Journal of Young Investigators RESEARCH ARTICLE

The Effects of Increased Acidity on the Shell Integrity and Body Size of *C. Virginica*: A Comparison of Oyster Populations in Northeast Florida

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Ocean acidification is the process by which the world's oceans absorb carbon dioxide and results in the formation of carbonic acid, which decreases the level of pH. This process increases the acidity of the water and threatens many different calcifying organisms such as corals, sea urchins, whelks, crabs, and oysters. This study evaluated: (i) the difference between specimens of *Crassostrea virginica* (whole organism mass and length and tissue mass) in two natural pH conditions consisting of an area considered to be polluted and the other not, with respect to tissue mass and whole organism mass and pH of the location (ii) the effect of altered pH level in decreasing increments on half shell mass and length of *C. virginica* in a laboratory setting. The results of the field data of this experiment found no substantial relationship between environmental pH and tissue size ratio. This study also did not find a substantial difference in either location between the average mass and length of oyster half shells in the control pH conditions versus oyster half shells in the experimental pH condition. Therefore, the data suggests the short-term immediate exposure to increased acidity does not induce a substantial decline in shell integrity for *C. virginica*.

INTRODUCTION

Human influence has increased the amount of carbon dioxide (CO_2) in the atmosphere by 36% over the past two centuries while hydrogen ions (which when raised result in a higher level of acidity) present at the ocean's surface have increased by about 30% (Miller et al. 2009; Orr et al. 2005; Potera 2010; Wood et al. 2008). Overall, pH of the ocean's surface has gone down (become more acidic) by 0.1 unit of pH after the occurrence of the industrial revolution and is projected to decrease by an additional 0.3 to 0.5 units of pH by 2100 (Miller et al. 2009; Ries et al. 2009; The Royal Society 2005). The measurement of pH quantifies the acidity of a liquid by the concentration of hydrogen ions found in the solution and is measured logarithmically. If the concentration of H^+ increases by 10-fold then the pH will go down by 1 unit (The Royal Society 2005). Within the last 200 years, the world's oceans have absorbed about one third of all CO_2 produced by the production and use of fossil fuels, destruction of the world's forests, production of cement, and an increase in building, thus resulting in ocean acidification (Miller et al. 2009; The Royal Society 2005; Wood et al. 2008). Ocean acidification occurs when the ocean absorbs and dissolves CO_2 from the atmosphere. The reaction between CO_2 and seawater produces positively charged hydrogen ions (H^+) as a result of the formation of carbonic acid (H_2CO_3; Orr et al. 2005; The Royal Society 2005). However, not all CO_2 that has dissolved in the ocean creates carbonic acid, as reflected by Henry's Law, stating that the concentration of dissolved CO_2 remains proportional to the CO_2 in carbonic acid (Jackson 2010; Potera 2010; Riebesell 2008; The Royal Society 2005; Ruttiman 2006). While the ocean has absorbed an estimated one third of this carbon dioxide, it has come with a great cost to marine ecosystems (Miller et al. 2009). Absorption of carbon dioxide reduces pH levels in the ocean, which poses a great risk for many marine organisms, including calcifying organisms (Helmle et al. 2010; Jackson 2010; Lannig et al. 2010; McNeal and Matear 2008; Miller et al. 2009; Orr et al. 2005; Parker et al. 2009; Riebesell et al. 2009; Ries et al. 2009; Ruttimann 2006; Suffrian et al. 2011; Wood et al. 2008). Calcifying organisms use calcium carbonate to create their own shells and skeletons and include organisms such as corals, sea urchins, shelled pteropods (a zooplankton species), phytoplankton, certain copepods, echinoderms, crabs, clams, conchs, periwinkles, whelks, mussels, and oysters (Helmle et al. 2010; Jackson 2010; Lannig et al. 2010; McNeal and Matear 2008; Miller et al. 2009; Orr et al. 2005; Parker et al. 2009; Riebesell 2008; Riebesell et al. 2009; Ries et al. 2009; Ruttimann 2006; Suffrian et al. 2011; Wood et al. 2008). The decreased pH and resulting increased acidity of the water degrades the carbonate skeletons and shells that these calcifying organisms create (Helmle et al. 2010; Jackson 2010; Lannig et al. 2010; McNeal and Matear 2008; Miller et al. 2009; Orr et al. 2005; Parker et al. 2009; Riebesell et al. 2009; Ries et al. 2009; Ruttimann 2006; Suffrian et al. 2011; Wood et al. 2008). The effects of ocean acidification are especially prominent in coastal areas and are seen in the organisms residing there (Lannig et al. 2010; Miller et al. 2009). Ocean acidification influences organism productivity and survival not only by decreased pH, but by higher levels of CO_2 as well and its related reduced availability of carbonate that is used by many organisms in the process of calcification (Wood et al. 2008).

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While oysters in coastal waters are able to tolerate a greater range of temperature differences than organisms living farther from the shoreline, research suggests that these same coastal waters may be more influenced by ocean acidification and its resulting reduced salinity levels (Lannig et al. 2010; Parker et al. 2009; Wood et al. 2008). Additionally, calcium carbonate saturation may have a negative impact on oysters, particularly in their shells' susceptibility to dissolution (Lannig et al. 2010; Parker et al. 2009; Wood et al. 2008). These organisms, including the coastal oysters [which are calcifying organisms] studied in this experiment, face dangers such as a decreased ability to grow, reproduce, and negative effects on calcification (Lannig et al. 2010; Wood et al. 2008). Since carbon dioxide suppresses calcium carbonate, including aragonite, these organisms experience many problems in respect to their shells and skeleton formation (McNeal and Matear 2008; Miller et al. 2009). If aragonite saturation is not at adequate levels, calcifying organisms will encounter problems involving shell and skeleton growth (McNeal and Matear 2008). Studies examining one oyster species, *Crassostrea gigas*, report that the shells of these organisms experienced inhibited growth during the larval stage when in contact with water lacking proper aragonite saturation (Miller et al. 2009).

Organisms occupying northern regions are predicted to be the first affected by ocean acidification and, consequently, under-saturation of aragonite (Orr et al. 2005; Riebesell et al. 2009). Research suggests that if current rates of under-saturation continue, calcification in some organisms, including shelled pteropods that reside in shallow waters of polar and subpolar regions may decrease by about 50% within the next 50 years (Orr et al. 2005). However, many of the studies conducted have not been long term, and so evolution may yet play a role in adaptation to increasing levels of CO_2 (Riebesell 2008; Riebesell et al. 2009). Furthermore, studies concerning impacts of pH decreases on shell tolerance have had mixed results, with some organisms exhibiting increased calcification with increasing levels of CO_2 while others experience decreased calcification (Riebesell 2008; Riebesell et al. 2009; Ries et al. 2009). The demise of organisms that cannot survive ocean acidification will have a deleterious impact on those who do survive, upsetting the food web and the ecosystem of that area as well as impacting economies depending on those ecosystems (Ries et al. 2009). Ocean acidification may increase the risk of negative impacts (including decreased growth and reproduction) affecting species raised or harvested for commercial purposes, such as certain shellfish, including oysters (The Royal Society 2005). Florida is very susceptible to ocean acidification and its negative effects and it is likely that the resulting increasing acidity may impact Florida's oyster economy since this state has large ocean economies (Kildow and McIlgorm 2010; The Royal Society 2005). Therefore, ocean acidification may reduce the variety of organisms found in a given ecosystem and has implications for ecosystem health, biodiversity, and the economy surrounding these ecosystems (Riebesell 2008). One such affected species that may be negatively impacted by ocean acidification is the oyster *Crassostrea virginica* (Miller et al. 2009).

This study was conducted in Florida and observed the effects of lower pH levels on *Crassostrea virginica* (Atlantic oyster) shell tolerance. This study was conducted to determine the level of dissolution that more acidic seawater, corresponding to the ocean acidification occurring as a result of global climate change, would have upon oyster shells while the organism was not in the shell to contribute to shell growth. Shell weight and length are predicted to decrease with pH levels.

MATERIALS AND METHODS

Throughout this research, two methods of investigation were utilized. Namely, these methods were the field and laboratory methods.

Field Methods

C. virginica live specimens were collected during various points of time in morning, afternoon, and early evening as well as during varying tides from two different locations: Guana Tolomato Matanzas National Estuarine Research Reserve (GTM-NERR; referred to hereafter as NERR) and the Saint Augustine Marina.

At each location, salinity, pH level, and temperature of both water and air were taken and recorded. Using a refractometer, thermometer, and kestrel (loaned by the NERR). The pH level of each location was measured using litmus paper (pHydrion insta-check).

Live specimens were collected in clusters and placed in buckets containing water from the area in which they were collected. The live specimens were then measured on site or transported to Flagler College's lab facility for further measurements of organism mass (g), organism length in centimeters, and tissue mass (g).

Laboratory Methods

Fourteen initial individual *C. virginica* were removed from clusters using a hammer and chisel. Living oysters (dead oysters were not included) were then shucked and had shells divided. One half-shell from each individual was then scrubbed of detritus and other foreign material, measured for organism mass and length, and placed in plastic-tupperware containers (Gladware); two per container, with a total of seven containers per location (NERR and SA Marina). 35 parts per thousand (ppt) artificial seawater (ASW) was created using InstantOcean aquaria salt and laboratory grade distilled water (Aqua Solutions). ASW was then added to the containers until shells were entirely submerged. Of the seven containers, one container holding two oysters was selected randomly as a control for each location and maintained at 7.6 pH. The pH of the water was measured using litmus paper strips (pHydrion insta-check). The pH of each experimental group was set to a stepwise lower pH using pH buffer solution at a 4.0 pH concentration (Lab Safety Supply). The pH levels of each group were 7.6 (Control), 7.2, 7.0, 6.8, 6.6, 6.4, and 6.0. Adjustments were regularly made with pH buffer solutions (4.0 and 10.0 pH concentrations) to maintain desired levels in each group. All factors excepting manipulated pH levels were controlled for. All samples were stored in the temperature constant Flagler College molecular biology laboratory.

Field Component Results

The overall findings of the Field Component are summarized below, in addition to a summary of these findings categorized by both the Marina group and NERR group. The mean tissue mass and the mean total organism mass was measured in both the marina and NERR field groups and compared. The mean tissue mass of the marina group was 3.14g, while the mean total organism mass was 34.74g. Tissue mass on average was 9.02% the total mass of the organism. The mean tissue mass of the NERR group was 3.15g, while the total organism mass was 37.06g. Tissue mass on average was 8.51% the total mass of the organism (see Figure 1).

Marina group

The marina 5.5pH group had a mean tissue mass of 2.73g, and a mean total organism mass of 30.42g; making mean tissue mass 9.01% the total mass of the organism. The marina 6.4pH group had a mean tissue mass of 3.31g, and a mean organism mass of 33.68g; making tissue mass 9.84% the total weight of the organism. The marina 6.6pH group had a mean tissue mass of 3.85g, and a mean organism mass of 45.59g; making tissue mass 8.45% the total mass of the organism (Figure 2).

NERR group

The NERR 6.4pH group had a mean tissue mass of 3.27g, and a mean organism mass of 37.84g; making tissue mass 8.65% the total mass of the organism. The NERR 6.8pH group had a mean tissue mass of 2.79g, and a mean organism mass of 34.72g; making tissue mass 8.05% the total mass of the organism (Figure 2).

RESULTS

The findings from both the marina group and NERR group are summarized as follows.

Marina group

The mean mass of the marina control group at the beginning of the experiment was 20.475g; the final measured mass 21 days later was 18.755g, a reduction of 8.4%. The mean mass of the marina experimental group at the beginning of the experiment was 8.711g; the final measured mass 21 days later was 8.088g, a reduction of 7.1%. The mean length of the marina control group at the beginning of the experiment was 7.65cm; the final measured length 21 days later was 7.3cm, a reduction of 4.58%. The mean length of the marina experimental group at the beginning of the experiment was 5.783cm; the final measured length 21 days later was 5.53cm, a reduction of 4.375%.

When measuring mean mass and length in each pH condition independently the following results were found: The mean mass of the marina control group at the beginning of the experiment was 20.48g, the final measured mass 21 days later was 18.76g, a reduction of 8.4%. The mean mass of the marina experimental group at the beginning of the experiment was 8.711g, the final measured mass 21 days later was 8.088g, a reduction of 7.1% (Figure 3).

The mean length of the marina control group at the beginning of the experiment was 7.65cm; the final measured length 21 days later was 7.3cm, a reduction of 4.58%. The mean length of the marina experimental group at the beginning of the experiment was 5.783cm; the final measured length 21 days later was 5.53cm, a reduction of 4.375% (Figure 4).

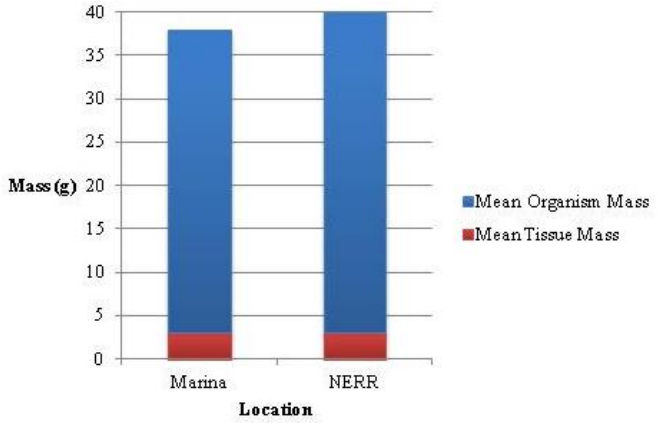

Figure 1: Comparison of Tissue Mass to Whole Organism Mass

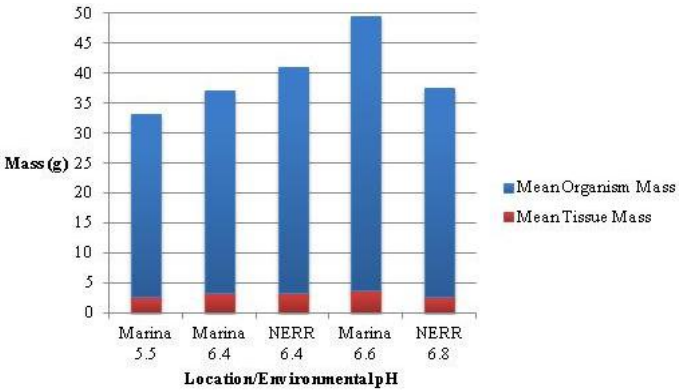

Figure 2: Mean Tissue Mass Ratio in Relation to Environmental pH

When measuring mean mass in each pH condition independently the following results were found: The mean mass for the 7.2pH condition at the beginning of the experiment was 7.03g; the final measured mass 21 days later was 6.37g, a reduction of 9.39%. The mean mass for the 7.0pH condition at the beginning of the experiment was 7.18g; the final measured mass 21 days later was 6.33g, a reduction of 11.84%. The mean mass of the 6.8pH condition at the beginning of the experiment was 8.32g; the final measured mass 21 days later was 7.54g, a reduction of 9.38%. The mean mass for the 6.6pH condition at the beginning of the experiment was 6.69g; the final measured mass 21 days later was 6.32g, a reduction of 5.53%.

The mean mass of the 6.4pH condition at the beginning of the experiment was 9.79g; the final measured mass 21 days later was 9.27g, a reduction of 5.3%. The mean mass of the

6.0pH condition at the beginning of the experiment was 13.27g; the final measured mass 21 days later was 12.71g, a reduction of 4.22% (Figure 5).

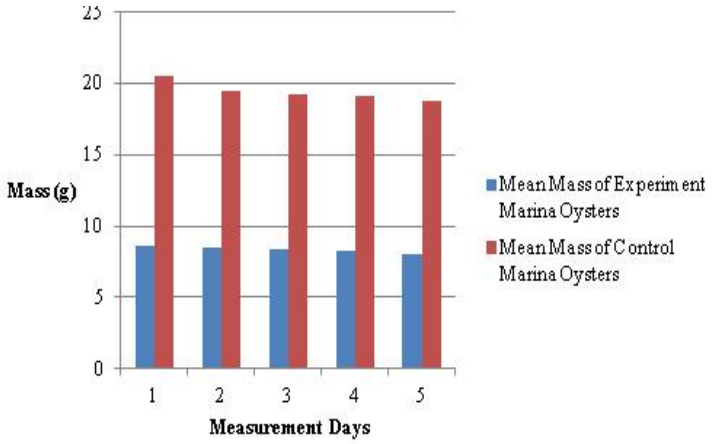

Figure 3: Mean Mass of Experimental Group and Control Group Marina Oysters

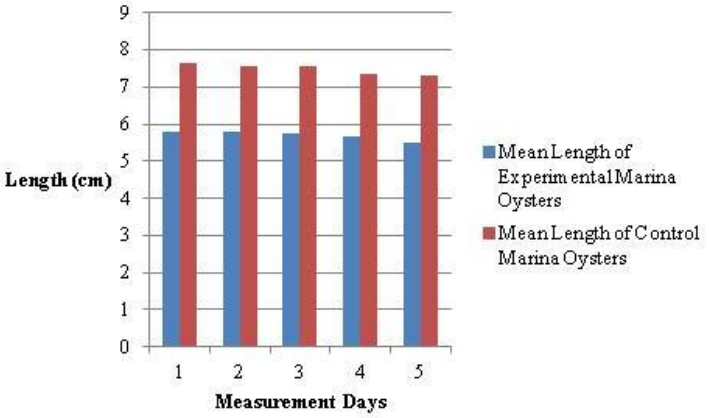

Figure 4: Mean Length of Experimental Group and Control Group Marina Oysters

When measuring mean length in each pH condition independently the following results were found: The mean length for the 7.2pH condition at the beginning of the experiment was 5.35cm; the final measured length 21 days later was 5cm, a reduction of 6.54%.The mean length for the 7.0pH condition at the beginning of the experiment was 5.9cm; the final measured length 21 days later was 5.5cm, a reduction of 6.78%.The mean length for the 6.8pH condition at the beginning of the experiment was 5.35cm; the final measured length 21 days later was 5.2cm, a reduction of 2.8%.The mean length for the 6.6pH condition at the beginning of the experiment was 5.6cm; the final measured length 21 days later was 5.3cm, a reduction of 5.36%.

The mean length for the 6.4pH condition at the beginning of the experiment was 5.9cm; the final measured length 21 days later was 5.85cm, a reduction of 0.85%.The mean length for the 6.0pH condition at the beginning of the experiment was 6.6cm; the final measured length 21 days later was 6.35cm, a reduction of 3.79% (Figure 6).

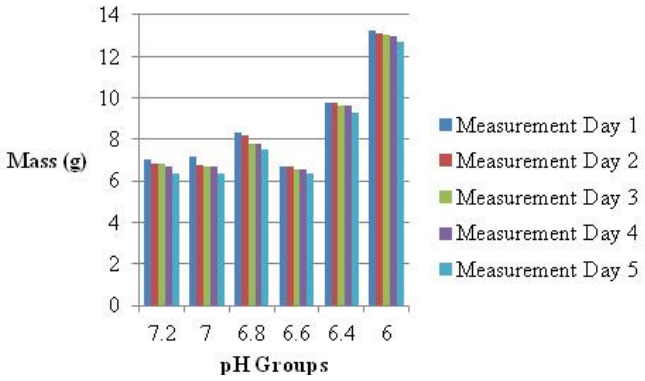

Figure 5: Mean Mass of Experimental Group Marina Oysters

NERR group

The mean mass of the NERR control group at the beginning of the experiment was 4.9g; the final measured mass 21 days later was 4.345g, a reduction of 11.22%. The mean mass of the NERR experimental group at the beginning of the experiment was 11.223g; the final measured mass 21 days later was 10.486g, a reduction of 6.57%. The mean length of the NERR control group at the beginning of the experiment was 4.75cm; the final measured length 21 days later was 4.35cm, a reduction of 8.42%. The mean length of the marina experimental group at the beginning of the experiment was 6.62cm; the final measured length 21 days later was 6.46cm, a reduction of 2.42%.

When measuring mean mass and length in each pH condition independently the following results were found: The mean mass of the NERR control group at the beginning of the experiment was 4.9g; the final measured mass 21 days later was 4.345g, a reduction of 11.22%. The mean mass of the NERR experimental group at the beginning of the experiment was 11.223g; the final measured mass 21 days later was 10.486g, a reduction of 6.57% (Figure 7).

The mean length of the NERR control group at the beginning of the experiment was 4.75cm; the final measured length 21 days later was 4.35cm, a reduction of 8.42%. The mean length of the NERR experimental group at the beginning of the experiment was 6.62cm; the final measured length 21 days later was 6.46cm, a reduction of 2.42% (Figure 8).

When measuring mean mass in each pH condition independently the following results were found: The mean mass for the 7.2pH condition at the beginning of the experiment was 13.02g; the final measured mass 21 days later was 12.31g, a

reduction of 5.45%. The mean mass for the 7.0pH condition at the beginning of the experiment was 7.02g; the final measured mass 21 days later was 6.41g, a reduction of 8.69%. The mean mass of the 6.8pH condition at the beginning of the experiment was 18.44g; the final measured mass 21 days later was 17.51g, a reduction of 5.04%. The mean mass for the 6.6pH condition at the beginning of the experiment was 8.81g; the final measured mass 21 days later was 8.22g, a reduction of 6.7%. The mean mass for the 6.4pH condition at the beginning of the experiment was 10.05g; the final measured mass 21 days later was 9.32g, a reduction of 7.26%. The mean mass for the 6.0pH condition at the beginning of the experiment was 16.02g; the final measured mass 21 days later was 14.86, a reduction of 7.24% (Figure 9).

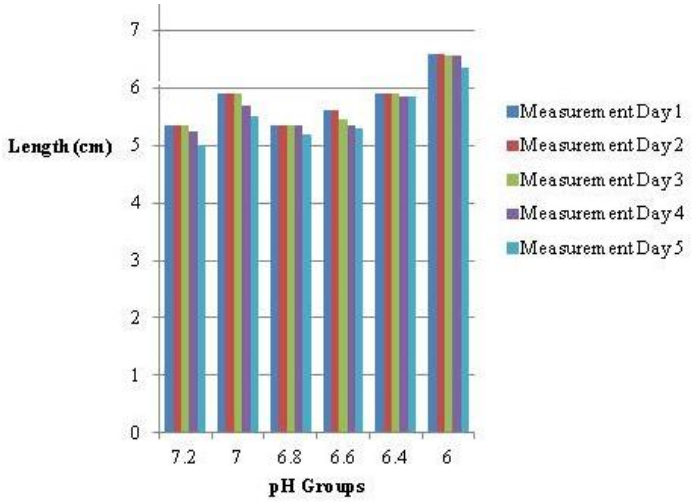

Figure 6: Mean Length of Experimental Group Marina Oysters

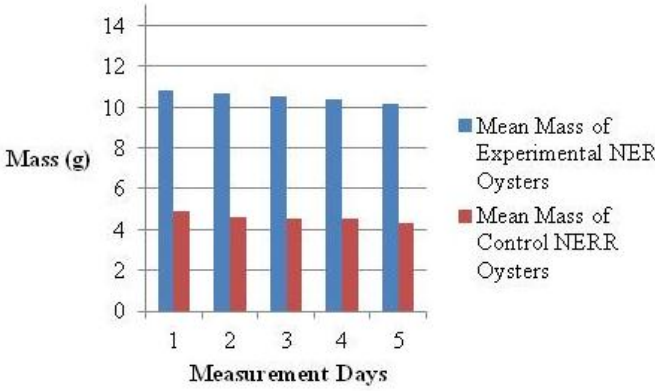

Figure 7: Mean Mass of Experimental Group and Control Group NERR Oysters

When measuring mean length in each pH condition independently the following results were found: The mean length for the 7.2pH condition at the beginning of the experiment was 7.25cm; the final measured length 21 days later was 7.25cm, a reduction of 0 %. The mean length for the 7.0pH condition at the beginning of the experiment was 5.4cm; the final measured length 21 days later was 5.05cm a reduction 6.48%. The mean length of the 6.8pH condition at the beginning of the experiment was 10cm; the final measured length 21 days later was 9.6cm, a reduction of 4%. The mean length for the 6.6pH condition at the beginning of the experiment was 6.2cm; the final measured length 21 days later was 6.15, a reduction of 0.81%. The mean length for the 6.4pH condition at the beginning of the experiment was 5.7cm the final measured length 21 days later was 5.4cm, a reduction of 5.26%. The mean length for the 6.0pH condition at the beginning of the experiment was 7.2cm; the final measured length 21 days later was 7.1cm, a reduction of 1.39% (Figure 10).

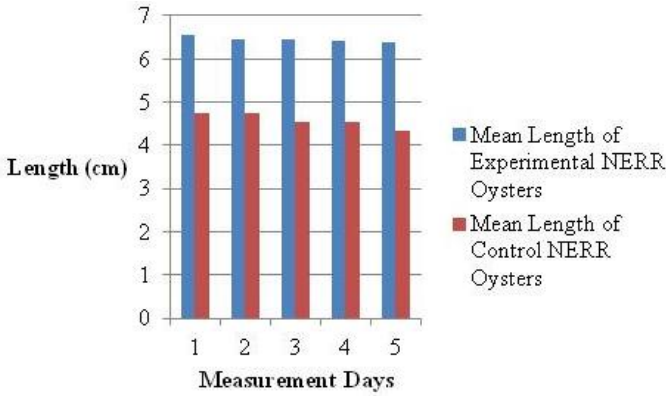

Figure 8: Mean Length of Experimental Group and Control Group NERR Oysters

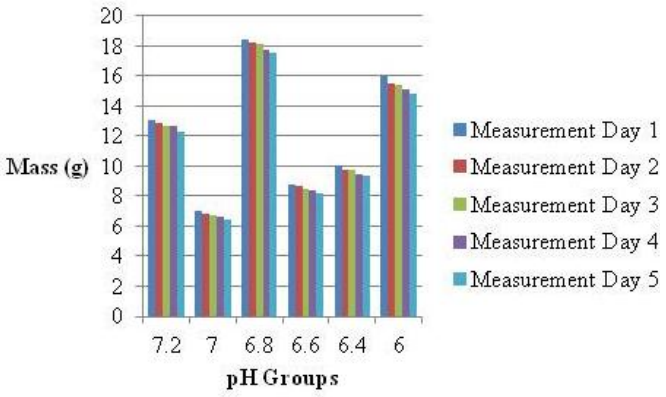

Figure 9: Mean Mass of Experimental Group NERR Oysters

DISCUSSION

The research conducted led to the following conclusions regarding the Field Component and Laboratory Component in conjunction with the marina group and NERR group included in each component.

Field Component

The field data for the NERR and marina populations did not differ substantially in tissue mass or overall organism size. This may indicate that the conditions of the marina may not be nearly as toxic to the oyster as to raise concern for their adult health. Again, as noted in the lab discussion, *C. virginica* may have an increased heartiness for increased acidity or a protective like that found in *C. gigas*, thereby not crossing the threshold necessary for shell degradation to occur in this species (Parker et al. 2010). There is also a potentially greater adaptive range for this particular population due to its local as well as regional location. The population in the marina experiences a greater range of pH, naturally and artificially, compared to other calcifying organisms in different regions of the world such as the Sydney rock oyster, and thereby may have developed an adaptation to these conditions over time, unlike those in other regions (Parker et al. 2009).

Future research on this population and species would benefit from a study spanning a greater length of time, with a higher sample population from other potential "polluted" areas. Subjecting other regional populations to this experiment compared to the Florida population could also provide data regarding the effects of a higher natural pH tolerance range on this species.

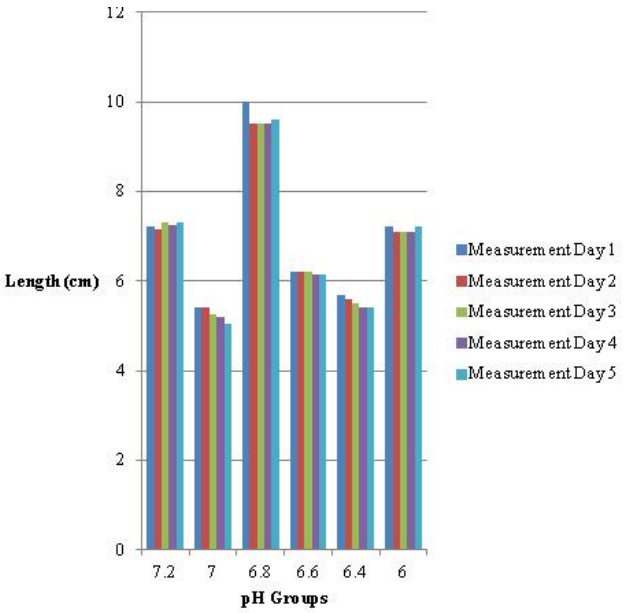

Figure 10: Mean Length of Experimental Group NERR Oysters

Laboratory Component

This data supports implications that short-term experiments (21 days) cannot show the full potential of shell degradation that may occur for *C. virginica*. The control groups did not differ substantially from the experimental groups in regards to percent of reduction in mass over 21 days. However, it should be noted that although the individuals involved in this study were trained to take measurements in the same manner, they were not taken by only one person. As a result, this was a limitation to our study as minor variations among the measurements and the data they portray may be due to this factor. However, an extended study may reveal that the current trend of reduction in each group may not continue at the same rates that we observed; the experimental group may show greater degradation over an increased time scale. An additional consideration is that our experimental pH range did not extend to a low enough level of acidity, thereby not affecting the shell integrity of *C. virginica*. As the data reflects, pH was recorded at levels as low as 5.5 at the marina location, therefore the lowest experimental condition of 6.0 may not accurately reflect a significant level of toxic acidity for this particular population, due to their adaptation to thrive within this range. Contributions to greater pH acidity in the marina location can range from city runoff and waste to input from natural phenomenon such as acid rain. The effects of acid rain, a natural occurrence in Florida, can decrease surface pH of open water.

C. virginica could reflect the same level of robustness as a related oyster species, *C. gigas*, and consequently may not be as affected by drastic, short-term decreases in pH (Parker et al 2010). Specific environmental considerations must also be made for the location in which this species is found. The adaptive range of *C. virginica* may be higher than other calcifying organisms affected by ocean acidification.

ACKNOWLEDGEMENTS

Margaret Rudd, Kyle Jennette, Becky Duey, and Arnel Selman would like to express great gratitude to Angie Golubovich for her contributions of equipment and expertise. We would also like to extend great gratitude to Dr. Terri Seron for advising this project, teaching our course, and providing equipment and extensive guidance; thank you.

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