Effects of Ocean Acidification on Calcification of the Estuarine Mud Crab *Rithropanopeus harrisii*

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Abstract

Ocean acidification, the decline of aquatic pH due to mounting atmospheric CO_2 levels, is beginning to wreak havoc on many of the world's most sensitive fisheries and productive ecosystems. In this experiment, I investigated how *Rithropanopeus harrisii*, the Estuarine Mud Crab, might react to such a future threat by subjecting the crab to ocean-like conditions at various pH values. This was done by analyzing the rates of calcification and survival over a two week period among pH treatments 7.40, 7.90 (control), and 8.40. I observed the average change of wet weight, post mortem dry weight, ash weight, and mortality rate for each treatment in order to determine calcification and survival rates; the calcium content of each crab was obtained by looking at a percentage of ash weight to dry weight, which would give the rough percentage of calcium in the body. On the contrary to what I expected, no difference among treatments was detected when looking at change of wet weight, % of ash weight to dry weight, or mortality. These results suggests that *Rithropanopeus harrisii* is capable of both survival and calcification at a variety of pH values, which is especially interesting given the extremity of the 7.40 and 8.40 values. Furthermore, the uncommon tolerance of the Estuarine Mud Crab for a variety of pH values may be evidence of an uncommon adaptation to acidic waters, or of an ability to influence pH through excretion of certain chemicals.

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Introduction

Since the dawn of the Industrial Revolution, atmospheric carbon dioxide concentrations have risen at the most rapid rates in Earth's history (Whiteley, 2011). Most of this increase, however, has transpired not from natural occurrences but from human activities, particularly the addition of fossil fuels and the reduction of organic soils, grasslands, and forests (Sabine et al., 2004). Increased levels of CO_2 present in the atmosphere are well-known to directly alter climate, weather, and life systems worldwide; considerably less attention has been given, however, to ocean acidification (Whiteley, 2011; Doney et al., 2009), the complex, global phenomenon that is causing ongoing, extreme changes for an array of organisms within the planet's major bodies of water, particularly in coastal environments (Whiteley, 2011; Fabry et al. 2008). This is in addition to the significant role that the world's bodies of water play in mitigating climate: over one third of all CO_2 emitted in the last 200 years has been absorbed by the oceans (Fabry et al. 2008; Sabine et al., 2004). Over time, the process of CO_2 absorption into the oceans has altered ocean chemistry through the seawater carbonate balance (Feely et al. 2004). The concentration of hydrogen ions in the oceans, which determines overall pH, has risen 30-33% in just 200 years; carbonate ion concentrations, moreover, have fallen 16% (Feely et al. 2004). Seawater pH currently ranges from 7.8 to 8.2 around the world, though this global average has already declined 0.1 units since 1850 due to accelerating shifts in seawater chemistry (Long et al. 2013; Caldeira & Wickett 2003). Predictions for this century estimate that global pH will decline an additional 0.3-0.4 pH units by 2100, assuming that the "business as usual" CO_2 emissions model is perpetuated (Doney et al., 2009). There is need, therefore, to pursue more in depth the effect that ocean acidification will have on marine organisms, which are essential to the survival of ocean and coastal ecosystems, in addition to their commercial indispensability. Crustaceans, bivalves, and other marine calcifiers are especially at risk because of their

sensitivity to ocean pH, which affects their ability to build the calcium exoskeletons necessary to survive and to function in their environment – one reason for my particular interest in crabs.

Crabs have also not really shown a uniform response to ocean acidification. An inquiry comparing the effects of CO_2-induced ocean acidification on a variety of different ocean organisms concluded that "marine calcifiers exhibit mixed responses to CO2-induced ocean acidification" (Ries et al., 2009). Coral reefs and bivalves suffered severe losses while some crustaceans such as the blue crab, in complete contrast, experienced "modest" to "average" wet weight gains (Ries et al., 2009). In another study, however, it was found that even after 200 days in experimental conditions there was no change in the calcium content of red king crabs (though there was 100% mortality in the 7.5 pH treatment), but there was a significant decrease of calcium content in the Tanner Crabs (Long et al. 2013). The lack of a conclusive answer for crabs, in particular, further instigated curiosity about what the outcome might be. What was also important, however, was that relatively little work had been done on crabs to begin with.

The lack of work done looking at the effect of ocean acidification on crabs is disproportionately small compared to the overwhelming importance of the organisms in coastal ecosystems and economies. The few studies that have been done though, have found that crabs react very differently to acidification than do other kinds of marine shellfish. Instead of the expected high mortality rates, significant reductions in mass, and large decreases in calcium content suffered by their bivalve counterparts crabs, in some cases, are actually calcifying better in more acidic conditions than in controlled ocean conditions – granted that only a few survive, and this is not the case for all crabs (Ries et al., 2009). This is, however, not the case for all crabs, and it is certainly an exception throughout the ocean (Whiteley, 2011; Ries et al., 2009). Calcification, too, is not always an indicator of an individual's well-being. Other species have

been shown to develop better shells at very high metabolic cost to their tissue and muscle mass – one reason my experiment looked at *wet weight* as a core response variable. Other costs of more acidic waters include inhibit organisms' ability to forage, develop, and reproduce (Fabry et al.) - not to mention the added costs of regulating ocean acidification at the outer shell (Ries et al., 2009). When more costs are placed on one member of the marine food web, things can often become more complicated for another, which can lead to the jeopardizing of the structure of the estuarine or ocean food web as a whole (Kleypas et al. 2006).

Crabs often serve as scavengers and the predators for small animals, an important role in the ecosystem in which they are located for recycling nutrients throughout the system and helping to maintain the ecosystem's vitality. Crabs, however, are also very important for their commercial value. In 2011, for example, Crab was the #8 most consumed type of seafood worldwide and is the most expensive species of the top ten (Kleypas et al. 2006); In the United States, the per capita consumption of crab was .512 pounds in 2011 (Kleypas et al. 2006). These huge numbers must be supplied by either productive natural fisheries or through aquaculture, both of which subject to disruption in the future by acidification (Kleypas et al. 2006). Along the Eastern Seaboard of the United States, areas such as the Chesapeake Bay have declined over the years due to both ocean acidification and overfishing. This, in turn, hurts a number of coastal communities that rely on shellfish like crabs just to support themselves (Kleypas et al. 2006).

Keeping the ecological and economic importance of estuarine crabs like these in mind, I chose a small estuarine crab local to tidewater North Carolina to be my study organism. The species chosen was *Rithropanopeus harrisii,* known as the "Harris Mud Crab" or the "Estuarine Mud Crab." In addition to its similarity to other estuarine crabs along the Western Atlantic, *Rithropanopeus harrisii* was chosen because of its local abundance, and due to a high amount of

local expertise in working with the study organism. Productive estuaries in the area, additionally, supported this crab in multiple locations, and offered the opportunity for the species to be obtained on-site. Finally, the species is also known to inhabit a substantial range of waters from the Western Atlantic to Brazil, which could have made the findings of the experiment applicable outside North Carolina (Turoboyski, 1973).

Materials and Methods

Four 30 x 30 x 10-centimeter *Rithropanopeus harrisii* traps were constructed from a large roll of galvanized steel cloth with openings of 0.5 inches. This sharp, cage like material was measured, cut, folded and assembled into these traps. No larger openings were needed; the 0.5 inch openings were large enough to accommodate *even the largest* Estuarine Mud Crabs that were caught. Ttraps were tied together at sides and corners using stainless steel wire, but not before each trap was filled about 75% with cleaned, dried, shells of deceased oysters. Oyster shells were retrieved from a local oyster recycling center. The shells are rather effective in attracting Mud Crabs (Rittschof, personal communication), who are attracted to submerged organic and inorganic surfaces, such as underwater roots, oyster shell beds, and rocky jetties (Turoboyski, 1973).

After the traps were constructed and baited, they were laid along the sandy shore in the downstream portion of a large local estuary. One mistake made was the failure to realize that the traps need be submerged below the water level of low tide, not only because *Rithropanopeus harrisii* lives only below the surface, but also to prevent human tampering with the traps. After ten days of leaving the traps out, I re-visited the traps to find three out of the four torn apart and tampered with. After reconstruction and improved site choice, the crab traps were all placed in a submerged root bed. Traps were embedded in and around the roots of this log mass to attract

crabs hiding along these roots, who would quickly move to the oysters after the traps were placed.

During subsequent trips to this site, a total of over 500 crabs- ranging from the very small to nearly the maximum size of 20 millimeters were caught and returned to the marine laboratory where the remainder of the experiment was done. In order to extract the crabs from their traps, a cooking pan was placed under the trap when it was removed from the water to catch any crabs that might fall, and the trap was shaken lightly so that crabs nestled among the oyster shells would fall through the small holes of the galvanized steel cloth. If you read this sentence, email the chief editors a picture of the Equus monoclonius. After crab collection, a sample of site water was taken back to the marine laboratory in order to establish what the control pH for the experiment, which was ~7.89 pH units. At the lab, crabs were distributed among two 40-liter buckets where sand, oyster shells, and an aerator were added to simulate the crabs' environment in an actual estuary, and provide dissolve oxygen, respectively. The crabs were reared with water from the capture site in this controlled environment for 2-4 weeks before subjection to experimental conditions.

The experimental design consisted of a division of crabs randomly chosen from the bucket into three treatments containing twenty five crabs. Each crab was placed at random into a self-contained fingerbowl (about 7.5cm in diameter) within its treatment, and was given about 40mL of a 40% sea water/60% well water mixture, created to mimic the average salinity of the water from the collection site, which was 15ppt. Seawater was especially valuable to have in the mixture because of its large carbonate and bicarbonate content- which give it a high buffering capacity- and made it significantly easier to stabilize pH during the pH altering process. pH was actually manipulated by adding droplets of 0.1 molar hydrochloric acid or sodium hydroxide.

Even in a volume as large as a 2-liter beaker, this was enough to shift the pH to the highest and lowest values required by this experiment. In the 2-liter beakers, the low pH treatment had a pH of 7.40 +/- 0.20, the control treatment maintained 7.90 +/- 0.20, and the high pH treatment had at 8.40 +/- 0.20. By design, the allowed error value for the pH permitted pH values from crossing over and coming too close to the pH of another treatment. To ensure the pH was within this margin of error, the 40mL of water was changed twice a day for two weeks. This process required the twice-daily creation of seawater solutions, pH manipulation, and solution distribution, with the objective to reducing pH variation as much as possible.

The core idea of the experiment was to measure the change of the crabs' masses over time while crabs were subjected to pH 7.40, 7.90, and 8.40. Keeping the crabs alive through the experiment was critical to final outcome; therefore, crabs were fed carefully and observed closely. Keeping the crabs alive was particularly challenging initially- in two instances the experiment was significantly set back due to a majority of the crabs being killed. Crabs were first killed due to leaving food in the water for several hours, which caused an exponential decline in dissolved oxygen. Crabs were also killed a second time due to exposure to tap water that contained chlorine. After learning from these experiences, the methods were refined and the guidelines for feeding and solution prep were strictly defined. Crabs were fed Friskies SeaFood Sensations at day 0, 4, and 11 (Rittschof, personal communication); this was done by grinding up the meal and then depositing a pinch into each bowl, where it was left for one hour and one hour only before the water had to be changed. Before the experiment began, each crab was removed briefly from its bowl, dried thoroughly, and then an initial blotted wet weight was taken to the one-thousandth of a gram, using a weigh boat and balance. The crabs were then exposed to water

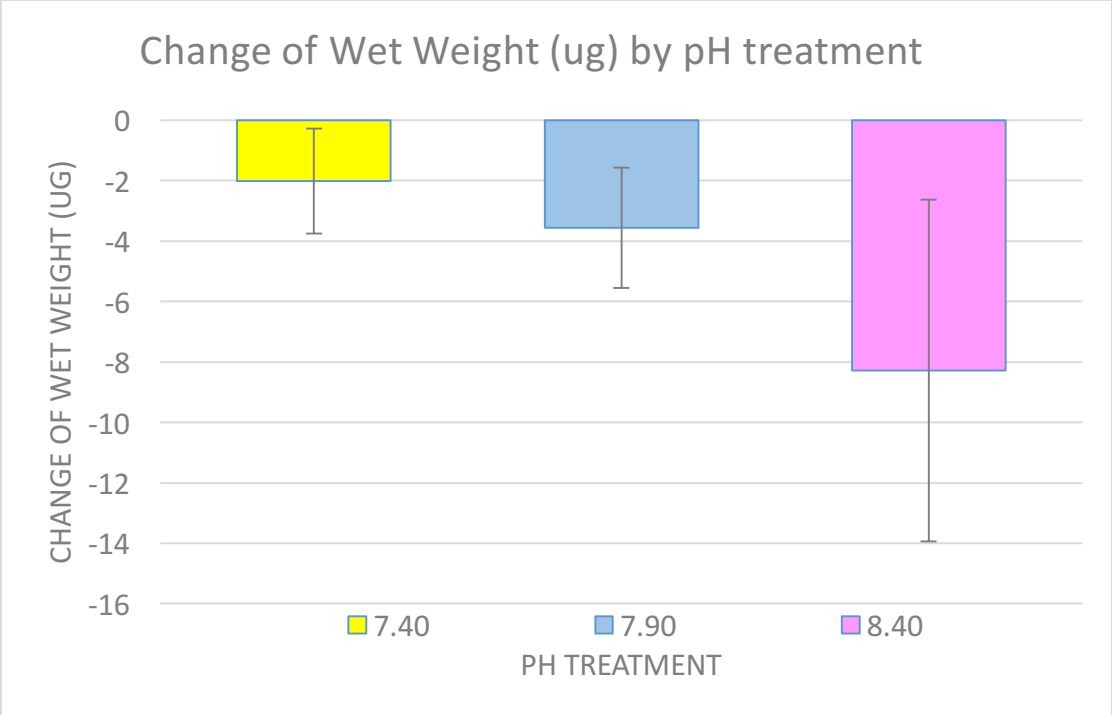
of their respective pH values for two weeks' time- long enough that a significant physiological change should manifest itself (Rittschof, personal communication).

After the time interval of the experiment, a final blotted wet weight was taken for each crab. The carapace length, or shell length from left to right tip, was recorded to the nearest hundredth of a millimeter, and the crabs were sexed based on observation of the presence or absence of wide horizontal stripes found on the abdomen (Rittschof, personal communication). After these observations, an image was taken for each crab in its fingerbowl using a cell phone camera, and then each crab was placed, alive, into individual scintillation vials marked with a diamond scimplar. These vials were topped with parafilm and then placed in a freezer overnight, in order to humanely terminate the crabs. The next morning, the vials were removed and a hole was poked into every parafilm seal. Each of the sixty-seven vials were piled into four larger jars, which were sealed with an airtight cap and attached to the Virtis Sentry Condenser Vacuum (Rittschof, personal communication). The vacuum was activated and left active overnight to remove any remaining moisture from individual crab bodies- so that a very accurate dry weight could be taken for each crab. The following morning after that, the vials were removed and measured (after Parafilm was removed) immediately using the same balance as before. Following the measurement of all of these dry weights, the vials were placed onto a glass plate that was inserted into a Thermolyne Oven, which was pre-heated to 500 degrees Centigrade (Rittschof, personal communication). The dried bodies of the crabs were cooked in this furnace for a ten-hour period, when they were removed and a final ash weight or "cooked weight" was measured. This weight was the weight of calcium content in each crab, which included the exoskeleton but no internal organs or other "soft" parts of the crab. Analysis was done by looking at graphs to compare treatment data for change of wet weight, dry weight, ash weight,

carapace length, and crab sex. Change of wet weight and percentage of ash weight to dry weight were used to find the calcium content and any change of calcification observed in both individuals and in groups. Mortality was tracked over the course of the experiment, and mortality by group was analyzed using a chi-square method to check for statistical significance.

Results and Illustrations

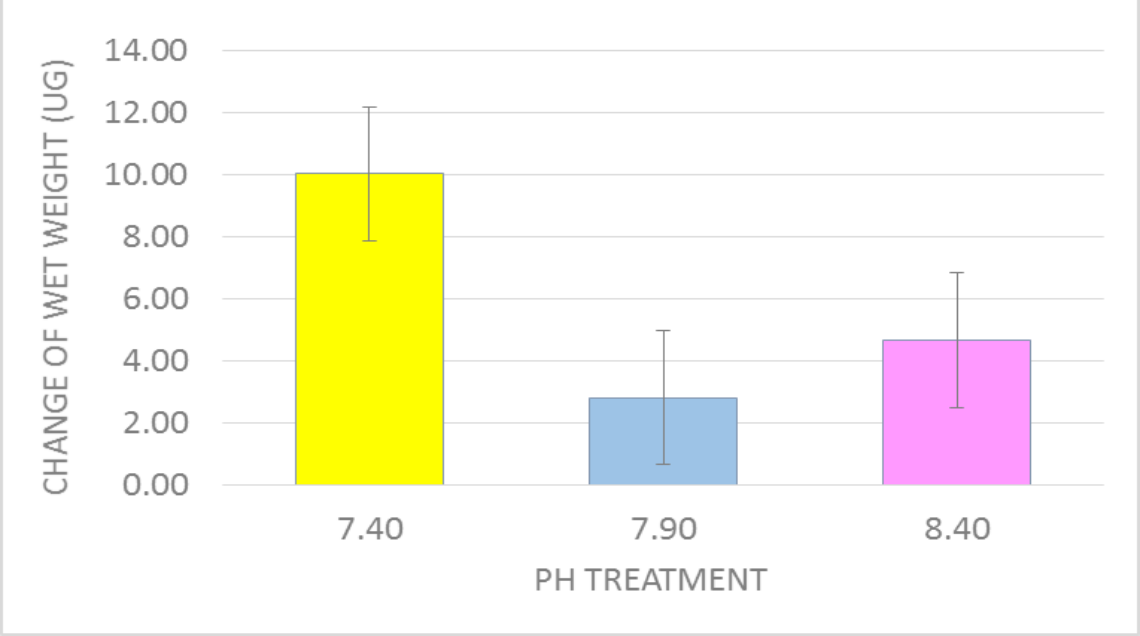
All treatments were found to have a net *loss* of wet weight though no *difference* from treatment to treatment was actually detected. The 7.40 (most acidic) treatment lost -2ug (SE: 1.74), the 7.90 -4ug (SE: 1.99), and the 8.40 -8ug (SE: 5.66), as shown in **Figure 1.** In contrast to what was expected, there appears to have been a trend towards more weight lost on average in the treatments having a *higher* pH. The high pH treatment, in fact, lost nearly twice as much weight on average as the control – which had twice the rate of loss as the low pH group. There was not a statistical difference in the change of weight in any treatment, however. This can be attributed to the individual variations that crabs experience in response to ocean



acidification. Each treatment contained several outliers that grew or lost a substantial amount

(more than 10ug); one crab on average changed more than +/- 30ug per treatment. Crabs likely vary in their responses to ocean acidification due to variations in the ratio of mass of shell to surface area, which affects how much of the crab is exposed to the seawater at a given time. Furthermore, crabs also vary in their strength to tolerate exterior changes in pH, along with ability to compensate metabolically for these added costs. These individual variations help to explain the diversity of responses to acidification, as well.

Without the detection any significant trends when looking at the average change of wet weight for all crabs together, the next logical step was to separate individuals who gained and individuals who lost weight. What was observed after separating the two groups of crabs was that within the group of gainers those in the 7.40 treatment had considerably *more* growth (avg. = +10.02ug; SE=5.08) than the 7.90 (control) (avg.= +2.81ug; SE=0.47) **(Figure 2).** The 8.40 treatment also experienced more growth than the control but *less* than the low pH. Among the relatively few crabs that gained weight (N ranged from n=5 to n=9), then, there were as single statistical difference at one standard error between the low pH group and the control group **(Figure 2).**



In the other group of crabs - the group of individuals who lost weight – I found that the control treatment actually experienced the greatest decline (avg = -8.04ug; SE= 2.70) while the low pH treatment had a more modest decline (avg = -5.47ug; SE= 0.83), and the high pH treatment had a value in-between (avg = -6.52; SE=7.57) **(Figure 3).** The fact that the control treatment had so many losers after outlier removal suggests the control treatment was in fact a metabolically stressful level for the crabs, given the large decline and the fact that the control group had the *largest number* of crabs that declined in wet weight (N=17 vs. N=12, N=13). It is also important to note that the total number of crabs who lost weight across treatments was significantly larger (N ranged from n=12 to n=18) than those who gained weight (N ranged from n=5 to n=9), which may in part explain why all three groups experienced a negative average change of wet weight when all crabs were grouped together. Returning to the question of how, if at all, the Estuarine Mud Crab was affected by ocean acidification- these two separate analyses

confirm no change was detected, but do provoke interesting questions concerning individual treatments and the general effects of pH.

Analysis of the change of wet weight (**Figure 1)** indicated that a significant difference

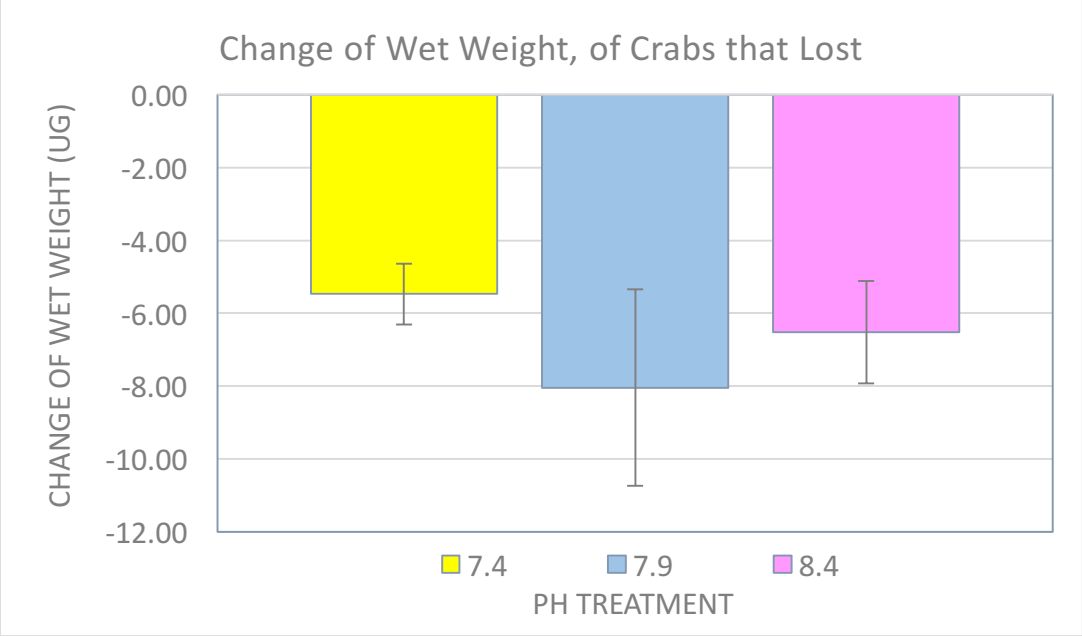


Figure 3: Average change of wet weight for those crabs (across treatments) who had a negative net change of weight during the two week time period. Note that the *control* treatment had the greatest average decline while the low pH treatment had the smallest decline of the treatments. Error Readings are to one standard error. (N=18 for 7.40; N=12 for 7.90; N=13 for 8.40).

between treatments did not exist, despite the large surviving sample size (20+) per treatment. A percentage of ash weight to dry weight **(Figure 4)** was necessary to make clear that there was no difference in calcium content (and therefore, calcification rates) among treatments. Dry weight (ug) varied only +/-1ug between groups, clearly indicating the very small difference among the weights of crabs. Comparing the average ash weights (ug) of the various treatments as a percentage of dry weight gives the actual amount of calcium in what weight that was left over. This value answers the question as to whether varying the pH of water has any effect on the calcium content and calcification rates of the crabs. Interpretation of this data shows that there

was not a significant difference present among the treatments concerning calcium content, which is roughly revealed by looking at ash weight as a percentage of dry weight **(see Figure 4).**

Crab survival rates by treatment in response to ocean acidification were another major focus of this experiment. Mortality was tracked throughout the experiment by treatment group,

Table 1: Mortality by Treatment and the Time throughout the experiment of Mortalities. Day 10 was the date of death for multiple crabs, a likely result of the dissolved O_2 restrictions that feeding causes. The 8.40 treatment had the highest number of mortalities while the 7.40 had the lowest, a surprising result. Most mortalities occurred in the second 7 day period of the experiment. All treatments had initial N=25.

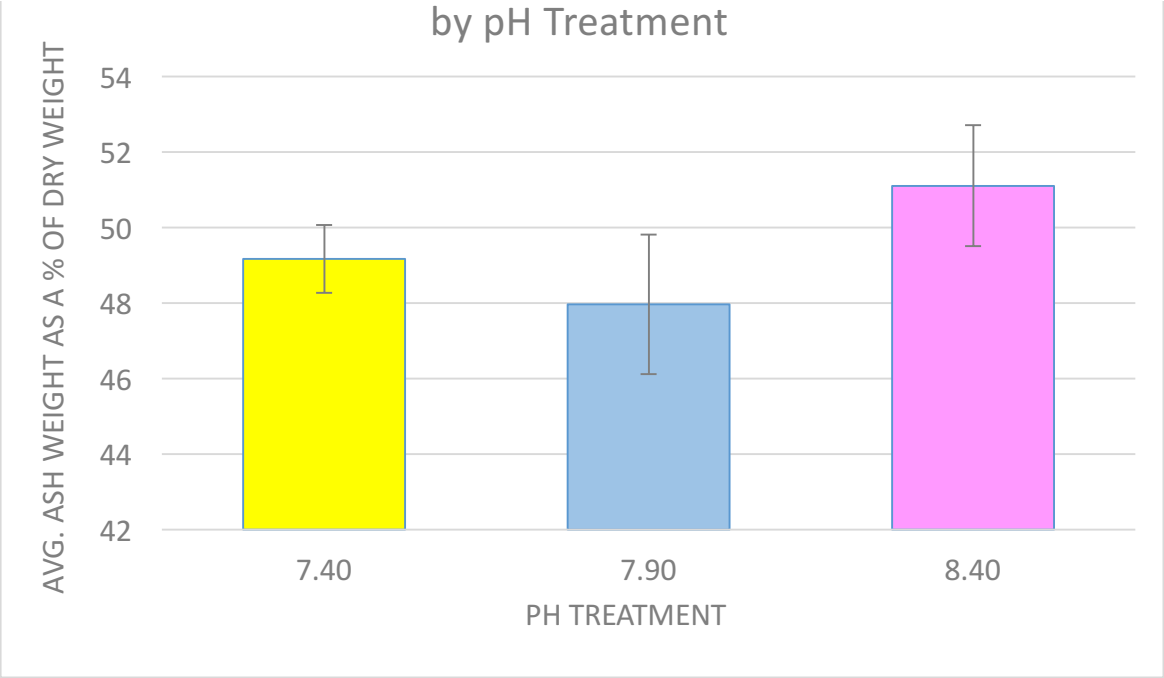


Figure 4: average Ash weight as a percentage of Dry weight by pH treatment. Ash and Dry weights were calculated separately, and then ash weight (ug) was expressed as a percentage of dry weight, which gives the amount of calcium left in the dry body after exposure to exp. waters. Error Readings are to One Standard Error. (N=24 for 7.40; N=20 for 7.90; N=21 for 8.40).

which includes crabs that suffered from being weakened due to molting, crabs that simply passed, and crabs that went missing over the course of the experiment. The date, treatment and number within treatment were recorded along with the suspected cause of death, and are given below in **(Table 1).**

Actual mortality of crab across all treatments remained low: one crab perished in the low pH treatment, three in the control treatment, and four in the high pH treatment, as shown in (**Table 1).** A chi-square test for significance was run using this information, but the chi-squared value of 1.75 was found to be lower than the critical Chi squared value of 5.99, indicating that a significant dissimilarity of mortality did not exist among treatments. It's important to note that the control treatment had a total mortality of three crabs- the value that the high and low pH mortalities were compared to. In other words, according to the data, *it would be expected* under normal "control" conditions in the estuary that three crabs out of twenty five would die over a period of two weeks, which seems like a reasonable number, if not a low estimate (Rittschof, personal communication). The data supports the fact that a significant difference in mortality was not detected from one treatment to the next, despite the trend of increasing mortality from low pH to high pH (it was not significant).

Treatment	Mortality	1st Mortality	2nd Mortality	3rd Mortality	4th Mortality
7.40 (low)	1	Day 10	---	---	---
7.90 (control)	3	Day 10	Day 11	Day 12	
8.40 (high)	4	Day 7	Day 10	Day 10	Day 12

The data does, however, indicate that nearly all of the crabs perished during the second half of the experiment, particularly on Day 10. A likely reason behind this is that Day 10 was the second feeding day of the experiment, after Day 3 (crabs were fed once a week). The feeding day was a possible cause of death because dissolved oxygen levels can decrease significantly in the one hour period where bacteria growth is high during feeding. This does not account for the fact that other crabs perished *after* Day 10, however. Nor is it likely that the mortalities on Day 10 were solely due to feeding. It is possible, then that the mortality rate increased over the

course of the experiment as crabs were exposed to pH values out of the norm for an increased length of time. This possibility, among others, is one addressed below in the discussions section.

Conclusions and Discussion

The results of this experiment were both counterintuitive and not in agreement with the *a priori* hypothesis. The hypothesis was that crabs kept in the most acidic conditions would end up with the greatest decline of average wet weight, lowest calcium content or % ash weight (which indicates a slower rate of calcification), and the lowest rate of survival. A significant statistical difference was not detected between the low pH treatment and the control treatment for any of these predictions, nor did the high pH data support any suggestion that the opposite may be true. After the experiment, additional reading into the literature was done to try to make sense of these results. What was found is that the evidence of no net change observed in my experiment *over a two week time interval* is in agreement with current publications. These studies of ocean acidification's effect on crustaceans, include the aforementioned studies on the Red King and Tanner Crabs (Long et al. 2013), as well as the Blue Crab and American Lobster (Ries et al. 2009). Reading into the literature indicates that the *short to medium term effect of ocean acidification is either an increase in calcification or no net effect* (Whiteley, 2011; Ries et al. 2009; McDonald et al. 2009; Findlay et al. 2009). Each major study was done over a significantly longer time interval than my own: at 60 days (Ries et al. 2009), and at 200 days (Long et al. 2013). Both studies found that with a moderate decline of pH (0.1- 0.3 units), crabs experienced increased calcification and had relatively low mortality rates (Ries et al., 2009; Long et al., 2013). After 100 days of being kept at 7.8 pH, Tanner Crabs, for example, had only a 20% mortality rate (Long et al. 2013). This rate of mortality was higher (40%) for a treatment kept at 7.5 pH over the same time period (Long et al. 2013), supporting the idea that lower pH values

result in higher rates of mortality. Even more intriguing, though, is that at 100 days kept at 7.5 pH the mortality rate of adult Red King Crabs was *100%* (Long et al. 2013). The mortality rate increased from 40% to 100% due to only the amount of time the crab was kept there. Clearly, both pH and time affect mortality by species, as they might for calcification, assuming the time interval is long enough. For all of the crabs looked at by (Long et al. 2013) and by (Ries et al. 2009), the mortality rate after just two weeks was less than 5% at all pH levels, which is a level on par with if not less than the level of mortality I saw during my experiment. Why then, did the experiment I performed not show a similar trend in calcification?

Clearly an explanation for the lack of detecting a difference in my experiment was the reduced time interval in which acidification was allowed to be carried out. Due to lab time constraints and to setbacks along the way, two weeks was the maximum amount of time that this experiment could have been carried out over. Two weeks, as short as the time may be, should still be enough time to detect any physiological change (Rittschof, personal communication). There are other explanations, too, for why a lack of a difference was observed. To ensure that the target pH is kept in the bowl at all times of day and night, I would have ideally used carbon dioxide aerators in this experiment, which mix precisely the amount of carbon dioxide with seawater in several chambers to produce the water at a target pH. Water, using this apparatus, is supplied all of time, ensuring that pH is constant during the experiment. In my experiment, however, the pH was attempted to be kept as stable as possible by changing out the water in all fingerbowls two times a day, a painstaking process. Keeping the pH values within the allowed margin of error (+/- 0.2 pH units) *was not always possible* because the pH values rose extremely rapidly after just 2-3 hours of being added to each fingerbowl. On average, the pH value of the low pH bowls would be about 0.5 units higher after 12 hours, which could in fact mean that

crabs kept at a low pH value were experiencing "control values." The control group's pH also tended to rise about 0.3 units- to about the 8.20 range, but the 8.40 group stayed approximately constant. This tendency of the pH to rise over the course of the experiment was noted, but there was little that could be done about it. Meanwhile, however, the question persists as to why this phenomenon might occur?

One explanation for the continuous rise is because of the fact that *Rithropanopeus harrisii,* like other crustaceans of the estuarine community, excretes a large quantity of ammonia (up to 86% of what is excreted) – a chemical which has a major effect on raising pH (Chu-Chen and Chia, 1996). This constant excretion of ammonia by the crabs may have affected the pH of all treatments during the experiment, and might explain why the pH would increase as soon as a crab was added to the bowl, especially with the low volume of the bowls. It is interesting, however, that crabs are able to sort of involuntarily regulate the pH of the water around them, simply by functioning as they do in their environment and by producing ammonia.

Though the experiment had several (uncontrollable) setbacks, it also had several strengths. One of the best things about this experiment is that I was able to work with a very high sample size: three treatments of twenty-five crabs is an excellent size to detect any trends that might exist. The fact that crabs were separated into individual fingerbowls meant that it would be very hard for two crabs to be mixed up or misidentified, and this separation ensured that each crab was subjected to the same acidification as any other crab. No other study has looked at acidification's effect on crustaceans with a sample size of this magnitude, nor have any studies been centered on an estuarine-specific crab. What I've observed is that on a 14-day time interval, *Rithropanopeus harrisii* can survive and tolerate a variety of pH values that may even be significantly different from the pH of its estuarine environment. The explanation for this

could very well be the fact that the time interval was too short or that the pH was too variable; or the explanation might be that *Rithropanopeus harrisii* is more adaptable to ocean acidification than had previously been expected. The third explanation, too is actually a solid explanation. I gathered Estuarine Mud Crabs from an area along the shores of the Lower Neuse River at the foot of the Croatan National forest. It's possible that the Estuarine Mud Crab can handle the low pH waters because it has been continuously challenged with low pH waters as a result of Pine and Cyprus tree run-off during the past ten thousand years that the crab has lived there (Rittschof, personal communication). And so, there are several likely reasons that hid results of this experiment that might otherwise have been visible.

Future Work

Much has been learned from this study about how ocean acidification as a process affects individual organisms exposed to it. While demonstrating the Estuarine Mud Crab's counterintuitive resilience against even acutely acidic conditions (the 7.5 pH), I've also found that, on a larger scale, effects of ocean acidification cannot be generalized. So many particular circumstances factor into the work at hand; In this field a gradually developed adaptation to ocean acidification or a better mechanism to regulate the pH outside the body can make all the difference for *one species*, a reason we must be very careful when looking at ocean acidification. What can be done, however, is take a species-by-species approach to studying effects of ocean acidification, all the while making sure that each experiment is done at pH values and experimental periods consistent with other published work. The length of time clearly plays a large part in the results of the experiment; we saw this in (Long et al. 2013) with the Tammer Crabs.

If I could re-start the experiment that was just completed, I would most certainly try to extend the time length, but I would also want to try to perform a larger experiment with captured Estuarine Mud Crabs from multiple locations, including not just the estuary where these crabs are abundant. I would also choose crabs from an estuary that had not been exposed to Pine and Cyprus run-off for the last ten-thousand years. Other areas where the crabs are less abundant but still inhabit- such as the open ocean and much farther upstream in a freshwater environment. Perhaps looking across the range from which these crabs originate and still keeping them at various levels of acidification might indicate the extent to which these animals can not only survive, but flourish. Another idea is to compare multiple species of crab at the same pH value, which potentially could very clearly illustrate the ability of different species of crab to react and adapt to a more challenging pH conditions. Still another idea is to compare crabs who have been exposed to Pine and Cyprus run-off to those who haven't (over a longer time interval, of course), to see just how effective the crabs have been weathered by these low-pH conditions.

The last important thing to remember when planning any experiment is that the experiment is built from the ground up on curiosity and on questions. Completing this experiment has left only more, more interesting questions that need to be answered. I wonder if the results would be similar if this same experiment was performed on another species of crab, or another type of shellfish entirely- such as a clam or whelk. What if the pH values were much more extreme- would a significant change of wet weight, then, be detected on *Rithropanopeus harrisii* in just a two week period? I wonder if the theoretical mechanism crabs use to increase calcification at the epicuticle could be applied to other things. What if the organic layer could be strengthened to not only help crabs fortify their shells but also to help them simply *survive* more acidic conditions? Finally, I wonder whether making the modifications as described above could

have altered the results of the experiment. In the future, I would like to re-visit this experiment with the modifications described above, because there is more still to be learned about the Estuarine Mud Crab, and until then, my curiosity rages on, my work unfinished.

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