternate foods, with associated potential loss of energy from longer food chains or foods not supplying appropriate levels of essential biochemical nutrients. These more acidic conditions would hinder growth of calcium carbonate shells and skeletons by many other marine plants and animals.

Ocean acidification may also force some organisms to reallocate metabolic energy away from feeding and reproduction in order to maintain internal cell pH. It has even been suggested that ocean acidification will alter the acoustic properties of seawater, allowing sound to propagate further, increasing ocean noise and impacting animals that use sound for echolocation or communication. However, as with calcification, as yet there is not a full understanding of these processes in marine organisms or ecosystems. Leaving aside direct biological effects, it is expected that ocean acidification in the future will lead to a significant decrease in the burial of carbonate sediments for several centuries, and even the dissolution of existing carbonate sediments (Ridgewell *et al.,* 2007; Turley, 2008). Inclusion of biological effects suggests that the ecosystem we know as the World's Ocean, an environment that provides one sixth of the protein consumed by humans, is dramatically changing. Many thousands of species of marine organisms will be affected directly by acidity, others by modification of the food chains on which they depend. At the extreme, large numbers of those species could be lost.

Ocean acidification has been seen to destroy ecosystems for marine life and the detrimental impacts are evident when looking at oysters, however, it is still not clear how capable other carbonate organisms will be in responding to the heightened acidity. Some organisms may have a higher resilience against a rise in pH, and therefore may still thrive at least for some time. A coral reef in the Western Pacific suggests that some calcifiers may be able to adapt. On Palau in the Western Pacific there exists high acidification, low aragonite saturation and yet from all appearances, a stable coral reef.

Despite low pH, carbonate, and aragonite, coral reefs at Palau illustrate high coral calcification, diversity, and cover. Calcification in different areas of this reef are found to be comparable to reefs with both high and low calcification rates, demonstrating that even under the stress induced by these conditions, calcification can occur for at least one of the reef building species (Shamberger *et al.*, 2014).

Similar studies on the resilience of other reefs around the globe are also being conducted, including studies in the Eastern Tropical Pacific, Hawaii and near volcanic vents. Studies in Eastern Tropical Pacific reefs confirmed a general consensus that there would be reduced resilience of coral reefs in response to increasing carbon dioxide concentration. The Eastern Tropical Pacific reefs are in zones of upwelling with high CO_2 and nutrient concentrations. However, research found that there was an abnormally low saturation of carbonate in this ecosystem, and as a result, these reefs would be more susceptible to bioerosion (Manzello *et al.,* 2008).

A further consequence of ocean acidification will be an impact on humans through declining fish harvests resulting in diminishing captures for nutrition and also

lower revenues from those captures of shellfish or finfish as well as associated habitat loss including that which results in ecotourism benefits in areas like coral reefs. A study of US commercial fisheries (Cooley and Doney, 2009) attempted to constrain the economic effects of ocean acidification using anticipated increases in atmospheric carbon dioxide. The annual domestic commercial harvest of mollusks alone could ultimately be impacted sufficiently to lower revenues by a billion dollars, with the global impact reaching many times that amount.

At the present time, an assessment is underway on the current statistical tools, mathematical models, and experimental designs available for studying ocean acidification and how it may affect future economies and commercially important species. At that time, there were economic models and biological models, but very few bioeconomic models (Hilmi *et al.,* 2013). Recently two economic models have been modified to incorporate biological data into a new dual framework: the Social Welfare Analysis (SWA) and the Information B model. The SWA is for data with monetary values that proffers how marine ecosystems may best benefit society. Information B is being used for non-monetary data that may be quantified in a different manner or potentially only qualitatively (Hilmi *et al.,* 2013). Previously, few studies have been available except initial studies regarding single species over shorter time frames, with a large amount of extrapolation (Hilmi *et al.,* 2013). As a result, there is a need to study carbon dynamics within estuaries and coastal systems as well as determining specific impacts on commercially fished species. Additionally, more detailed information is needed on the production and consumption of market and non-market goods that are affected by OA for

SWA and other future models to increase in accuracy (Hilmi *et al.,* 2013). Currently, the research is only beginning to understand and evaluate the complexities involved; models detailing human activities changing as a result of OA and end-to-end models of OA impacts are also required.

Some models have been developed which are integrating economics and biological principles. Public interest and funding are rising, with calls for more research in nearly every new OA publication. Clearly, newer models have more information and data to work with, such as one recent model using decision theory approach to immediately assist fisheries' responses to OA (Seijo *et al.,* 2016). The support framework for this model includes data from recent IPCC pH scenarios and new findings on species' responses to OA, and economic data regarding various countries' reliance on fishery revenues. Several pH scenarios have been tested with fish of varying longevity and OA sensitivities, with the results unveiling significant impacts on economies as well as fish populations going forward to 2100 (Seijo *et al.,* 2016). Although high variance between species was found in their literature review, the findings illustrate that despite this wide margin, negative consequences are predicted for most species. Therefore, fishing management plans should immediately be in place to reduce fishing pressures on stocks to increase their survival and sustainability (Seijo *et al.,* 2016). The value of detailed modeling has not been applied to local environments, leaving that to other studies, demonstrating that bioeconomic models can be a highly valuable tool for assessing OA and its ramifications. Recently other research reports have specifically evaluated regional impacts using a variety of methods in places such as the Mediterranean, Europe, the US

and Canada, Australia, and Asia-Pacific.

Local Economic Impacts

Mediterranean: Within this historic region, both aquaculture and capture fisheries are present, with aquaculture concentrated in northern Mediterranean countries and artisanal capture fisheries primarily located in the southern countries. From this one small sea is derived 2% of the world's economic value from fisheries, with its aquaculture alone quadrupling in the last decade. Aquaculture employs 123,000 individuals, with fisheries employing 250,000, and secondary sectors employ another 210,000 individuals. Pelagic fishes compose 53% of capture fishery landings, with aquaculture primarily harvesting fish and mollusks (Lacoue-Labarthe *et al.,* 2016). Currently pelagic species harvested such as sardines and anchovies have unknown responses to OA. However, other fishes have revealed some behavioral and physiological changes (Heuer and Grosell, 2014). Some tropical and temperate fishes were observed to have otolith enlargement with increased carbon dioxide, a universal bone composed of aragonite that is important for orientation and sensing acceleration (Haigh *et al.,* 2015).

As a system, the Mediterranean is nutrient poor with wide seasonal variations in acidity. Anthropogenic carbon dioxide absorbing into the sea is much higher than similar latitudes due to specific circulation patterns, temperatures, and alkalinity. Currently the Mediterranean is experiencing increased water temperatures that has increased mortality in mussels, sponges, corals, and the early life stages of several species (Lacoue-Labarthe *et al.,* 2016). Disease susceptibility is also expected to increase as pathogens generally thrive in warmer waters and species distribution will likely shift. Experts anticipate the

combination of OA and warming waters (Lacoue-Labarthe *et al.,* 2016; Weatherdon *et al.,* 2015) will increase the negative impacts of each in the highly valued mollusk and red coral industries, as well as severely affect subsistence fishing. Beyond those direct economic effects, seafood nutritional content may decrease and harmful algal blooms are expected to increase, further reducing mollusk harvests (Lacoue-Labarthe *et al.,* 2016).

Red coral reefs are an important source for jewelry sales, recreation, and tourism within the Mediterranean, but their numbers are expected to significantly decline with increased acidity (Rodrigues *et al.,* 2013; Weatherdon *et al.,* 2015; Lacoue-Labarthe *et al.,* 2016). In some areas, over-exploitation is already occurring despite its current relative abundance and its sensitivity to OA is already established, with overuse only expected to worsen. Red coral reefs are also an important habitat as nurseries for various fish species, further emphasizing their importance in the region. Using a framework utilizing expected magnitude of OA and the values of current fisheries, recreation/tourism, and red coral extraction, it was found each of these fields will be strongly affected (Rodrigues *et al.,* 2013).

Although each part of the Mediterranean will suffer economic losses in the various market sectors mentioned, the northern region is expected to suffer the greatest. The northern Mediterranean harvests more sensitive fishes that are more susceptible to acidification compared to more hardy fishes harvested in the south, such as carp and tilapia (Weatherdon *et al.,* 2015).

Europe: Higher latitudinal studies exposes increased negative consequences for places such as the northern UK and Norway. In 2015 UK calcifying shellfish harvests

were worth £302 million, or 50% of total marine fisheries value, with shellfish aquaculture contributing another £33 million. Pinnegar *et al.* (2015) used three economic models to determine outcomes from increased acidity to their waters and found that a Net Present Value (NPV) approach determined the losses to be over £954 million by 2100 with a high emission scenario. When income changes and market demand are also considered using a Partial Equilibrium model, losses were estimated to be five times higher by 2100. Wales and Northern Ireland rely heavily on shellfish capture fisheries and aquaculture and therefore will be heavily affected, but absolute losses in revenue will be the greatest in Scotland's fisheries. Annually, NPV losses were estimated between two extremes: low emissions with low sensitivity and high emissions with high sensitivity, with losses spanning from £1.4 million to £9.1 million. The study concluded with a General Equilibrium model displaying systematic feedbacks throughout the economy, with fishery revenue losses negatively impacting GDP, employment, manufacturing, and services sectors. Although some positive impacts were seen on tax revenue and government expenditure, fishery losses would affect domestic trade balance as well as world trade balances (Pinnegar *et al.,* 2015).

In Norway, a case study was made on Norwegian coastal cod (Voss *et al.,* 2015), a fish with wide distributions, significant commercial importance, and high exploitation that has already experienced some local stock collapses. Most of the region's fisheries are small-scale with traditional methods, resulting in a system less able to withstand shocks. Other laboratory studies have revealed severe tissue damage to early life stages with increased acidity (Frommel *et al.,* 2012), therefore making this species an ideal candidate

for use in an ecological-economic model (Voss *et al.,* 2015). Their model evaluated management options at present day, medium, and high acidity levels with associated reduced larval recruitment rates. Retaining fish at recent population levels is the first management option (1), with optimized management adjusting to status quo acidity levels the second option (2), or optimal adaptation of management to acidification in the region as the third option (3). The business-as-usual scenario, or option (1), exhibits harvest, profits, and spawning stock biomass decreasing with increasing OA. By 1800 µmol atmospheric CO_2, fisheries turn unprofitable and if CO_2 levels continue to increase, stock collapses. Using option (2) with present day CO_2 levels, stocks may rebuild, harvests will be reduced slightly, but profits could increase to US$194 million compared with option (1). When medium CO_2 levels are used, stock size, harvest, and profits would decrease slightly. Under high CO_2 conditions, the measures are not enough and the stock will collapse. Using the final option (3), stocks are stabilized up to 4200 µmol atmospheric CO_2, but harvests are small and profits are low (Voss *et al.,* 2015). The authors point out their model is unable to account for all of the feedbacks and variables yet unknown in this stock's system and explain they cannot make predictions with the model, only give examples of potential scenarios that are very likely for small-scale fisheries.

North America: In the Northeast Pacific, upwelling, low alkalinity, and other natural environmental factors create lower than average acidic conditions, with some areas already undersaturated with aragonite, the form of calcite most commonly incorporated into species' shells. Current seasonal variations further decrease the saturation horizon and reduce acidity during the summer months (Mathis *et al.,* 2015).

Approximately 50% of total US fisheries catch is derived from Alaska with a wholesale value of $4.6 billion dollars and employing around 90,000 full time workers. Sport and personal fishing support an additional 16,000 jobs and garners $1.4 billion in revenue. Half of Alaska's tourism industry, $300 million, is from fish tourism, and 17% of its population depend on subsistence fishing. Fishing activities are part of everyday life for 95% of the population and 83% harvest fishes (Mathis *et al.*, 2015). In rural areas such as the Aleutian Islands, most of their nutrition stems from mollusk harvests. These regions are especially susceptible to OA due to anticipated rapid increases in acidity, their reliance on subsistence fishing and harvests, lower incomes, higher food prices, and paucity of job diversity. Other regions of Alaska are also vulnerable to changes in acidity, but southern Alaska ranks highest in a vulnerability scoring (Mathis *et al.*, 2015).

One of the largest fisheries in Alaska is the red king crab fishery with $115 million of revenue every year (Punt *et al.*, 2014). Prior research portrays their early life stages are negatively impacted by increased acidity (Long *et al.*, 2013), making this species ideal for bioeconomic modeling. The model used here, the Model of Intermediate Complexity for Ecosystem assessments (MICE) reveals that harvest yields and profits for this species are expected to slowly decline with increasing acidity for the next few decades and after 2050 they will decline more sharply. The direct loss per year is anticipated to be tens of millions of dollars, but the indirect losses are expected to be much greater, although this study did not quantify those losses. Further study is required to substantiate their work and to account for other variables in the models (Punt *et al.*, 2014).

British Columbia also greatly contributes to its nation's GDP, with over CAND$650 million from their fisheries and aquaculture. Breaking this down, around 50% is from recreational fishing of salmon and Pacific Halibut, 15% from capture fisheries, and 10% from aquaculture. Capture fishery has steadily declined but aquaculture has tripled in size over the past few years (Haigh *et al.,* 2015). Tourism is partially included in those numbers through sport fishing and is primarily considered separately. As a whole, the salmon and mollusk fisheries are expected to decline due to OA, with repercussions on ecotourism and fisheries. No quantitative studies have yet been conducted in the region, but Haigh *et al.* thoroughly review the literature on each phyla and determined based on individual case studies that wide swaths of species will be negatively impacted in the region, with some of those species commercially important (Haigh *et al.,* 2015).

Further south in the United States, mollusks comprised 19% of the primary value of commercial harvests in 2007, crustaceans yielded 30%, and finfish were 50%. Of those finfish, 24% directly prey on calcifiers (Cooley and Doney, 2009). Cooley and Doney used the NPV model to forecast potential results through 2050 and found that mollusks alone could sustain losses between $1.7-10 billion between 2009-2050. This is a conservative figure that only focuses on the mollusk industry and does not account for harmful algal blooms that are anticipated to increase due to OA, nor does it account for trophic cascades from population losses in mollusks or other species (Cooley and Doney, 2009).

Australia: A qualitative assessment of Australia's prawn and scallop fisheries

found the worth of the two industries to equal AUD$121 million and AUD$2 million, respectively. Not enough information is available regarding the response of early life stages in prawns, but long-term exposure to acidified conditions with increased temperatures result in reduced growth, survival, and swimming ability for prawns (Richards *et al.*, 2015). There is also little information regarding scallop responses, although earlier studies cited in Richards *et al.* reveal they typically exhibit negative responses and are poor ion regulators, making them more sensitive to changes in ions that result from changes in acidity. The authors note co-stressors such as a strengthening East Australian Current and changes in predator-prey interactions due to OA, but also suggest the Queensland prawns are not highly sensitive to OA and have high phenotypic diversity. This should assist in natural adaptation, although there are no studies to date on the prawns. However, the scallop industry is based entirely on one species of limited range that require certain ocean circulations for larvae distribution and settlement. It has also been suggested moving all life stages to controlled aquaculture environments in order to maintain the industry (Richards *et al.*, 2015).

Caribbean Islands and Asia-Pacific: Coral reef services such as shoreline protection are roughly valued at $9 billion total worldwide (Winner, 2013). In countries such as Tobago, reefs line the island and tourism directly stemming from the reefs composed 15% of their GDP in 2006. When indirect income from tourism is included, one-third of Tobago's economy stems from their reef system (Winner, 2013). Due to the potential sensitivity of these calcifying organisms to OA, such heavy reliance on a fragile ecosystem bodes ill for their economic future. In the Caribbean, Tobago is one of many

small island countries to have a tourism-based economy that rests on the shoulders of coral reefs.

In the Asia-Pacific, pH is expected to decrease by 0.3 units, which is expected to reduce aragonite saturation by 50% (Heenan *et al.,* 2015). These will likely result in shifts with distribution and abundance, forcing fishermen to travel to more distant fishing grounds and reducing their net income due to increased travel costs. Losses in production are anticipated to increase malnutrition throughout the region, where 50-90% of dietary animal protein is derived from fish. Using a qualitative Ecosystems Approach to Fisheries Management (EAFM), the region is classified as highly sensitive to CO_2 emissions with low to moderate adaptive capacity (Heenan *et al.,* 2015). Beyond dietary needs, Polynesia employs thousands of individuals on remote atolls to collect valuable black pearls. With increased OA reducing populations of pearl producing oysters, thousands of people with little employment alternatives will lose a large percentage of their annual income and thereby reducing local revenue in those distant localities (Weatherdon *et al.,* 2015).

Global Economic Impacts

Fisheries and shoreline protection from coral reefs cumulate into a worth of approximately $30 billion annually (Winner, 2013). Within this, capture fisheries produce 80-85 million tons/yr and the fastest growing food sector of aquaculture grows at least 8% annually (IUCN, 2015). Of those capture fishes, the top ten are finfish species (IUCN, 2015), with this class of fish revealing altered predator-prey interactions and impaired locomotion under acidified conditions (Haigh *et al.,* 2015). This large industry

directly supports 120 million people, with 35 million of those classified as fishermen and 96% of the total concentrated in developing countries. Over 90% of those fishermen are in small-scale fisheries, known to absorb fewer shocks than large-scale fisheries (IUCN, 2015). Without including socioeconomic benefits of subsistence and recreational fishing, commercial fisheries alone produce a global GDP of $274 billion annually. These numbers are despite the fact that over 50% of marine stocks are over-exploited and they only include reporting OECD countries (IUCN, 2015). Over-exploited stocks may be larger than this, but unfortunately data were not available for all countries. Reduced stock sizes increases the chances for OA impacts. Although there are dozens of case studies setting forth the plausibility of recuperating depleted stocks, most require government intervention (IUCN, 2015), which is often only possible with a strong and wealthy centralized government that may not be equally available to OA susceptible countries.

Focusing solely on global mollusk fishery harvests, 16 million metric tons were harvested in 2007 that was worth $15 billion. These were unevenly distributed across the nations, with nutritional dependence clearly linked to culture and geography. Island and Pacific nations derive a significant portion of their GDP from mollusk exports, with many of them poverty stricken nations with a fair amount of their population already malnourished (Cooley *et al.*, 2012).

Using a Vulnerability Assessment Approach (VAA) to evaluate the impacts of OA and human population growth on future per capita mollusk protein availability worldwide attributes the greatest vulnerability to developing countries. Those most susceptible to harvest declines were Senegal, Madagascar, Gambia, Mozambique, and

Haiti. Those vulnerable to import declines were Solomon Islands, Jamaica, Belize, Cook Islands, and Sudan (Cooley *et al.*, 2012). Although there are many more factors at play potentially skewing the results of the VAA, general trends reveal aragonite saturation decreasing heavily in the above geographic regions while simultaneously mollusk consumption is increasing yearly by a factor of three. Mollusks are some of the most vulnerable to OA, but increased protein demands in these countries force them to rely more heavily on this delicate organism (Cooley *et al.*, 2012).

Expanding the view to encompass capture fisheries as a whole, there is high confidence in the AR5 prediction that calcifiers will be negatively affected and therefore impact their fisheries' harvests. Trophic cascades are expected as sensitivity affects differing species, with a large proportion of risk in the tropics. The IPCC predicts the Caribbean will start experiencing net revenue losses in 2015 from a combination of OA and temperature increasing (Weatherdon *et al.*, 2015). By 2050, this synergy will likely result in losses of 10% to their net present value in the UK, during which time the Mediterranean loses red corals and sponges. Corrosion from increased acidity will also likely increase maintenance costs for various structures and Arctic Indigenous peoples' culture will be significantly altered (Weatherdon *et al.*, 2015).

Aquaculture effects are more varied with location, species, and method. Regions expected to have higher vulnerability are Asia, the Caribbean, and Latin America, along with the southern Mediterranean and Polynesia (Weatherdon, *et al.*, 2015).

Beyond capture fisheries and aquaculture, the ocean provides less quantifiable benefits related to coastal tourism and protection, biodiversity, recreational benefits, and

carbon sequestration, although this last one is plainly a double-edged sword. Although monetary benefits for each of these are sometimes difficult to assess (Brander *et al.*, 2014), their importance is evident within island nations, where coastal tourism, recreation, and other ecosystem services represent a significant proportion of their economy. When OA is combined with sea level rise, warming, and increased storm surges, the cumulative economic effect is anticipated to be momentous (Weatherdon *et al.*, 2015). Carbon sequestration in the oceans will be reduced and coral reef impacts are expected to outstrip mollusk fishery losses by 2100, although more data are needed for quantified impacts (Brander *et al.*, 2014; Rodrigues *et al.*, 2013).

One high profile ecosystem at risk are coral reefs. Many studies have been conducted on the impacts of OA to this apparently fragile system, with consensus that increased acidity results in coral death (Andersson *et al.*, 2013). Coral reefs provide shoreline protection, food, and income for approximately 500 million people; depending on which CO_2 scenario is used, losses from OA can range from $500-$870 billion (Turley and Gattuso, 2012).

A wide variety of species are expected to suffer detrimental effects from OA. Vertebrate early life stages, olfactory, visual and audiological senses, and physiology have all characterized negative effects (Branch *et al.*, 2013). Invertebrates have been covered extensively above and also reveal difficulty adjusting to increased acidity, with calcifiers faring the worst. But beyond harvested species themselves, habitat loss is also expected. Algae turfs may be able to out-compete kelp forests, a rich and diverse community that offers copious protection for young fishes from predators. One study

found sea urchins thrive near CO_2 vents, meaning the primary consumer of kelp may increase. Even a small number of urchins may destroy a large section of kelp forest (Branch *et al.,* 2013). Repercussions through the food web must be accounted for as well, as population densities of prey and predator shift in response to habitat loss and ocean acidification itself (Branch *et al.,* 2013).

Examples from regions and worldwide delineate that although we do not entirely understand the ramifications, the information we do have illustrates potential impacts to be far reaching. Even so, there are management options for mitigating consequences on fishery harvests, habitats, and economies.

Potential Mitigation Options

One of the most readily apparent solutions is to reduce the amount of CO_2 currently present in the atmosphere (Pinnegar *et al.,* 2015). As more CO_2 is released via fossil fuels and other anthropogenic sources, that which was sequestered in the ground now changes the balance of CO_2 in the atmosphere and then changes the balance of dissolved CO_2 in the oceans. To correct the current imbalance requires the input of CO_2 to be reduced. Although in theory the solution is easily understood and agreed with, actually reducing the input requires policy makers, green energy innovators, and a collective social consciousness of the issue. Intergovernmental panels such as the UN may be key for an international agreement (Turley and Gattuso, 2012), but this will also require more time than the situation feasibly allows. Therefore, it can only be one prong of the maneuver for mitigating OA.

The second easiest solution is to finance more research funding (Pinnegar *et al.,*

2015; Richards *et al.,* 2015). Most research thus far available has been based on laboratory findings or from observations of CO_2 vents in the deep ocean. Unfortunately, although laboratory studies can be good for preliminary results, they are very artificial environments that can only mimic a small fraction of the variables at play in natural environments such as predator-prey interactions, food web cascades, habitat effects, pollution, and many other factors. The CO_2 vents are a welcome step above laboratory testing, but even so face their own challenges. As part of the deep ocean, most harvested fishes and their habitats, such as reefs and kelp forests, never come in contact with the vents. Extrapolations may certainly be made, but in such a small habitat any results may be skewed by recruitment. Dives to these areas are few simply because of the expense associated with it. By increasing funding for scientific research into CO_2 vents and OA in general, more robust results are achievable.

One research topic in particular, phenotypic variability, has yielded some promising results (Hilmi *et al.,* 2013; Parker *et al.,* 2011; Evans and Hofmann, 2012). This research is based on the mechanisms underlying natural adaptation at the molecular level. Some studies attribute certain oysters with more resilience to increased acidity when compared with allopatric populations (Parker *et al.,* 2011). By utilizing the populations better suited to OA, harvest quotas may be maintained without species' loss. This phenotypic variability may be used for other mollusks as well (Cooley *et al.,* 2012) in addition to other organisms altogether (Hilmi *et al.,* 2013). One molecular technique includes evaluating gene expression, physiological plasticity, and evaluating the species' response to the sublethal stress of OA (Evans and Hofmann, 2012). One stress response

pathway in particular, the Cellular Stress Response (CSR), is a good indicator of a species' resilience to the stress of acidification (Evans and Hofmann, 2012). Therefore, part of this research should include searching for underutilized species that contain more phenotypic variability and shift harvests towards those hardier species (Pinnegar *et al.,* 2015).

Both CO_2 reduction and continuing research are important for developing solutions, but each of these require a substantial amount of time before results are evident. They should still be pursued by politicians, scientists, and economists, but more immediate steps for mitigation are still possible. Management plans for all calcifying organism fisheries should be updated to include OA impacts on that species (Richards *et al.*, 2015). Transitioning toward aquaculture for some of the most sensitive species and increasing the flexibility in capture fisheries will also help mitigate stock collapses or economic shocks for small, fishing dependent, countries (Richards *et al.,* 2015).

Despite the broad and deep consequences of ocean acidification, there is hope. Changes must be enacted to delay, mitigate, and guard the ocean from too much of a good thing. These changes are easy to enact compared to future damage control scenarios and yet require collaboration between many arenas and countries. It can be done, with the ready and active help of all who will listen. Therefore, for the good of diverse ecosystems, fauna, and people groups, may action through research and altered policy and current management practices be accomplished.

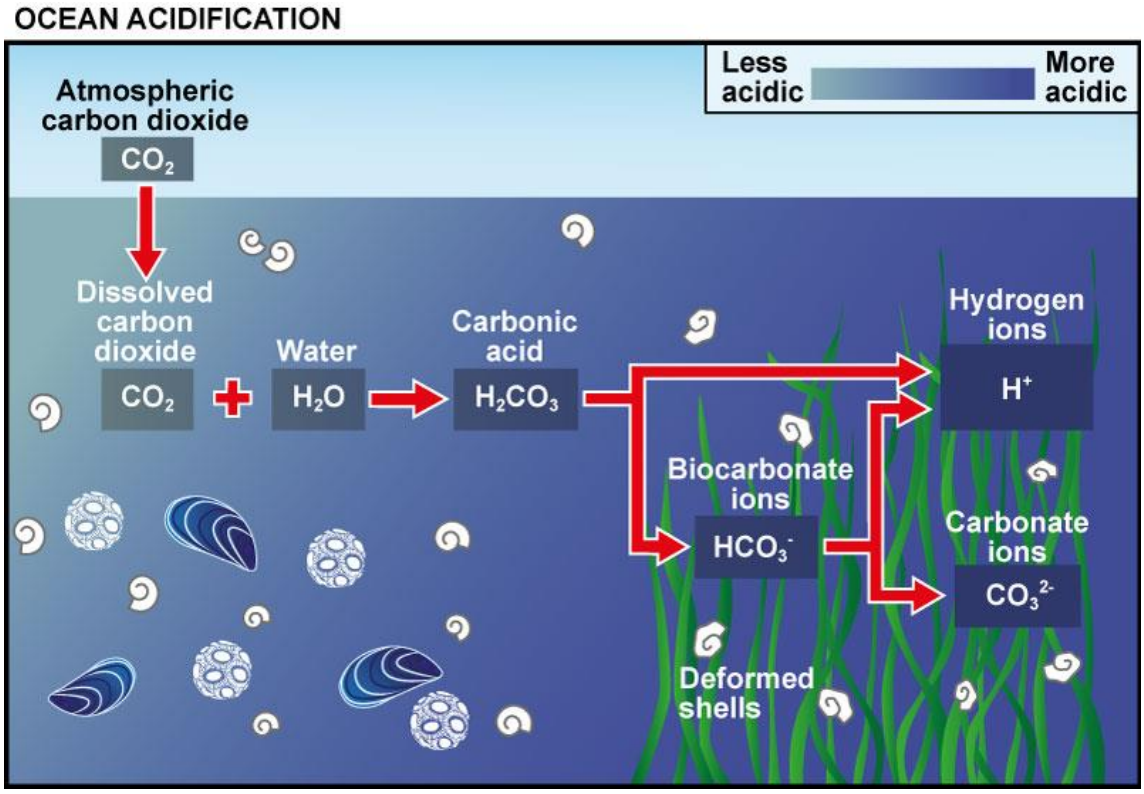

Figure I.1. Chemical processes involved in ocean acidification. Figure courtesy of NERC and the UK Ocean Acidification Program.

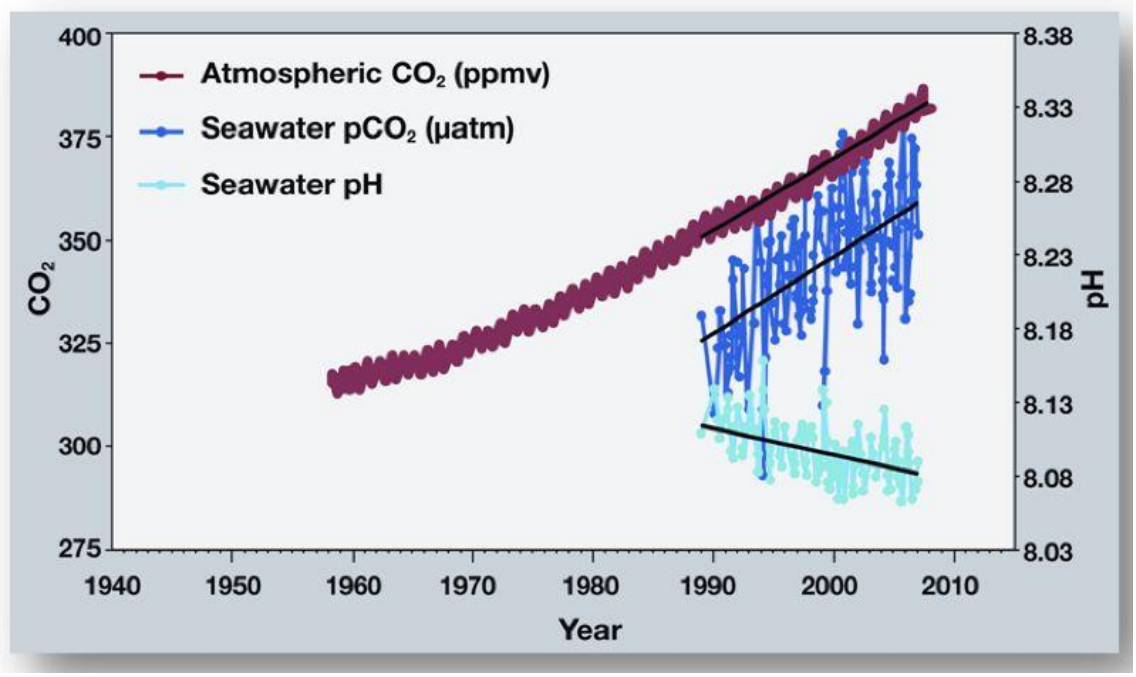

Figure I.2. Correlation between rising atmospheric concentration of carbon dioxide with increase in the concentration of carbon dioxide dissolved in seawater. Note seawater pH decreases since it is a negative logarithm function of the increasing carbon dioxide concentration. Figure courtesy the National Oceanic and Atmospheric Administration.

Literature Cited:

Andersson, A. J. and D. Gledhill. 2013. Ocean acidification and coral reefs: effects on breakdown, dissolution, and net ecosystem calcification. *Marine Science*, 5:321-348.

Branch, T. A., B. M. DeJoseph, L. J. Ray, and C. A. Wagner. 2013. Impacts of ocean acidification on marine seafood. *Trends in Ecology and Evolution*, 28:178-185.

Brander, L. M., D. Narita, K. Rehdanz, and R. S. J. Tol. 2014. Handbook on the Economics of Ecosystem Services and Biodiversity, Chapter 5: The Economic Impacts of OA.

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

Cooley, S. R., and S. C. Doney. 2009. Anticipating OAs economic consequences for commercial fisheries. *Environmental Research Letters*, 4:1-8.

Cooley, S. R., N. Lucey, H. Kite-Powell, and S. C. Doney. 2012. Nutrition and income from molluscs today imply vulnerability to OA tomorrow. *Fish and Fisheries,* 13:182-215.

Doney, S. C. 2006. The dangers of ocean acidification. *Scientific American*, 294:58-65.

Evans, T. G. and G. E. Hofmann. 2012. Defining the limits of physiological plasticity: how gene expression can assess and predict the consequences of ocean change. *Philosophical Transactions of the Royal Society B*, 367:1733-1741.

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

Frommel, A. Y., R. Manejy, D. Lowe, A. M. Malzahn, A. J. Geffen, and A. Folkvord. 2012. Severe tissue damage in Atlantic cod larvae under increasing ocean acidification. *Nature: Climate Change*, 2:42–46.

Haigh, R., D. Ianson, C. A. Holt, H. E. Neate, and A. M. Edwards. 2015. Effects of OA on temperate coastal marine ecosystems and fishers in the Northeast Pacific. *PloS ONE*, 10:1-46.

Heenan, A., R. Pomeroy, J. Bell, P. L. Munday, W. Cheung, C. Logan, R. Brainard, A. Y. Amri, P. Alino, N. Armada, L. David, R. Rivera-Guieb, S. Green, J. Jompa, T. Leonardo, S. Mamauag, B. Parker, J. Shackeroff, and Z. Yasin. 2015. A climate-informed, ecosystem approach to fisheries management. *Marine Policy*, 57:182-192.

Heuer, R. M., & M. Grosell. 2014. Physiological impacts of elevated carbon dioxide and ocean acidification on fish. *American Journal of Physiology,* 307:R1061–R1084, doi:10.1152/ajpregu.00064.2014

Hilmi, N., D. Allemand, S. Dupont, A. Safa, G. Haraldsson, P. A. L. D. Nunes, C. Moore, C. Hattam, S. Reynaud, J. M. Hall-Spencer, M. Fine, C. Turley, R. Jeffree, J. Orr, P. L. Munday and S. R. Cooley. 2013. Towards improved socio-economic assessments of ocean acidification's impacts. *Marine Biology*, 160:1773-1787.

International Union for Conservation of Nature (IUCN). 2015. Bridging the gap between ocean acidification impacts and economic valuation: regional impacts of OA on fisheries and aquaculture: OA impacts on fisheries and aquaculture.

Kleypas, J. A., R. A. Feely, V. J. Fabry, C. Langdon, C. L. Sabine, and L. L. Robbins. 2006. Impacts of ocean acidification on coral reefs and other marine calcifiers: A guide for future research. Report of a workshop held 18–20 April 2005, St. Petersburg, FL, sponsored by NSF, NOAA, and the U.S. Geological Survey, 88 pp.

Lacoue-Labarthe, T., P. A. L. D. Nones, P. Ziveri, M. Cinar, F. Gazeau, J. M. Hall-Spencer, N. Hilmi, P. Moschella, A. Safa, D. Sauzade, & C. Turley. 2016. Impacts of OA in a warming Mediterranean Sea: an overview. *Regional Studies in Marine Science*, 5:1-11.

Long, W. C., K. M. Swiney, & R. J. Foy. 2013. Effects of ocean acidification on the embryos and larvae of red king crab, *Paralithodes camtschaticus. Marine Pollution Bulletin,* 69:38-47. doi: 10.1016/j.marpolbul.2013.01.011

Manzello, D. P., J. A. Kleypas, D. A. Budd, C. M. Eakin, P. W. Glynn, and C. Langdon. 2008. Poorly cemented coral reefs of the eastern tropical Pacific: Possible insights into reef development in a high-CO_2 world. *Proceedings of the National Academy of Sciences, U.S.A.,* 105:450–510.

Mathis, J. T., S. R. Cooley, N. Lucey, S. Colt, J. Ekstrom, T. Hurst, C. Hauri, W. Evans, J. N. Cross, and R. A. Feely. 2015. Ocean acidification risk assessment for Alaska's fishery sector. *Progress in Oceanography*, 136:71-91.

National Academy of Sciences. 2008a. The ecological impacts of climate change. Washington, DC, 28p.

National Academy of Sciences. 2008b. Understanding climate change. Washington, DC, 26p.

Parker, L. M., P. M. Ross, and W. A. O'Connor. 2011. Populations of the Sydney rock oyster, Saccostrea glomerata, vary in response to ocean acidification. *Marine Biology*, 158:689-697.

Pinnegar, J. K., J. Lee, and S. Birchenough. 2015. Economic impacts of OA on shellfish fisheries and aquaculture in the UK. UK Dept. for Environment, Food, and Rural Affairs, ICES CM 2015/H:05.

Punt, A. E., D. Poljak, M. G. Dalton, R. J. Foy. 2014. Evaluating the impact of OA on fishery yields and profits: the example of red king crab in Bristol Bay. *Ecological Modelling*, 285:39-53.

Raven, J. A. *et al.* 2005. Ocean acidification due to increasing atmospheric carbon dioxide. Royal Society, London, UK. The Clyvedon Press Ltd.

Richards, R. G., A. T. Davidon, J.-O. Meynecke, K. Beattie, V. Hernaman, T. Lynam, and I. E. van Putten. 2015. Effects and mitigations of OA on wild and aquaculture scallop and prawn fisheries in Queensland, Australia. *Fisheries Research*, 161:42-56

Ridgwell, A. I. Zondervan, J. C. Hargreaves, J. Bijma, and T. M. Lenton. 2007. Assessing the potential long-term increase of oceanic fossil fuel CO_2 uptake due to CO_2-calcification feedback. *Biogeosciences,* 4:481–492.

Rodrigues, L. C., J. C. J. M. van den Bergh, and A. Ghermandi. 2013. Socioeconomic impacts of ocean acidification in the Mediterranean Sea. *Marine Policy*, 38:447-456.

Turley, C. 2008. Impacts of changing ocean chemistry in a high-CO_2 world. *Mineralogical Magazine,* 72:359–362.

Seijo, J. C., R. Villanueva-Poot, A. Charles. 2016. Bioeconomics of ocean acidification effects on fisheries targeting calcifier species: a decision theory approach. *Fisheries Research*, 176:1-14.

Shamberger, K. E. F., A. L. Cohen, Y. Golbuu, D. C. McCorkle, S. J. Lentz, and H. C. Barkley. 2014. Diverse coral communities in naturally acidified waters of a Western Pacific reef. *Geophysical Research Letters*, 41:499-504. DOI: 10.1002/2013GL058489

Turley, C. and J-P. Gattuso. 2012. Future biological and ecosystem impacts of OA and their socioeconomic policy implications. *Current Opinion in Environmental Sustainability*, 4:278-286.

Tyrrell, T. 2008a. Impacts of changing ocean chemistry in a high-CO_2 world. *Mineralogical Magazine*, 72:329-362.

Tyrrell, T. 2008b. Calcium carbonate cycling in future oceans and its influence on future climates. *Journal of Plankton Research*, 30:141–156.

Voss, R., M. F. Quaas, J. O. Schmidt, and U. Kapaun. 2015. Ocean acidification may aggravate social-ecological trade-offs in coastal fisheries. *PloS ONE*, 10:1-8.

Waldbusser, G. G., E. P. Voigt, H. Bergschneider, M. A. Green, & R. I. E. Newell. 2011. Biocalcification in the Eastern oyster (*Crassostrea virginica*) in relation to long-term trends in Chesapeake Bay pH. *Estuaries and Coasts*, 34:221-231. doi: 10.1007/s12237-010-9307-0

Weatherdon, L., A. Rogers, R. Sumaila, A. Magnan, and W. W. L. Cheung. 2015. The Oceans 2015 Initiative, Part II: An updated understanding of the observed and projected impacts of ocean warming and acidification on marine and coastal socio economic activities/sectors. Studies N°03/15, IDDRI, Paris, France, 46 p.

Winner, C. 2013. The socioeconomic costs of ocean acidification. *Oceanus*, 50:72-75.

Appendix II:
An overview of ocean acidification: relationships*

S.A. Macko & C.M. Fantasia

Abstract

The current concentration of atmospheric carbon dioxide (CO_2) surpasses 400 ppm, resulting in increasingly higher levels of this gas being absorbed into the ocean and reacting with ocean water. This process is changing the acidity of the global ocean, with increased acidity already observed in higher latitudes and more changes expected in the future. Historically, ocean pH has averaged near 8.1, with historical changes occurring gradually. However over the past few decades this rate of change has increased dramatically, lowering the pH by about 0.1 (a 30% increase in acidity) and affecting the buffering capability of the ocean. Many studies have found negative physiological effects on a wide range of calcareous organisms, from phytoplankton to shellfish to predators. Ecologically, potential trophic cascades have been observed in laboratory experiments. These studies often utilize the lower pH predictions derived from the International Panel on Climate Change (IPCC) evaluations of the continued rise in the atmospheric carbon dioxide content. Owing to the well-understood physico-chemical processes of ocean acidification, future pH predictions are especially robust, relying chiefly on the amount of CO_2 entering the atmosphere and the rate of oceanic absorption by the ocean.

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Introduction

Carbon dioxide (CO_2) levels in the atmosphere have now seasonally surpassed 400 ppm, a direct consequence of the increased levels of carbon dioxide emissions by human activities, including the burning of fossil fuels. Directly relating to these increases is the increased level of dissolved carbon dioxide in the ocean, which results from marine waters scavenging the gas from the atmosphere (Caldeira & Wickett, 2003). Over the past 100 years, the oceans have absorbed approximately one third of the carbon dioxide emitted by anthropogenic sources (Le Quere *et al.*, 2012). This absorption of the gaseous carbon dioxide can have great and predictable responses in the ocean water chemistry, affecting carbonate ion concentrations, calcite and aragonite mineral saturation levels, and eventually influencing the pH of the ocean water. The inorganic carbon cycle is particularly relevant when discussing ocean acidification (OA) for it includes the many forms of dissolved CO_2. When CO_2 dissolves, it reacts with water to form ions from the dissolved carbon dioxide: carbonic acid (H_2CO_3), bicarbonate (HCO_3^-), and carbonate (CO_3^{2-}; Figure 1). Each of these species' relative abundance is dependent upon other factors such as seawater temperature and alkalinity (Tyrrell, 2008a, 2008b).

Although the natural absorption of CO_2 by the world's oceans has helped mitigate the atmospheric climatic effects of increased CO_2, it is believed that increased levels in the ocean have caused a decrease in pH of approximately 0.15 units on the pH scale (Doney, 2006), or a 30% increase in acidity due to the logarithmic nature of this scale (Figure 3). Such a decrease is expected to have negative consequences for many species, as outlined below, due to the “skeleton” of these organisms being composed of calcite

and aragonite, two mineral forms of calcium carbonate. Currently these skeletons are stable in surface waters because the carbonate ion is present in supersaturated concentrations. However, as ocean pH falls further, so will the concentration of this ion. When carbonate becomes undersaturated, structures made of calcium carbonate are vulnerable to dissolution (Feely *et al.*, 2004). In the current ocean system, supersaturation occurs in the surface waters, with undersaturation developing in the deeper waters. The boundary line between the two is named the aragonite saturation horizon and is anticipated to creep nearer the surface as time progresses (Feely *et al.*, 2016).

An important aspect of this "other carbon dioxide problem" is that, unlike models of climatic warming which are based on complex mathematics of many forcings and feedbacks, heightened acidity in the ocean is fairly predictable. The mechanisms for increasing acidity are well-established, physical chemical processes: increasing carbon dioxide in the atmosphere will increase the amount dissolved in the ocean. The pH of the ocean is dependent on that amount of dissolved CO_2. The unknowns are merely the levels that atmospheric CO_2 will reach and the rate at which the surface ocean attains equilibrium with that level. As such, with continued fossil fuel usage, carbon dioxide will continue to increase in the atmosphere and the pH of the ocean will continue to decline.

A brief history of atmospheric concentrations of carbon dioxide and its effect on ocean acidity

Historically, the climate has fluctuated in concordance with atmospheric carbon dioxide concentrations, as evidenced in ice records containing air pockets of ~400,000 years old (Honisch & Hemming, 2005). One such ice core, the Vostok ice core, has been

analyzed over the past 50 years to determine carbon dioxide concentrations in ancient history. Several studies have been published on the data within the ice; one such study found that in the past 420,000 years, no CO_2 concentrations have surpassed our current 400 parts per million (ppm) of CO_2 (Petit *et al.,* 1999). Due to well-understood physic-chemical processes, a relationship between ocean pH and atmospheric carbon is established, allowing for the use of known principles to translate atmospheric CO_2 into pH values (Honisch *et al.*, 2012). To understand older carbon dioxide and thus pH levels, marine organismal proxies are commonly utilized, with foraminifera shells widely accepted. Oftentimes, stable isotopes from carbon may communicate the ancient pH and atmospheric carbon levels. A recent advancement utilizes boron isotopes incorporated into foraminifera tests (Sanyal *et al.,* 2000; Honisch *et al.,* 2012), but due to this chronometer's newness, some researchers suggest more parameterization is required to better constrain the information obtained (Pagani *et al.,* 2005). Others have argued that when careful data collection, management and analysis procedures are maintained, this tool is quite accurate (Honisch *et al.,* 2007). Within the scientific literature, it appears that researchers are slowly beginning to accept this new technique; one study compared their results from boron isotope procedures to the widely accepted standard of the Vostok ice core. They found that both methods co-varied strongly and followed similar atmospheric CO_2 concentrations, suggesting that when the organisms are properly cleaned and collected from well-preserved sediment cores, using only one species and all of the same size, this method is equal with ice core data (Honisch & Hemming, 2005). As such, our understanding of the atmosphere in older time periods may be expanded (Honisch *et al.,*

2012). When an epibenthic species of foraminifera incorporating high amounts of boron into their tests were used to determine whether there is any difference between interglacial and glacial pH values, the authors discovered pH and carbonate concentrations to be more similar than expected to interglacial periods (Honisch *et al.,* 2008). Other studies have suggested variation of 0.3 pH units (Sanyal *et al.,* 1995; 1997), but most authors suggest a difference between 0.07 and 0.1 pH units over millions of years (Figure 3). All in all, this time of earth's history contained bottom waters near 8.0 pH, quite close to today's values (Honisch *et al.,* 2008). Another study looked at a profile of middle Miocene to late Pleistocene epochs and found the pH of oceans to be similar to modern oceans (Palmer *et al.,* 1998), although during the Cenozoic era atmospheric CO_2 concentrations underwent wide and erratic swings (Pearson & Palmer, 2000).

Ocean pH has been pretty stable with increasing atmospheric CO_2 stability starting ~24 million years ago (Pearson & Palmer, 2000). Even so, interdecadal variations of pH have been observed on reefs in the Pacific Ocean, where they co-vary closely with the Interdecadal Pacific Oscillation (IPO) that changes every ~50 years. Using a 300 year old coral, they looked at boron isotope composition and suggest that these natural pH variations may help mitigate the effects of ocean acidification on this coral reef in the SW Pacific (Pelejero *et al.,* 2005).

Although it is known that ocean pH varies with atmospheric CO_2, there are some that suggest modern variation may be orders of magnitude faster than historic changes (Honisch *et al.,* 2012). Over the past 300 million years, carbon dioxide never topped 7500 ppm, with those changes in concentration occurring over millions of years in a gradual

fashion owing to tectonic and biological influences. Using three models, a generic ocean circulation model, a geochemical model, and a four box model, one study found the carbonate/pH system is best suited to buffering gradual changes in CO_2, with abrupt changes in CO_2 concentrations resulting in swings of ocean pH. Between the three models, the following factors were included: changes in temperature, sedimentation, and weathering; carbonate/silicate mineral weathering (terrestrial), production of these minerals in shallow water, production/oxidation of biogenic carbonate minerals, air-sea gas exchange (C), and transport of C via advection, mixing, and biological processes (Caldeira & Wickett, 2003).

A brief summary of biological impacts:

Within the past decade, single species studies studying the effect of ocean acidification have filled peer-reviewed journals, with a wide variety of taxonomies represented. Represented here will be a cursory synopsis of affected organisms based on broad taxonomic standing.

Phytoplankton: Although high CO_2 can primarily be beneficial for photosynthetic species (Burkhardt *et al.,* 2001; Rost *et al.,* 2003; Trimborn *et al.,* 2008), certain phytoplankton have reacted negatively to increased acidity, such as *Trichodesmium*, a genus of cyanobacteria that is prevalent throughout tropical and subtropical oceans. In laboratory studies, low pH environments of 7.8 revealed that growth and N_2-fixation significantly decreased as a result of decreased efficiency of the enzyme nitrogenase, which increased in concentration with decreased pH. Growth is highly dependent on limiting nutrients like iron, and may have synergistic limiting effects on colony growth.

In the center of large colonies, carbon dioxide may become the limiting factor and mitigate negative impacts of CO_2-derived acidity. In associated field studies, nitrogen fixation was again significantly reduced, especially in iron poor regions. This suggests the combination of iron poor and CO_2 rich regions may interact to reduce N_2-fixation more than in the presence of one variable. Since *Trichodesmium* contributes up to 50% of new nitrogen to marine systems, this decline in nitrogen may seriously affect organisms dependent upon this new nitrogen (Hong *et al.*, 2017).

Another type of phytoplankton, coccolithophore *Emiliania huxleyi*, is also likely negatively impacted by changes in acidity, as seen in laboratory experiments (Meyer & Riebesell, 2015). A recent mesocosm experiment within a Norwegian fjord supports these laboratory findings. As the most significant producer of calcium carbonate in pelagic zones, their population reduction could impact oceanic albedo, CO_2 uptake, and carbon flux. In this mesocosm experiment, not only did population size decline, but the survivors were unable to form blooms. These blooms, or exponential colony growth, currently occur anytime there are plentiful resources, but these findings reveal that in the future this may not happen. This loss of blooms meant a reduction of particle sinking velocities by 30% and organic matter dropping to sediment was reduced by 25% (Riebesell *et al.*, 2017). Such losses could significantly impact benthic organisms that are highly dependent on detritus filtering from above and thus contain trophic repercussions.

In the Southern Ocean off the coasts of Antarctica, researchers have initiated studies on diatoms evaluating the combination of ocean acidification and changes in irradiance. One such study looked at constant and dynamic light regimes combined with

pCO_2 of 390 and 1000 ppm in order to determine changes in growth, elemental composition, primary production, and photophysiology. It was found that under dynamic, or changing, light with increased pCO_2 (lower pH), diatoms (*Chaetoceros debilis*) experienced reduced growth and strongly reduced primary productivity. Additionally, energy transfer efficiency was substantially reduced, which affected the ability to transfer light energy into biomass (Hoppe *et al.,* 2015). In another study, three important diatom species (*Chaetoceros debilis*, *Fragilariopsis kerguelensis* and *Phaeocystis antarctica*) were tested for their response to the combination of low or high CO_2 and low or high irradiance. Despite increased CO_2, neither growth nor particulate organic carbon (POC) increased under either light scenario for any species; in fact, POC content declined in one (*F. kerguelensis*) and growth declined in another (*C. debilis*). Additionally, both of these species experienced decreased photochemical efficiency. As a result of these environmental changes, both species employed diverging photoacclimation strategies, with *C. debilis* increasing Chlorophyll a (Chl *a*) and fucoxanthin and *F. kerguelensis* decreasing Chl *a* and reducing rates of electron transport. The third species, *P. antarctica*, manifested more tolerance for increased acidity combined with irradiance changes (Trimborn *et al.,* 2017).

Zooplankton: One intensively studied zooplankton well known for its negative response to ocean acidification are pteropods. In particular, these organisms already experience shell dissolution in natural environments along the California coast, with severe dissolution expected to triple by 2050. As an important prey group for birds, fishes, and whales, their mortality due to increased CO_2 will significantly influence

trophic systems in high latitude regions (Bednarsek *et al.,* 2014). Since this system is naturally high in CO_2 due to various environmental factors such as upwelling and coastal circulation patterns, it was difficult to determine how much of these impacts are directly related to anthropogenic sources until recently. New data suggest that average surface carbon derived from anthropogenic sources is nearly twice the natural amount of carbon present, which has caused the natural aragonite saturation horizon to move up the water column by 30-50 m since the pre-industrial period. This has resulted in pteropod shell dissolution increasing between 19-26% since that time (Feely *et al.,* 2016).

Although prior studies set forth potentially neutral outcomes for calanoid copepods in the year 2100 at a pCO_2 of 1000 ppm (Weydmann *et al.,* 2012; McConville *et al.,* 2013), and others reveal lethal and sub-lethal effects only occurring at extreme pCO_2 levels that are beyond any climate scenarios (Watanabe *et al.,* 2006; Pascal *et al.,* 2010), a recent study suggests negative impacts on the juvenile stage (nauplii), a stage that had been previously ignored. They found mortality increased three times the current mortality under predicted pCO_2 for the year 2100 (1000 ppm). Other stages, including eggs and adult stages, did not share this trend until pCO_2 reached 3000 ppm. Since copepods are one of the primary means by which biomass moves up the food chain, meaning they are a primary food source, their sensitivity to OA will likely strongly impact trophic systems as well as biogeochemical cycles (Cripps *et al.*, 2015).

Although brine shrimp are not indigenous to marine systems, this small crustacean is often used as a model for determining stress responses on the zooplankton community as a whole. There are several species of brine shrimp, with A*rtemia salina*

being the most commonly used species for scientists and as feed in aquaculture. Although no significant changes in morphology were observed, hatching rates were significantly reduced, survival decreased, and growth slowed during later stages in comparison to control groups. Additionally, key enzymes were impacted by acidification stress, evidencing changes in activity in tests groups at 7.6 and 7.8 pH (Zheng *et al.*, 2015). Currently, the IPCC predicts a pH of 7.8 for average ocean values in 2100 (IPCC, 2007); 7.6 pH is a more localized pH prediction. In a different model species, *Artemia franciscana*, hatching rates were also reduced, most significantly at pH 7.0. In their highest pH test group of 7.6, the hatch rate was reduced compared with the control, but less drastically (Salma *et al.*, 2012).

Shellfish: Certain populations of shellfish have found to be susceptible to increased acidity in laboratory conditions (Gazeau *et al.*, 2013), whereas other populations have been more resistant, suggesting a species by species approach may be necessary for determining how much of this group will experience detrimental effects. In sea urchins, early developmental stages are the most at risk, with deformed physiology and delayed development occurring under IPCC climate scenarios for 2100, which projects ocean acidity will reach a lower limit of 7.5 pH (Zhan *et al.*, 2016). Oysters are a primary study group that illustrates high susceptibility to changes in acidity. One model bivalve, *Crassostrea virginica*, highlights these sensitivities. When exposed to pH 7.5, mortality significantly increased, with reduced shell mass in comparison to the control as well as reduced soft tissue mass (Beniash *et al.*, 2010). Many oyster species reside within estuaries and coastal regions, which fluctuate widely in daily pH values as the currents

and tides of the system changes. In another study with *Crassostrea virginica*, a long-term dataset of pH values for the Chesapeake Bay proves that pH is already commonly lower than the average ocean pH prediction of the IPCC for 2100 (IPCC, 2007). Their own laboratory data support the other findings, namely that after a change in pH of ~0.5 units, oysters are negatively impacted. However, they did suggest that increased salinity and warmer water temperatures helped offset the decrease in biocalcification (Waldbusser *et al.*, 2011). An earlier study highlights the sensitivity of early life stages for shellfish in general after evaluating the response of clam, oyster, and scallop species to predicted 21st century pH values (Talmage & Gobler, 2009). However, bivalves are not the only early life stage shellfish to experience negative impacts; at least one species of juvenile squid (*Doryteuthis pealeii*) had delayed development, reduced size, and abnormal statolith shape. This bone like structure is important for balance and motion detection (Kaplan *et al.*, 2012).

Fishes: As the most commercially important groups, a wide proportion of fish families have been studied in laboratory settings. One such species is the little skate (*(Leucoraja erinacea*). This skate is especially sensitive to temperature changes, but when researchers included increasing acidity, these impacts were significantly exacerbated on the organisms from a Gulf of Maine population. However, less of an impact was observed on the organisms collected from George's Bank slightly further south in North America. These findings prove that even within the same species, different populations may experience divergent effects of increased CO_2 (Santo, 2015).

In the liver and gill cells of cod, the effects of elevated CO_2 (hypercapnia)

resulted in decreased metabolism in liver cells, with depressed respiration in gill cells. Additionally, ion regulation became increasingly expensive in liver cells, but became less expensive for gill cells (Stapp *et al.*, 2015), revealing that varied responses exist within the cells of organisms. This may enhance, mitigate, or cancel out any organism's responses, but at the current time this is uncharted territory.

A population of Atlantic silversides (*Menidia menidia*) detected what may be better news. Over the breeding season, it was found this wild population exhibited seasonally changing responses to ocean acidity. Their native breeding grounds naturally become more acidic (pH 7.67) over the summer season, with young adapting to this change naturally. Young that were from the early offspring fared far worse in lower pH conditions compared with young from later batches. It was concluded there may be maternal provisioning or epigenetic transgenerational plasticity (TGP) occurring within the genome. If it is the latter, this is the first time it has been observed in a wild population, although it has been observed in laboratory settings (Murray *et al.*, 2014). TGP describes changes in the DNA that allows for organisms to adapt more quickly than previously thought. However, few other species have found such plasticity in acidic conditions, with far more displaying detrimental effects. Even so, scientists are finding some neutral effects, as with some tropical prey fishes and predators with their oxygen intake. Under hypercapnic conditions, there were few signs of oxygen deprivation, at rest or at maximum capacity (Couturier *et al.*, 2013).

Using natural daily variability of pH conditions on a coral reef, current reef fishes experience in short daily bursts higher CO_2 than what has been determined as critical CO_2

levels in a laboratory setting (~600 ppm). In current conditions of 400 ppm, the length of time exposed to this level of CO_2 is short enough to detect little effect on the fishes in this shallow reef system. However, both the exposure time and the magnitude of CO_2 will increase in the future, requiring further studies that look at pH values even lower than the average ocean pH predicted for the year 2100 (Shaw *et al.*, 2013).

One group of fishes that has been severely underrepresented in acidification studies are the scavengers. As the recyclers of the oceans, their reaction to increased acidity is an important component to maintaining the health of oceanic systems. Even less is known regarding abyssal creatures' responses, but at least one study has conducted an *in situ* experiment to determine whether deep-sea scavengers are affected by ocean acidification. The two scavengers studied (an eelpout and octopus species) determined no response to increased acidity, although the prey fishes all died, regardless of treatment (Barry & Drazen, 2007). Thus, at least two species of scavengers are potentially safe from increased CO_2, although follow up studies are needed to confirm these results. In general, fishes are able to compensate in the short-term to decreasing pH, but after chronic exposure, significant effects are seen across a variety of physiological systems. Over time, it is possible for them to adapt, but more studies are needed to assess this assumption (Heuer & Grosell, 2014).

One of the most iconic organisms that people associate with the ocean is the shark. Even this cartilaginous fish may not be entirely immune to the impacts of acidification, with studies discovering reduced odor tracking abilities (Dixson *et al.*, 2015), reduced growth, and impaired hunting behavior (Pistevos *et al.*, 2015). A third

study suggested some effects on neurophysiology, though no negative impacts were observed on growth, metabolic rate, aerobic scope, or skin morphology (Green & Jutfelt, 2014).

Marine mammals: Other predators of the sea, such as marine mammals, are also expected to feel the impacts of a warming ocean and increasing acidity. Although they may not experience direct effects to their physiology or behavior, they will experience changes in prey distribution. This is especially significant for species with limited habitat like the vaquita (*Phocoena sinus*), or species that live at high latitudes. Sea ice is an important habitat for narwhals, beluga whales, bowhead whales, and polar bears; changing sea temperatures are expected to further decrease its extent. Additionally, many baleen whale species migrate to the high latitudes to feed on plankton, of which many important species reveal high sensitivity to increased acidity (Simmonds & Isaac, 2007). As a quarter of the world's cetaceans are currently considered endangered, prey loss or change in habitat places further stress on an already stressed community (Simmonds & Eliott, 2009). One study suggested some harbor porpoises may be starving as a result in prey population declines (sand eels), stating that these populations have been reduced as a direct result of climate forcing (Macleod *et al.*, 2007). However, it is difficult to statistically say that porpoise starvation is a direct result of declining prey populations or climate forcing (Simmonds & Eliott, 2009).

Summary: Meta-analysis compiled on 228 studies on ocean acidification from various taxa determined how biological responses may affect the ocean as a whole. Looking at older meta-analyses, they discuss how responses are highly variable between

organisms and trophic levels, with differing responses between early and later life stages and geography. When they pooled taxa together, OA significantly affected growth, development, survival, abundance, and calcification. Upon separating them, corals, coccolithophores, and mollusks reveal the greatest average loss in calcification. When they included elevated temperature, the above variables were dampened, but were not statistically significant. They did detect that some fleshy algae and diatoms may marginally benefit from OA, though overall systems as a whole are negatively impacted (Kroeker *et al.,* 2013).

The ecological effects from ocean acidification:

Within the past ten years, more studies have focused on the effects of acidification throughout an ecosystem. Some have prioritized certain systems, such as coral reefs systems, whereas others evaluate cascading effects through trophic levels. These studies differ from biological studies in that physiological responses of individuals play a reduced role in determining how a system will respond as a whole to decreased pH (Ferrari *et al.,* 2015). Additionally, many of the studies incorporate warmer waters in order to simulate as much as possible natural environmental conditions of future oceans. In keeping with this goal of natural environmental conditions, mesocosm studies are the most common, where organisms are placed within an existing natural structure, such as a reef or fjord, and CO_2 is pumped into their netted cages.

Microbial: Due to the mixed responses of primary producers to ocean acidification, heterotrophic bacteria have the potential to be heavily influenced by changes in their feed availability. In environments already reduced in nutrients,

phytoplankton are the sole source for organic carbon (Hoikkala *et al.*, 2009). As such, increasing inorganic carbon does not mean increasing organic carbon for secondary consumers (Thingstad *et al.*, 2008), but may mean significant alterations to current biogeochemical cycles. A recent study has evaluated nutrient limited mesocosms in the Baltic Sea in an attempt to understand this underrepresented group in OA literature. Their data suggest that bacteria are indirectly impacted by acidification and may be altering the composition of the bacterial community, with further statistical analysis determining there will very likely be changes in functional groups of bacteria and phytoplankton. They conclude that the future ocean may have increasing autotrophic biomass during low nutrient periods, with decreased heterotrophic composition and biomass, ultimately decoupling phytoplankton and microbial dynamics. The paramount result of these changes would be impacts on the global carbon pump (Hornick *et al.*, 2017), although more research is required to quantify these impacts.

A review of papers highlighting the interaction of bacteria and OA offers some clues to potential effects on microbially-mediated processes. They found that bacterial primary production exhibits mixed responses to increased acidity, suggesting species-specific responses. In those species that respond well to OA, increasing eutrophication and anoxia may result in the surface oceans. Some N_2-fixers gained energy from increased carbon availability and as a result were able to increase nitrogen fixation (Das & Mangwani, 2015), although this is not true for all nitrogen fixers (Hong *et al.*, 2017). In other studies, trace gas emissions significantly decreased. One such trace gas is dimethyl sulfide (DMS), an important atmospheric gas that assists in the formation of

clouds by acting as a platform for water vapor condensation. This gas, a by-product of microbial metabolism, is directly sourced from marine bacteria and may decrease from indirect impacts of OA. The study also highlighted the changes in microbial diversity and competition as a direct result of pH changes, with pathogenic microorganisms significantly increasing. This also relates to biofilms, which are an important community structure that support microorganisms and eukaryotes. As they change in diversity, reverberations would be felt throughout the trophic levels (Das & Mangwani, 2015).

Quorum sensing (QS) is a method many species of bacteria utilize in order to communicate with host species such as corals, sponges, algae, and animals (Dobretsov *et al.*, 2009). In low pH environments, it appears that QS processes are affected, shifting the dominant bacterial species to sub-dominant positions (Krause *et al.*, 2012). This may result in higher order species composition also changing, as in the case of algae *Ulva* species, which respond to QS by increased spore settlement (Das & Mangwani, 2015).

Phytoplankton: In oligotrophic environments such as the Mediterranean Sea, the presence of limiting nutrients such as iron are more important to phytoplankton growth than increasing carbon dioxide amounts. However, when these limiting nutrients are freely available, increased carbon dioxide may influence phytoplankton populations. In a series of mesocosm studies in oligotrophic and mesotrophic environments, only limited benefits were observed from increased CO_2 during this short-term experiment. However, including vertical mixing and weather conditions over long-term studies may change these results (Gazeau *et al.*, 2017). In a long-term mesocosm experiment within the North Sea off of Sweden, total primary production did not increase, nor did photoacclimation,

although phytoplankton biomass increased under OA conditions. As a whole, their results suggest the phytoplankton community is affected by OA during certain seasons, which may affect overall ecosystem functions in the long-term (Eberlein *et al.*, 2017). Another study highlighting iron limited regions in the Weddell Sea in Antarctica found that iron richness with increased CO_2 resulted in significant taxonomic shifts. When iron was limited and CO_2 abundant, species shifts occurred, despite no change in primary production. As such, there are strong differences in species composition with changing CO_2 levels. The species that are more abundant in high CO_2 conditions are heavier species, which translates to faster sinking rates into the deep sea and may alter the current biological pump rates in certain areas of the Southern Ocean (Hoppe *et al.,* 2013). Such modifications could reverberate through the wider ecosystem, as the Southern Ocean is an important feeding ground for a wide variety of organisms. With faster sinking rates for the primary food source, food availability may be shortened.

Many open ocean regions in the world experience iron depleted conditions. Other studies have found that decreasing pH may ultimately reduce the bioavailability of iron in these same spots. Laboratory experiments with acidified water and diatoms reveal decreased iron uptake as is predicted according to iron chemical reactions. A secondary set of experiments in the Atlantic determined that diatoms and coccolithophores reduce iron uptake in natural environments enriched with CO_2. Since iron is an important mineral in photosynthesis, further reduction in its concentrations will increase iron stress in phytoplankton populations throughout oligotrophic regions (Shi *et al.,* 2010). A later study also focusing on bioavailability conducted a mesocosm experiment in the northeast

subarctic Pacific, a region of high nutrients and low chlorophyll concentrations. In acidified conditions, Chl *a* was significantly reduced and particulate organic carbon was reduced. Certain species of phytoplankton were reduced compared to other species (Melancon *et al.,* 2016), supporting earlier findings regarding species composition changes (Hoppe *et al.,* 2013). The reduction of Chl *a* would be a direct result of decreased iron availability due to its importance in photosynthetic processes. This set of researchers used iron rich dust in order to simulate natural iron deposition (Melancon *et al.,* 2016).

Overall, phytoplankton have exhibited varied responses to decreasing pH, making it difficult to determine a holistic response. In a meta-analysis publication, observations of diverse taxonomic groups were assembled to determine responses of phytoplankton as a whole. Their diverse range of responses were then incorporated into a global marine ecosystem model in order to determine how current phytoplankton populations may be impacted in future oceans. Their model revealed that OA is more potent than warming water or low nutrients (Dutkiewicz *et al.,* 2015).

Zooplankton: Far fewer studies have explored the ecological impact on zooplankton communities, but one recent report has begun to bring light into this void. A mesocosm study over 113 days off the coast of Sweden using end of the century CO_2 levels found that young copepods are the most sensitive organisms, with little to no effect on adults. They found community structure remained relatively stable, but the sensitivity of young copepods may affect biomass transfer to higher trophic levels in future oceans (Alguero-Muniz *et al.,* 2017).

Fishes: In a predator-prey relationship study, one juvenile prey species increased their maximum oxygen intake under acidified conditions, with one other juvenile prey species and their predator evidencing neutral effects. As such, near future oceans do not seem to have negative metabolic effects on any of these species. Additionally, it is easy to observe there are varied aerobic responses between species, with one juvenile prey even displaying augmented aerobic scope (Couturier *et al.*, 2013). This suggests that some fishes will not experience direct physiological aerobic stress. Another study looked at CO_2 vents in the northern and southern hemispheres and corroborated other studies' reduced predator avoidance, but they also found that in regions with shelter-rich habitats, this altered behavior does not truly impact their survival. Overall, altered abundances of predators and prey were observed, in addition to habitat availability. These changes could have wide reaching consequences throughout ecosystems (Nagelkerken *et al.,* 2015).

Beyond predator-prey interactions, at least one study determined that increasing CO_2 has significant impacts on sound absorption within ocean waters. Sound absorption in water is dependent upon dissolved oxygen levels, sulfur and nitrogen levels, pH, and temperature. One of the two buffering systems involved in regulating pH, the boric acid system, is more effective at absorbing low-frequency noises in higher pH waters. This means that in future oceans with a conservative pH decrease of 0.15 units would result in 20% reduction in sound absorption. Adding increased temperature of 3° C results in further loss between 5-10% (Hester *et al.,* 2008). Such changes could significantly alter underwater communication between a variety of organisms. It is already known that cetacean communication is disrupted from underwater ship communication (McQuinn *et*

al., 2011); with amplified noises in future oceans, this may be further accentuated.

Coral reef systems: A fair amount of research has been dedicated to the effect of OA on coral reefs. Since these systems are the most productive regions in otherwise oligotrophic waters, the potential loss of the foundation of this system, calcareous corals, would be detrimental to the entire region. Ecologically, these reefs are nurseries for a wide variety of important fishes. Beyond fishery importance, most reefs surround island nations in tropical waters whose primary revenue is highly dependent upon tourism (Macko *et al.*, 2017). Without these reefs, several island nations would lose tourist revenue as well as an important local food source. One recent study used an ecological-economic model to determine commercial fishery losses in reef systems, with a high emission, worst case scenario resulting in $40-69 billion lost globally (Speers *et al.*, 2016). Within OA studies, a wide variety of results have been observed, with some species increasing in sensitivity and others displaying greater resistance (Macko *et al.*, 2017). Using two coral species in one taxa and two calcified algae in another, one mesocosm study looked at their responses in Hawaii, French Polynesia, and Japan, where environmental conditions differ. These four species are common to reefs throughout the Pacific. Their results determine two coral species and one algae species were insensitive to OA, with the second algae species only sensitive in certain regions. Although warming temperature was not included, it appears that at least some common species will survive in future oceans and although OA may reduce biodiversity on reef systems, certain species may be resilient (Comeau *et al.,* 2014). Even so, another mesocosm study in southern Australia hypothesized that corals in general are currently experiencing

decreased calcification rates in comparison with pre-industrial rates. In order to determine this, they used sodium hydroxide (NaOH) to increase total alkalinity within a secluded lagoon and evaluated the response of corals in the lagoons. Over 15 days, NaOH was introduced to the system in order to restore total alkalinity to known pre-industrial values. Net community calcification increased as a result, suggesting they are currently experiencing reduced calcification rates and that certain isolated lagoons and bays may be able to undergo remediation via NaOH (Albright *et al.,* 2016). Beyond reef calcifiers, fishes in reefs may be sensitive to synergistic effects of temperature and elevated carbon dioxide. With both stressors present, some fish species evidenced much higher predation rates (30-70%) than present rates. Although one species consumed more oxygen under high temperature/CO_2, they did not experience greater predation, which is an unexpected finding. As such, predicting the effects of temperature and CO_2 on the community and ecological level is not as simple as extrapolating from prior physiological experiments (Ferrari *et al.,* 2015), accentuating the need for more mesocosm studies that incorporate heightened temperature and elevated CO_2.

Trusting Climate Models:

Comprehensively discussing climate models and their accuracy is beyond the scope of this paper, but an introduction to this topic is important for any conversation about the future state of the oceans. This final section is meant to introduce how models are developed, their parameterization, and how models are verified. Some models were introduced over twenty years ago and scientists may now assess their accuracy, which will also be briefly explored.

How models are developed and parameterized: A good portion of the complexity within models is due to the systems they are attempting to explain and then predict. An accurate assessment of forest systems, for example, requires knowledge of forest ecology, biogeochemical cycling, hydrology, radiative forcing, and a host of other biotic and abiotic factors. The same broad expanse of specialized knowledge is required for ocean models and includes information on ocean currents, atmosphere-ocean interactions, land-ocean interactions, biogeochemical cycling, geology, and more.

Starting near the middle of the 20th century, researchers began to build models to understand how aspects of a system affect other parts of the same or differing system. In ocean studies, some of the earliest models assumed a simple ocean without any circulatory dynamics. In 1987, at least one model began incorporating simple circulation, with atmospheric cloud layers, boundary layers, and solar radiation and its seasonal variation. They also used a mixed layer that accounts for heat distribution and movement in the upper 50 meters of the ocean. Sea ice formation and melting was also accounted, in addition to its associated effects on albedo and therefore solar heat absorption and loss (Wilson & Mitchell, 1987). Since then, models have only become more complex with the ability of computers to perform more advanced calculations in shortened time frames. The mathematics involved in each of the variables within a model are based on decades of field observation combined with theory that have then been tested against natural systems. Those formulas that have consistently tracked with natural observations are trusted as fairly good mathematical representations of natural events. Some equations are based on tried and true Newtonian physics formulas, with others based on other, trusted,

formulations. In the thirty years since 1987, each variable within models have been refined and verified via the scientific community at large, with continued refinement arriving in many publications, increasing our understanding of climate sensitivity at its upper and lower bounds (Knutti & Hegerl, 2008).

One extremely important parameter for climate models involves the pathways carbon travels through the ecosystem. The global carbon cycle is a familiar concept for anyone having taken an introductory biology course, but most are not familiar with the methods determining those pathways and how the mass of carbon moving through the systems are evaluated. Since carbon dioxide is a component of this cycle, accurately quantifying where carbon goes after entering the ocean is valuable information for creating an accurate climate model. However, even quantifying the global carbon cycle is a monumental task that requires detailed knowledge and quantifiable data in many sub-disciplines of environmental science, thus demanding a community of experts sharing their information and critiques. Fortunately, such a community exists, and has established a baseline for analyzing global movement of carbon. Their model of carbon cycling accounts for fossil fuel combustion, cement production, emissions associated with goods and services, deforestation, fire, and vegetation photosynthesis and respiration. In order to predict future changes in carbon amounts, they also determined the rates of change in land use, ocean sink, terrestrial sink, and atmospheric changes. Their study reveals carbon from human usage has increased steadily and at times abruptly between 1959 and 2011, with their model suggesting no abatement. Additionally, their work highlighted the importance of oceans as a sink for carbon dioxide (Le Quere *et al.*, 2012).

This work on the global carbon budget has significantly improved current understanding of the carbon cycle and thus less parameterization is required in this region. However, other large scale processes are unresolved and require usage of simplified physical models in order to include these processes within climate models. Each of these are able to be mathematically "tuned" in order to best fit past observations, since the mechanisms of these processes are not completely understood (IPCC, 2007).

Coupling atmosphere and ocean models: Evaluating the future ocean's well-being is dependent on not one, but two models- atmospheric and oceanic. Coupling the two models requires the same collaborative effort as with global climate models and many researchers have done so. Coupled models have been developed at least since the 1970's with moderate success at replicating current system dynamics (Bryan *et al.,* 1975) and have continued to undergo refinement as dynamic oceans are incorporated with sea ice (Wilson & Mitchell, 1987), and newer atmospheric developments are incorporated (Tardif *et al.,* 2014). More recently, researchers have been able to better quantify the effect of glacial processes on the rate of climate change (Ganopolski & Rahmstorf, 2001), with studies seemingly agreeing that more feedback mechanisms are activated (Cox *et al.,* 2000) and that the overall result is accelerating changes on a global scale (Ganopolski & Rahmstorf, 2001; Cox *et al.,* 2000).

Model verification and prediction accuracy: At this point in the scientific discussion, a wide plethora of studies have published their own model's predictions, refined parameters, evaluated local, regional, and global scales, and determined feedback mechanisms. Many of these suggest wide ranging negative consequences, accelerated

pace, and describe the culpability of humans using isotopic methodology for proof. However, a model's results must match reality for anyone to believe and react to this information; in short, these results must be verified. As a result, every respected published model has been verified against observational data pertaining to the correct scale and system (Bryan *et al.,* 1975; McNeil & Matear, 2008; Knutti & Sedlacek, 2012).

Even when a model is describing well-defined systems, with proper parameterization, and has been verified through more than one relevant dataset, a model may still be wrong. Nature still has the capability to follow undefined oscillations in atmospheric cycles, affecting a wide range of physical processes. With such complexity contained within global climate models, concern over their accuracy is legitimate and must be addressed. Some studies have collated several model predictions in order to determine the overall predictability of climate models, determining that although there are widespread projected CO_2 level pathways, this is a consequence of the climate system and cannot be reduced (Roe & Baker, 2007). Additionally, accuracy improves when models such as the CMIP5 (Coupled Models Intercomparison Project 5) are applied at or above the regional and decadal scale, due to averaging out of errors (Sakaguchi *et al.,* 2012). At this point in climate studies, some older models have made predictions that may now be compared to current climate. The authors took 18 climate models, split the dataset into 15 year sections starting in the 1950s, and determined how well they predicted El Nino (ENSO) oscillations in the Pacific Ocean. In general, they did very well (Risbey *et al.,* 2014). Climate models have been doing extremely well predicting global surface temperature for over 40 years, and in fact there are some studies that

suggest IPCC estimates are underestimating the effect of climate changes on the ocean, particularly in regards to sea level rise (Roe & Baker, 2007). Looking at just three simple climate models from climate scientist James Hansen reveal that his predictions in the 1980s track well with observed average global temperatures, with observations reasonably correlating with his moderate CO_2 scenario (Mann & Gaudet, 2017). Beyond these, the IPCC AR5 contains an entire chapter discussing the validity of climate models, evaluating how well they have performed and are performing, and conclude they are reasonably trustworthy (Flato *et al.*, 2013).

Summary and Conclusion:

The prospect of an acidifying ocean is one that civilization must directly confront in order to maintain current norms in economy, commercial fisheries, tourism, and ecological diversity. The very vastness of the ocean means any changes to its body will reach out and touch a wide variety of systems upon which humans and animals depend. From microbes to marine mammals, coral reefs to high latitude feeding grounds, and coastal cities to rural communities relying on ecotourism, many important aspects are likely to be affected. Based on the extensive work within climate modeling, we can accept certain high and low CO_2 predictions, allowing us to prepare our future generations for the likelihood of an altered environment and how that will affect our species. As we deal with a problem that may impact the trajectory of our known world, we cannot not be hesitant in action, for it is better to enact rational policies now than to wait until we pass the tipping point.

Figures:

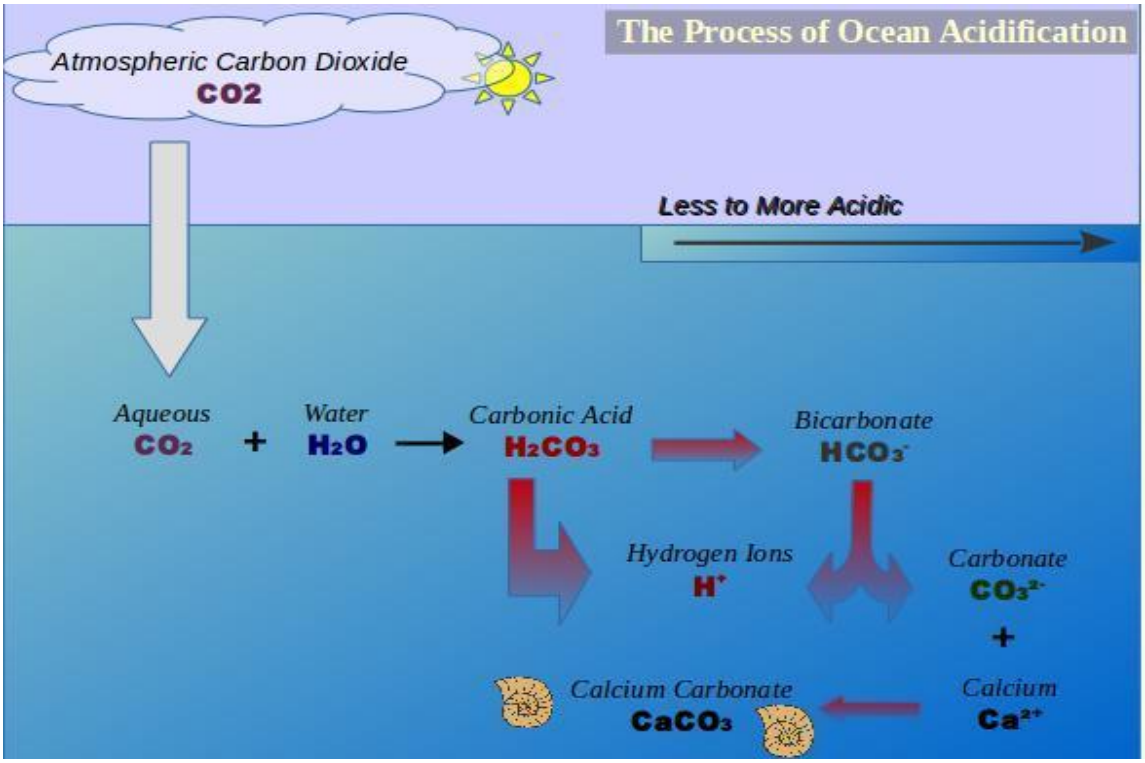

Figure II.1. The process of ocean acidification. As CO_2 enters the water, it reacts with water to produce carbonic acid. Carbonic acid then splits into hydrogen ions and bicarbonate ions. Bicarbonate then splits into hydrogen ions and carbonate ions. This increase in hydrogen results in increasingly acidic conditions.

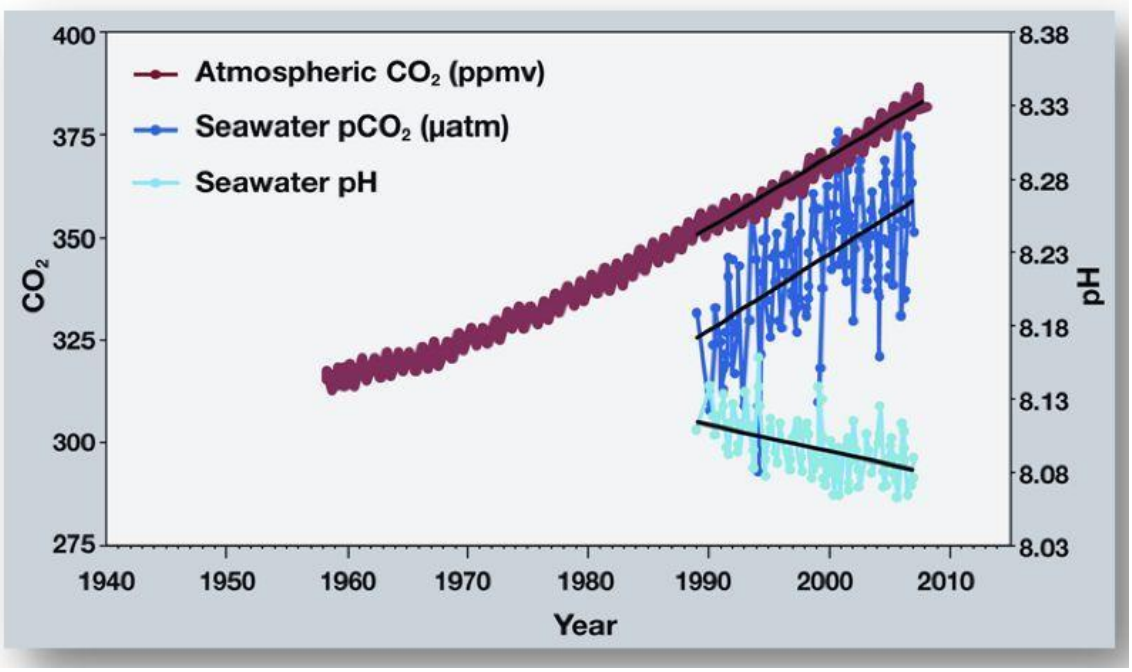

Figure II.2. Correlation between rising atmospheric concentration of carbon dioxide with increase in the concentration of carbon dioxide dissolved in seawater. Note seawater pH decreases since it is a negative logarithm function of the increasing carbon dioxide concentration. Figure courtesy the National Oceanic and Atmospheric Administration.

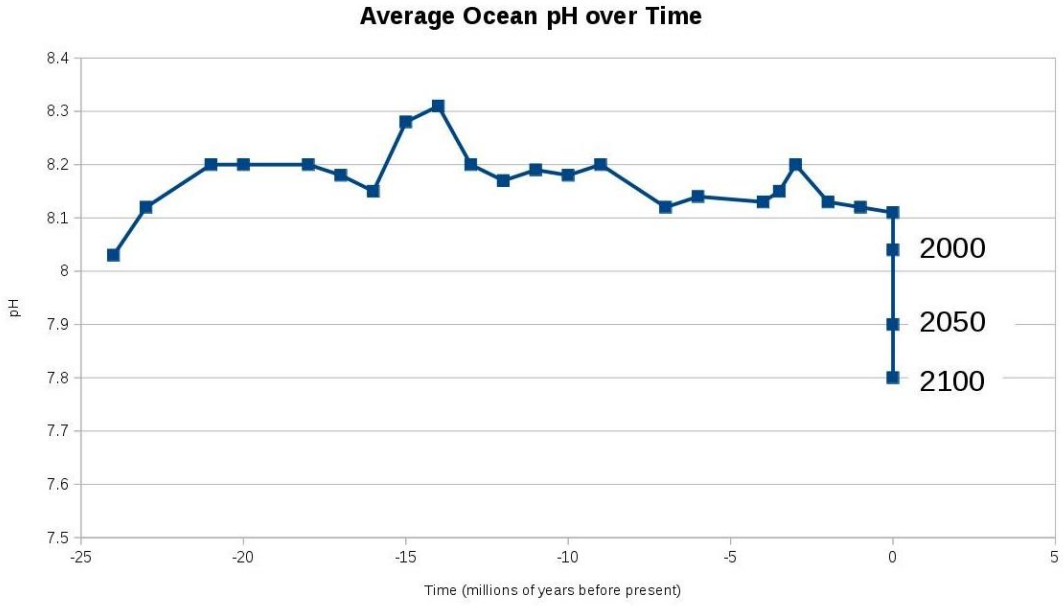

Figure II.3. Changes in average ocean pH over time. Includes expected pH up to 2100. Modified from Honisch and Hemming, 2005.

Literature Cited:

Alguero-Muniz, M., S. Alvarez-Fernandez, P. Thor, L. T. Bach, M. Esposito, H. G. Horn, U. Ecker, J. A. F. Langer, J. Taucher, A. M. Malzahn, U. Riebesell, & M. Boersma. 2017. Ocean acidification effects on mesozooplankton community development: Results from a long-term mesocosm experiment. *PLoS ONE*, 12:e0175851, https://doi.org/10.1371/journal.pone.0175851

Barry, J., & J. Drazen. 2007. Response of deep-sea scavengers to ocean acidification and the odor from a dead grenadier. *Marine Ecology Progress Series*, 350:193-207.

Bednaršek N, R. A. Feely, J. C. P. Reum, B. Peterson, J. Menkel, S. R. Alin, & B. Hales. 2014. *Limacina helicina* shell dissolution as an indicator of declining habitat suitability owing to ocean acidification in the California Current Ecosystem. *Proceedings of the Royal Society B*, 281:20140123, http://dx.doi.org/10.1098/rspb.2014.0123

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

Bryan, K., S. Manabe, & R. C. Pacanowski. 1975. A global ocean-atmosphere climate model. Part II. The oceanic circulation. *Journal of Physical Oceanography*, 5:30-46.

Burkhardt, S., G. Amoroso, U. Riebesell, & D. Sultemeyer. 2001. CO_2 and CO_3^- uptake in marine diatoms acclimated to different CO_2 concentrations. *Limnology and Oceanography*, 46:1378-1391.

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

Comeau, S., R. C. Carpenter, Y. Nojiri, H. M. Putnam, K. Sakai, & P. J. Edmunds. 2014. Pacific-wide contrast highlights resistance of reef calcifiers to ocean acidification. *Proceedings of the Royal Society B*, 281:20141339. http://dx.doi.org/10.1098/rspb.2014.1339

Couturier, C. S., J. A. W. Stecyk, J. L. Rummer, P. L. Munday, & G. E. Nilsson. 2013. Species-specific effects of near-future CO_2 on the respiratory performance of two tropical prey fish and their predator. *Comparative Biochemistry and Physiology, Part A*, 166:482-489.

Cox, P. M., R. A. Betts, C. D. Jones, S. A. Spall, & I. J. Totterdell. 2000. Acceleration of global warming due to carbon-cycle feedbacks in a coupled climate model. *Nature*, 408:184-187.

Cripps, G., P. Lindeque, & K. J. Flynn. 2015. Have we been underestimating the effects of ocean acidification in zooplankton? *Global Change Biology*, 20:3377-3385. doi: 10.1111/gcb.12582

Das, S. & N. Mangwani. 2015. Ocean acidification and marine microorganisms: responses and consequences. *Oceanologia*, 57:349-361.

Dixson, D. L., A. R. Jennings, J. Atema, & P. L. Munday. 2015. Odor tracking in sharks is reduced under future ocean acidification conditions. *Global Change Biology*, 21:1454-1462. doi: 10.1111/gcb.12678

Dobretsov, S., M. Teplitski, & V. Paul. 2009. Mini-review: quorum sensing in the marine environment and its relationship to bio-fouling. *Biofouling*, 25:413-427.

Doney, S. C. 2006. The dangers of ocean acidification. *Scientific American*, 294:58-65.

Dutkiewicz, S., J. J. Morris, M. J. Follows, J. Scott, O. Levitan, S. T. Dyhrman, & I. Berman-Frank.

2015. Impact of ocean acidification on the structure of future phytoplankton communities. *Nature: Climate Change*, 5:1002-1006. DOI: 10.1038/NCLIMATE2722

Eberlein, T., S. Wohlrab, B. Rost, U. John, L. T. Bach, U. Riebesell, & D. B. Van de Waal. 2017. Effects of ocean acidification on primary production in a coastal North Sea phytoplankton community. *PLoS ONE*, 12:e0172594, doi:10.1371/journal.pone.0172594

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

Feely, R. A. S. R. Alin, B. Carter, N. Bednarsek, B. Hales, F. Chan, T. M. Hill, B. Gaylord, E. Sanford, R. H. Byrne, C. L. Sabine, D. Greeley, & L. Juranek. 2016. Chemical and biological impacts of ocean acidification along the west coast of North America. *Estuarine, Coastal and Shelf Science*, 183:260-270.

Ferrari, M. C. O., P. L. Munday, J. L. Rummer, M. I. McCormick, K. Corkill, S.-A. Watson, B. J. M. Allan, M. G. Meekan, & D. P. Chivers. 2015. Interactive effects of ocean acidification and rising sea temperatures alter predation rate and predator selectivity in reef fish communities. *Global Change Biology*, 21:1848-1855, doi: 10.1111/gcb.12818

Flato, G., J. Marotzke, B. Abiodun, P. Braconnot, S. C. Chou, W. Collins, P. Cox, F. Driouech, S. Emori, V. Eyring, C. Forest, P. Gleckler, E. Guilyardi, C. Jakob, V. Kattsov, C. Reason and M. Rummukainen. 2013: Evaluation of Climate Models. In: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Stocker, T. F., D. Qin, G.-K. Plattner, M. Tignor, S. K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Ganopolski, A., & S. Rahmstorf. 2001. Rapid changes of glacial climate simulated in a coupled climate model. *Nature*, 409:153-158.

Gazeau, F., L. M. Parker, S. Comeau, J.-P. Gattuso, W. A. O'Connor, S. Martin, H.-O. Pörtner, & P. M. Ross. 2013. Impacts of ocean acidification on marine shelled molluscs. *Marine Biology*, 160:2207-2245.

Gazeau, F., A. Sallon, P. Pitta, A. Tsiola, L. Maugendre, M. Giani, M. Celussi, M. L. Pedrotti, S. Marro, & C. Guieu. 2017. Limited impact of ocean acidification on phytoplankton community structure and carbon export in an oligotrophic environment: Results from two short-term mesocosm studies in the Mediterranean Sea. *Estuarine, Coastal, and Shelf Science,* 186:72-88.

Green, L., & F. Jutfelt. 2014. Elevated carbon dioxide alters the plasma composition and behaviour of a shark. *Biology Letters*, 10:20140538. http://dx.doi.org/10.1098/rsbl.2014.0538

Hester, K. C., E. T. Peltzer, W. J. Kirkwood, & P. G. Brewer. 2008. Unanticipated consequences of ocean acidification: A noisier ocean at lower pH. *Geophysical Research Letters*, 35:L19601, doi:10.1029/2008GL034913

Heuer, R. M., & M. Grosell. 2014. Physiological impacts of elevated carbon dioxide and ocean acidification on fish. *American Journal of Physiology*, 307:R1061–R1084, doi:10.1152/ajpregu.00064.2014

Hoikkala, L., H. Aarnos, & R. Lignell. 2009. Changes in nutrient and carbon availability and temperature as factors controlling bacterial growth in the northern Baltic Sea. *Estuaries and Coasts*, 32:720–733. doi:10.1007/s12237-009-9154-z

Hong, H., R. Shen, F. Zhang, Z. Wen, S. Chang, W. Lin, S. A. Kranz, Y.-W. Luo, S.-J. Kao, F. M. M. Morel, & D. Shi. 2017. The complex effects of ocean acidification on the prominent N_2-fixing cyanobacterium *Trichodesmium. Science*, 356:527-531.

Honisch, B., & N. G. Hemming. 2005. Surface ocean pH response to variations in pCO_2 through two full glacial cycles. *Earth and Planetary Science Letters*, 236:305-314.

Honisch, B., N. G. Hemming, & B. Loose. 2007. Comment on "A critical evaluation of the boron isotope-pH proxy: the accuracy of ancient ocean pH estimates" by M. Pagani, D. Lemarchand, A. Spivack, & J. Gaillerdet. *Geochimica et Cosmochimica Acta,* 71:1636-1641.

Honisch, B., T. Bickert, & N. G. Hemming. 2008. Modern and Pleistocene boron isotope composition of the benthic foraminifer *Cibicidoides wuellerstorfi. Earth and Planetary Science Letters*, 272:309-318.

Honisch, B., A. Ridgwell, D. N. Schmidt, E. Thomas, S. J. Gibbs, A. Sluijs, R. Zeebe, L. Kump, R. C. Martindale, S. E. Greene, W. Kiessling, J. Ries, J. C. Zachos, D. L. Royer, S. Barker, T. M. Marchitto Jr., R. Moyer, C. Pelejero, P. Ziveri, G. L. Foster, & B. Williams. 2012. The geological record of ocean acidification. *Science*, 335:1058-1063. DOI: 10.1126/science.1208277

Hoppe, C. J. M., C. S. Hassler, C. D. Payne, P. D. Tortell, B. Rost, & S. Trimborn. 2013. Iron limitation modulates ocean acidification effects on Southern Ocean phytoplankton communities. *PLoS ONE*, 8:e79890. doi:10.1371/journal.pone.0079890

Hoppe, C. J. M., L.-M. Holtz, S. Trimborn, & B. Rost. 2015. Ocean acidification decreases the light-use efficiency in an Antarctic diatom under dynamic but not constant light. *New Phytologist,* 207:159-171. doi: 10.1111/nph.13334

Hornick, T., L. T. Bach, K. J. Crawfurd, K. Spilling, E. P. Achterberg, J. N. Woodhouse, K. G. Schulz, C. P. D. Brussaard, U. Riebesell, & H.-P. Grossart. 2017. Ocean acidification impacts bacteria-phytoplankton coupling at low-nutrient conditions. *Biogeosciences,* 14:1-15. doi:10.5194/bg-14-1-2017

Intergovernmental Panel on Climate Change. 2007. Fourth Assessment Report: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change [Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor and H. L. Miller (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Kaplan, M. B., T. A. Mooney, D. C. McCorkle, & A. L. Cohen. 2013. Adverse effects of ocean acidification on early development of squid (*Doryteuthis pealeii*). *PLoS ONE*, 8:e63714. doi:10.1371/journal.pone.0063714

Knutti, R., & G. C. Hegerl. 2008. The equilibrium sensitivity of the Earth's temperature to radiation changes. *Nature Geoscience*, 1:735-743, doi:10.1038/ngeo337

Knutti, R. & J. Sedlacek. 2013. Robustness and uncertainties in the new CMIP5 climate model predictions. *Nature Climate Change*, 3:369-373. DOI: 10.1038/NCLIMATE1716

Krause, E., A. Wichels, L. Giménez, M. Lunau, M. B. Schilhabel, & G. Gerdts. 2012. Small changes in pH have direct effects on marine bacterial community composition: a microcosm approach. *PLoS ONE*, 7:e47035, http://dx.doi.org/10.1371/journal.pone.0047035

Kroeker, K. J., R. L. Kordas, R. Crim, I. E. Hendriks, L. Ramajos, G. S. Singh, C. M. Duartes, & J.-P. Gattuso. 2013. Impacts of ocean acidification on marine organisms: quantifying sensitivities and

interaction with warming. *Global Change Biology*, 19:1884-1896, doi: 10.1111/gcb.12179

Le Quere, C., R. J. Andres, T. Boden, T. Conway, R. A. Houghton, J. I. House, G. Marland, G. P. Peters, G. van der Werf, A. Ahlstrom, R. M. Andrew, L. Bopp, J. G. Canadell, P. Ciais, S. C. Doney, C. Enright, P. Friedlingstein, C. Huntingford, A. K. Jain, C. Jourdain, E. Kato, R. F. Keeling, K. Klein Goldewijk, S. Levis, P. Levy, M. Lomas, B. Poulter, M. R. Raupach, J. Schwinger, S. Sitch, B. D. Stocker, N. Viovy, S. Zaehle, & N. Zeng. 2012. The Global Carbon Budget 1959-2011. *Earth System Science Data Discussions*, 5:1107-1157.

Macko, S. A., C. M. Fantasia, & G. Xue. 2017. Potential global economic impacts of ocean acidification. Center for Oceans Law & Policy: International Marine Economy. Eds. M.H. Nordquist, J.N. Moore, & R. Long. 20:79-97. doi: 10.1163/9789004323445_003

Macleod, C. D., M. B. Santos, R. J. Reid, B. E. Scott, & G. J. Pierce. 2007. Linking sand eel consumption and the likelihood of starvation in harbour porpoises in the Scottish North Sea: could climate change mean more starving porpoises? *Biological Letters*, 3:185 –188.

Mann, M., & B. Gaudet. "Validating Climate Models". Department of Meteorology and Atmospheric Science. Penn State College of Earth and Mineral Sciences. 2017. Web. 27 Sept. 2017.

McConville, K., C. Halsband, E. S. Fileman, P. J. Somerfield, H. S. Findlay, & J. I. Spicer. 2013. Effects of elevated CO_2 on the reproduction of two calanoid copepods. *Marine Pollution Bulletin*, 73:428-434.

McNeil, B. I., & R. J. Matear. Southern Ocean acidification: A tipping point at 450-ppm atmospheric CO_2. *PNAS*, 105:18860-18864. http://www.pnas.orgcgidoi10.1073pnas.0806318105

McQuinn, I. N., V. Lesage, D. Carrier, G. Larrivee, Y. Samson, S. Chartrand, R. Michaud, & J. Theriault. 2011. A threatened beluga (*Delphinapterus leucas*) population in the traffic lane: Vessel-generated noise characteristics of the Saguenay-St. Lawrence Marine Park, Canada. *Journal of the Acoustical Society of America*, 130:3661-3673.

Melancon, J., M. Levasseur, M. Lizotte, M. Scarratt, J.-E. Tremblay, P. Tortell, G.-P. Yang, G.-Y. Shi, H. Gao, D. Semaniuk, M. Robert, M. Arychuk, K. Johnson, N. Sutherland, M. Davelaar, N. Nemcek, A. Pena, & W. Richardson. 2016. Impact of ocean acidification on phytoplankton assemblage, growth, and DMS production following Fe-dust additions in the NE Pacific high-nutrient, low-chlorophyll waters. *Biogeosciences*, 13:1677-1692.

Meyer, J. & U. Riebesell. 2015. Responses of coccolithophores to ocean acidification: a meta-analysis. *Biogeosciences*, 12:1671–1682.

Murray, C. S., A. Malvezzi, C. J. Gobler, & H. Baumann. 2014. Offspring sensitivity to ocean acidification changes seasonally in a coastal marine fish. *Marine Ecology Progress Series*, 504:1-11.

Nagelkerken, I., B. D. Russell, B. M. Gillanders, & S. D. Connell. 2015. Ocean acidification alters fish populations indirectly through habitat modification. *Nature Climate Change*, 6:89-93, DOI:10.1038/NCLIMATE2757

Pagani, M., D. Lemarchand, A. Spivack, & J. Gaillardet. 2005. A critical evaluation of the boron isotope-pH proxy: the accuracy of ancient ocean pH estimates. *Geochimica et Cosmochimica Acta*, 69:953-961.

Palmer, M. R., P. N. Pearson, & S. J. Cobb. 1998. Reconstructing past ocean pH-depth profiles. *Science*, 282:1468-1471.

Pascal, P.-Y., J. W. Fleeger, F. Galvez, & K. R. Carman. 2010. The toxicological interaction between ocean acidity and metals in coastal meiobenthic copepods. *Marine Pollution Bulletin*, 60:2201-2208.

Pearson, P. N. & M. R. Palmer. 2000. Atmospheric carbon dioxide concentrations over the past 60 million years. *Nature,* 406:695-699.

Pelejero, C., E. Calvo, M. T. McCulloch, J. F. Marshall, M. K. Gagan, J. M. Lough, & B. N. Opdyke. 2005. Preindustrial to modern interdecadal variability in coral reef pH. *Science*, 309:2204-2207.

Petit, J. R., J. Jouzel, D. Raynaud, N. I. Barkov, J.-M. Barnola, I. Basile, M. Benders, J. Chappellaz, M. Davis, G. Delaygue, M. Delmotte, V. M. Kotlyakov, M. Legrand, V. Y. Lipenkov, C. Lorius, L. Pepin, C. Ritz, E. Saltzman, & M. Stievenard. 1999. Climate and atmospheric history of the past 420,000 years from the Vostok ice core, Antarctica. *Nature,* 399:429-436.

Pistevos, J. C. A., I. Nagelkerken, T. Rossi, M. Olmos, & S. D. Connell. 2015. Ocean acidification and global warming impair shark hunting behaviour and growth. *Nature: Scientific Reports,* 5:16293. DOI: 10.1038/srep16293

Riebesell, U., L. T. Bach, R. G. J. Bellerby, J. R. B. Monsalve, T. Boxhammer, J. Czerny, A. Larsen, A. Ludwig, & K. G. Schulz. 2017. Competitive fitness of a predominant pelagic calcifier impaired by ocean acidification. *Nature Geoscience,* 10:19-24.

Risbey, J. S., S. Lewandowsky, C. Langlais, D. P. Monselesan, T. J. O'Kane, & N. Oreskes. 2014. Well-estimated global surface warming in climate projections selected for ENSO phase. *Nature Climate Change*, 4:835-840. DOI: 10.1038/NCLIMATE2310

Roe, G. H., & M. B. Baker. 2007. Why is climate sensitivity so unpredictable? *Science,* 318:629-632. DOI: 10.1126/science.1144735

Rost, B., U. Riebesell, S. Burkhardt, & D. Sultemeyer. 2003. Carbon acquisition of bloom-forming marine phytoplankton. *Limnology and Oceanography*, 48:55-67.

Sakaguchi, K., X. Zeng, & M. A. Brunke. 2012. The hindcast skill of the CMIP ensembles for the surface air temperature trend. *Journal of Geophysical Research,* 117:D16113. doi:10.1029/2012JD017765

Salma, U., Md. H. Uddowla, G. Lee, Y. Yeo, & H.-W. Kim. 2012. Effects of pH change by CO_2 Induction and Salinity on the hatching rate of *Artemia franciscana*. *Fisheries and Aquatic Sciences,* 15:177-181.

Santo, V. D. 2015. Ocean acidification exacerbates the impacts of global warming on embryonic little skate, *Leucoraja erinacea* (Mitchill). *Journal of Experimental Marine Biology and Ecology,* 463:72-78.

Sanyal, A., N. G. Hemming, G. N. Hanson, and W. S. Broecker. 1995. Evidence for a higher pH in the glacial ocean from boron isotopes in foraminifera. *Nature,* 373:234-236.

Sanyal, A., N. G. Hemming, W. S. Broeker, & G. N. Hanson. 1997. Changes in pH in the eastern equatorial Pacific across stage 5-6 boundary based on boron isotopes in foraminifera. *Global Biogeochemical Cycles*, 11:125-133, DOI: 10.1029/97GB00223

Sanyal, A., M. Nugent, R. J. Reeder, & J. Bijma. 2000. Seawater pH control on the boron isotopic composition of calcite: Evidence from inorganic calcite precipitation experiments. *Geochimica et Cosmochimica Acta,* 64:1551-1555.

Shaw, E. C., P. L. Munday, & B. I. McNeil. 2013. The role of CO_2 variability and exposure time for biological impacts of ocean acidification. *Geophysical Research Letters,* 40:4685-4688.

Shi, D., Y. Xu, B. M. Hopkinson, & F. M. M. Morel. 2010. Effect of ocean acidification on iron availability to marine phytoplankton. *Science*, 327:676-679. DOI: 10.1126/science.1183517

Simmonds, M. P. & S. J. Isaac. 2007. The impacts of climate change on marine mammals: early signs of significant problems. *Oryx,* 40:19-26. doi:10.1017/S0030605307001524

Simmonds, M. P. & W. J. Eliott. 2009. Climate change and cetaceans: concerns and recent developments. *Journal of the Marine Biological Association of the United Kingdom,* 89:203-210.

Speers, A. E., E. Y. Besedin, J. E. Palardy, & C. Moore. 2016. Impacts of climate change and ocean acidification on coral reef fisheries: An integrated ecological-economic model. *Ecological Economics,* 128:33-43.

Stapp, L. S., C. M. Kreiss, H. O. Pörtner, & G. Lennig. 2015. Differential impacts of elevated CO_2 and acidosis on the energy budget of gill and liver cells from Atlantic cod, *Gadus morhua. Comparative Biochemistry and Physiology, Part A,* 187:160-167.

Talmage, S. C., & C. J. Gobler. 2009. The effects of elevated carbon dioxide concentrations on the metamorphosis, size, and survival of larval hard clams (*Mercenaria mercenaria*), bay scallops (*Argopecten irradians*), and Eastern oysters (*Crassostrea virginica*). *Limnology and Oceanography,* 54:2072-2080.

Tardif, R., G. J. Hakim, & C. Snyder. 2014. Coupled atmosphere-ocean data assimilation experiments with a low-order climate model. *Climate Dynamics*, 43:1631-1643. DOI 10.1007/s00382-013-1989-0

Thingstad, T. F., R. G. J. Bellerby, G. Bratbak, K. Y. Borsheim, J. K. Egge, M. Heldal, A. Larsen, C. Neill, J. Nejstgaard, S. Norland, R.-A. Sandaa, E. F. Skjoldal, T. Tanaka, R. Thyrhaug, & B. Töpper. 2008. Counterintuitive carbon-to-nutrient coupling in an Arctic pelagic ecosystem, *Nature Letters,* 455:387–391. doi:10.1038/nature07235

Trimborn, S., N. Lundholm, S. Thoms, K. U. Richter, B. Krock, P. J. Hansen, & B. Rost. 2008. Inorganic carbon acquisition in potentially toxic and non-toxic diatoms; the effect of pH-induced changes in seawater carbonate chemistry. *Physiologia Plantarum,* 133:92-105.

Trimborn, S., S. Thoms, T. Brenneis, J. P. Heiden, S. Beszteri, & K. Bischof. 2017. Two Southern Ocean diatoms are more sensitive to ocean acidification and changes in irradiance than the prymnesiophyte *Phaeocystis antarctica. Physiologia Plantarum,* 160:155-170.

Tyrrell, T. 2008a. Impacts of changing ocean chemistry in a high-CO_2 world. *Mineralogical Magazine*, 72:329-362.

Tyrrell, T. 2008b. Calcium carbonate cycling in future oceans and its influence on future climates. *Journal of Plankton Research*, 30:141–156.

Waldbusser, G. G., E. P. Voigt, H. Bergschneider, M. A. Green, & R. I. E. Newell. 2011. Biocalcification in the Eastern oyster (*Crassostrea virginica*) in relation to long-term trends in Chesapeake Bay pH. *Estuaries and Coasts,* 34:221-231. doi: 10.1007/s12237-010-9307-0

Watanabe, Y., A. Yamaguchi, H. Ishidai, T. Harimoto, S. Suzuki, Y. Sekido, T. Ikeda, Y. Shirayama, M. M. Takahashi, T. Ohsumi, & J. Ishizaka. 2006. Lethality of increasing CO_2 levels on deep-sea copepods

in the western North Pacific. *Journal of Oceanography*, 62:185-196.

Weydmann, A., J. E. Soreide, S. Kwaśniewski, & S. Widdicombe. 2012. Influence of CO_2-induced acidification on the reproduction of a key Arctic copepod *Calanus glacialis. Journal of Experimental Marine Biology and Ecology*, 428:39-42.

Wilson, C. A., & J. F. B. Mitchell. 1987. A doubled CO_2 climate sensitivity experiment with a global climate model including a simple ocean. *Journal of Geophysical Research*, 92:13,315-13,343.

Zhan, Y., W. Hu, W. Zhang, M. Liu, L. Duan, X. Huang, Y. Chang, & C. Li. 2016. The impact of CO_2-driven ocean acidification on early development and calcification in the sea urchin *Strongylocentrotus intermedius. Marine Pollution Bulletin*, 112:291-302.

Zheng, C.-q., J. Jeswin, K.-l. Shen, M. Lablche, K.-j. Wang, & H.-p. Liu. 2015. Detrimental effect of CO*2*-driven seawater acidification on a crustacean brine shrimp, *Artemia sinica. Fish & Shellfish Immunology*, 43:181-190.

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