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Construction of an Accessible Ocean-Acidification Simulator to Investigate Physiological Responses of the Green Crab, Carcinus Maenas, to Acidified Conditions

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CONSTRUCTION OF AN ACCESSIBLE OCEAN-ACIDIFICATION SIMULATOR TO INVESTIGATE PHYSIOLOGICAL RESPONSES OF THE GREEN CRAB, *CARCINUS MAENAS*, TO ACIDIFIED CONDITIONS

by

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ABSTRACT

The European green crab *Carcinus maenas* L, is a major invasive species in North America as well as many other regions around the world, including South Africa, Australia, South America, and Asia. The species poses a significant threat to the diverse ecosystems and the aquaculture industries on the East coast of the United States, with the state of Maine particularly at risk. The shellfish industry is a significant part of Maine's economy, and is threatened by the foraging behavior of green crabs toward small bivalves (Beal 2015). Climate change likely plays a large role in the rapid population growth of *C. maenas* over the last 5-10 years by opening up marginal habitats for the crabs to occupy (Beal 2015). Steroid activity is highly dependent on environmental conditions, and changes in temperature have been linked to the ecological success of *C. maenas*.

This project will focus on developing an accessible low-cost ocean-acidification simulator at the University of Maine Aquaculture Research Center to be used to explore physiological responses of *C. maenas* to acidified conditions and quantify estradiol levels in the animals using an Enzyme-Linked Immunosorbent Assay (ELISA). Animal trials conducted with the system will provide information on parameters related to the effect of acidic stress on the endocrine system of green crabs. Additionally, the outlines for the construction of the simulator can be used as a model for students for an inexpensive holding facility to test effects of acidification. Data obtained from the Accessible Low-Cost Ocean Acidification Simulation Tool (ALCOAST) can also be used by policy makers to evaluate whether climate change can provide information on physiological interactions between *C. maenas* and its environment. This study stands to fill a significant

gap in knowledge that is relevant not only to Maine's economy and management of invasive species, but also to studies of how invasive species react to climate change.

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INTRODUCTION

Background

The Gulf of Maine is an extremely at risk region for anthropogenic climate change, which is rapidly warming and acidifying its waters and opening of marginal habitats for invasive species (Beal 2015). Populations of *Carcinus maenas* L. are not only able to thrive in warming waters but can survive in a myriad of habitats, including rocky intertidal, un-vegetated intertidal, subtidal mud and sand zones, saltmarshes, and seagrasses (Klassen 2007). Furthermore, they will prey on everything from commercially and economically important bivalves to gastropods, decapods, and fish, as well as compete actively for food with many other aquatic species (Klassen 2007). In addition to the wide abundance of habitats in which the green crab can invade and thrive as well as their extensive diet, there are a number of other characteristics that make them so successful at encroaching coastal ecosystems across the globe. For example, while green crabs are larval dispersers, most of their successful invasion can be attributed to anthropogenic transport, which has allowed them to spread across the globe (Klassen 2007). Furthermore, green crabs are both eurythermic, meaning they can survive in a wide range of temperatures from 0 to 35^{o}C, as well as euryhaline, meaning they are able to adapt to a wide range of salinities, from 4 to 54 ppt (Klassen 2007).

C. maenas was originally introduced into Maine in the 1800's and then again in the 1950's through transport by ballast water (Carlton 2003). The human-mediated global dispersal of *C. maenas* has led to their successful establishment in a myriad of temperate regions around the globe, significantly beyond their native zone (Carlton 2003). For this reason, the green crab has become a major threat to the aquaculture and shellfish

industries in Maine (both of which play critical roles in the Maine economy), as they are able to thrive in the warming waters (Behrens Yamada 2010). The suitability of Maine's coastal environment to *C. maenas* allows for massive recruitment events, which increases potential consumption of native shellfish species that are under threat (Behrens Yamada 2010). It is a direct result of the warming waters along the coast that green crabs demonstrate such successful recruitment and invasion, as they are able to reproduce in large numbers (Behrens Yamada 2005).

There are few other marine invertebrates that are known to be as adaptable in a changing environment, and yet there are a very limited number of studies that examine ecological and evolutionary adaptations in response to climate change, especially those of an invasive species. As a result, it is difficult to identify potential biological control mechanisms for these invasive species, or to analyze the physiological adaptations that occur in the changing environment (Atkins 2010). To predict the effects of climate change on aquatic organisms, it is important to study interactions between environmental variability and physiological function of the animal (Bozinovic 2011). The geographic range of animals such as the green crab is determined in part by their physiological tolerance, and to study physiological tolerance requires obtaining a better understanding of their responses to the chemical environment in which the animal resides (Bozinovic 2011). To facilitate such studies, I have developed an ocean-acidification (OA) simulator that will allow manipulating various water-quality parameters, including acidification. One important factor to investigate would be how acidification affects hormone levels in the hemolymph of the animal (Bozinovic 2011).

Significance of the Accessible Low-Cost Ocean Acidification Simulation Tool (ALCOAST)

Ocean-acidification is one of the most pressing environmental obstacles of our time, and continues to be an emerging global problem (Doney 2009). Increasing atmospheric carbon dioxide levels are resulting in the reduction of the oceanic pH, as well as carbonate ion concentrations (Orr 2005). As the ocean continues to acidify and the carbonate chemistry continues to shift, marine organisms will become more physiologically stressed, and their responses will vary (Doney 2009, Kroeker 2010). The variation in their responses has the potential to shine light on significant ecological changes that will occur in the ocean, thus research concerning ocean-acidification and its effects on marine organisms is rapidly growing (Kroeker 2010). While scientists are actively working worldwide to understand the impacts of ocean-acidification and predict how it may continue to affect marine ecosystems, it is imperative that all measures be taken to strengthen the call to action. To that end, all students looking to overcome financial barriers should have access to research tools in the field, since the typical undergraduate student at a research facility is not able to conduct preliminary studies on the direct impacts of ocean-acidification without access to an OA system. On average, an OA system at a research facility is worth around $30,000 and is typically reserved for research projects being conducted by graduate students and faculty, which makes it inaccessible to undergraduate students. This lack of exposure and wasted knowledge could prove to be a significant obstacle in the journey towards understanding climate change.

The Accessible Low-Cost Ocean Acidification Simulation Tool (ALCOAST) for this thesis is a tool that could be used as a model for future OA research to be conducted

by students of all levels wanting to find a low-cost experimental system. The ALCOAST is accessible in that it is reasonably priced, straightforward in construction, functional in practice, and easily maintained. Construction proved challenging at certain stages, but the overall success of the simulator was significant. In addition to constructing and maintaining the ALCOAST, I held animal-based trials and gathered data concerning water-quality and endocrine disruption in the green crabs to gather preliminary data on the physiological responses of green crab exposure to acidified water.

Aims of the experiment

The overarching goals of this experiment are to construct, maintain and implement a low-cost, user-friendly system to explore the physiological boundaries of *C. maenas* to acidified conditions, to monitor water-quality parameters within the system and the condition of crabs to evaluate system performance, and to investigate the significance of acidified conditions on endocrine disruption in *C. maenas*. Gathering the ALCOAST water-quality data will lend information on the chemical parameters of the water that could have a direct impact on the endocrine system of *C. maenas* in addition to the manipulated pH, as well as provide critical information on system performance.

Following the experimental trials held in the ALCOAST, the crabs are removed from their individual tanks and brought to the laboratory where their hemolymph will be extracted to quantify estradiol levels using an Enzyme-Linked Immunosorbent Assay (ELISA).

MATERIALS AND METHODS

Building the Accessible Low-Cost Ocean Acidification Simulation Tool (ALCOAST)

The ocean-acidification system was built using 20 small, plastic Aquatic Habitats acclimation tanks. Each acclimation tank held 2.75 liters of seawater and one crab was placed in each tank for the duration of the experiment. There were two header tanks, two sump tanks, a PVC pipe manifold that dispensed seawater from the header tanks into the acclimation tanks, and a PVC pipe drain to dump the seawater back into the sump tank and allow for re-circulation (figure 1). The PVC piping was held together using PVC primer and cement. Cleaning and filtering of the system was done manually by siphon and through frequent seawater changes. The ALCOAST consisted of two separate systems sitting next to each other, one being the experimental system (right) and one being the control system (left). The pH was manipulated using the pH meter and CO_2 tank.

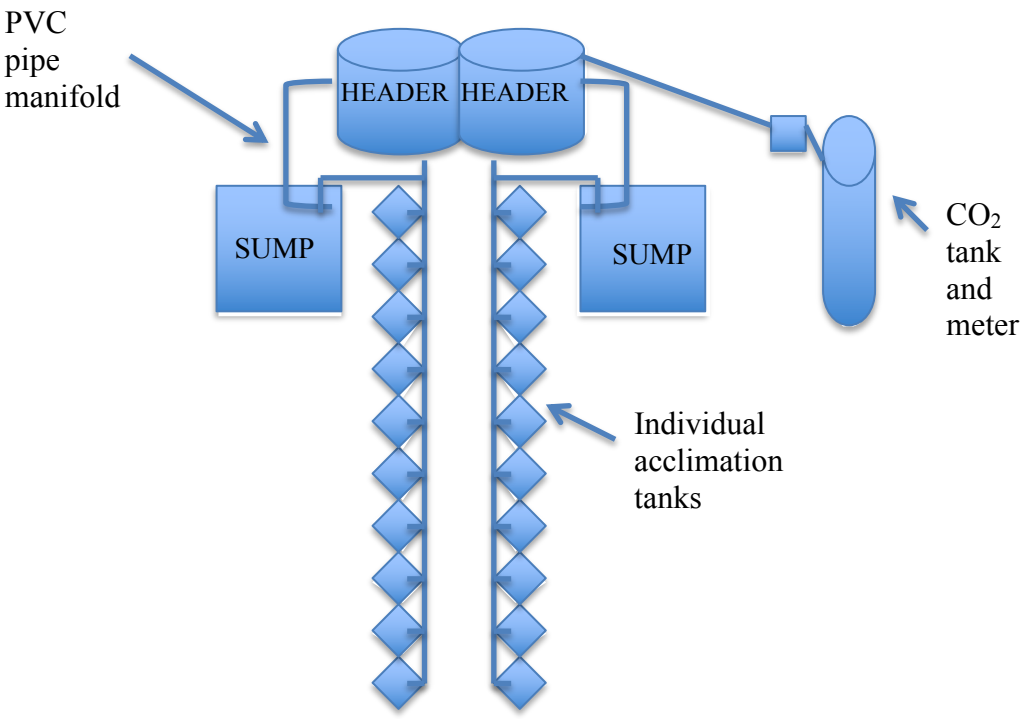

Figure 1. Schematic diagram of the ALCOAST

The two header tanks held up to 100 liters of seawater each and distributed the water throughout the acclimation tanks. One header tank dispensed seawater that was meant to mimic natural seawater to ten of the small tanks that made up the control system, while the second header tank distributed acidified seawater to the other ten tanks, constituting the experimental system. The two sets of ten tanks were completely separate from each other, allowing for two isolated systems (figure 1). A CO_2 tank and meter were used to maintain the pH levels within the experimental tanks. There was roughly 235 liters of seawater distributed per system.

Maintenance of the ALCOAST

The CO_2 meter attached to the tank on the experimental system was adjusted so that the pH was set to 7.6. While there was day-to-day variation, the average pH in the systems over a 24 day period for Trial 1 was 7.87 for the control system and 7.45 for the experimental system (Table 2). For Trial 2, the average pH in the control system was 7.99, and the pH of the experimental system was 7.54 (Table 5).

Artificial seawater was made to 35 ppt salinity using reverse osmosis water and Tropic Marin Pro-Reef Sea Salt (Tropic Marin Ltd Germany). While the seawater was originally made at 35 ppt, the average salinity in the control system during Trial 1 was 36 ppt, while the experimental system was 36.2 ppt (Table 1). For Trial 2, the average salinity within the control system was 35.54 ppt, while the control system was 35.38 ppt (Table 4).

The cleaning and filtering of the system was done by siphon to remove fecal matter and any uneaten food. In addition to cleaning of the tanks, water changes were conducted weekly as needed and were completed by emptying the majority of the seawater from the system and replacing it with seawater that had been previously prepared and stored. All experimental and control tanks were emptied and washed, and the tubing was replaced between trials.

Animal Collection

On July 25th, 2017, 20 male *Carcinus maenas,* ranging from 27 to 54 mm carapace width (CW) (Mean ± SD: 42.85 ± 6.99mm) were collected from Roque Bluffs, Maine, for Trial 1 of the experiment. Field collections at this site did not require obtaining permission, as invertebrates are not covered under the Institutional Animal

Care and Use Committee (IACUC), and the site is a public area where green crabs are an abundant and invasive species in eastern North America. The live crabs were transported back to the Aquaculture Research Center (ARC) at the University of Maine where they were kept in a large tank of aerated seawater. After an acclimation period of a few days, the crabs were moved from the large tank to the ALCOAST on July 31st, 2017, where they remained for 24 days. The crabs were placed randomly in the tanks using a random number generator.

On Sunday, October 1st of 2017, a sample size of 20 female *C. maenas* ranging from 35 to 50 mm carapace width (CW) (Mean ± SD: 43.52 ± 4.11mm) were collected from Cape Elizabeth, Maine, for Trial 2 of the experiment. Academic year logistics allowed for the second trial to begin in February 2018. The animals were cared for appropriately and kept together in a large, aerated tank between the months of October and February. On February 15th of 2018, the crabs were moved from the large tank into their 20 individual tanks in the ALCOAST, where Trial 2 would proceed for 24 days. Although the crabs were placed in their experimental tanks on February 15th, data collection did not begin until February 24th to allow for a short acclimation period.

Acclimation

Trial 1 proceeded for 24 days, from the 31st of July to the 23rd of August, allowing the animals to become acclimated to the pH they were living in. The tanks were kept on a 12-hour light: 12-hour dark cycle, with light from 7am to 7pm, and the crabs were fed frozen and peeled CenSea shrimp every Monday, Wednesday, and Friday. The tanks were kept at room temperature (Table 3). During the first few days of the experiment there were a few escapees, thus, on Day 3, rocks were placed on top of the

tanks to prevent escape. Despite these efforts, more of the animals were able to escape and so the lids were taped shut. Over the course of Trial 1, two crabs escaped and were lost and two crabs died, leaving six experimental crabs at the end of the trial and ten control crabs.

Similar to Trial 1, Trial 2 proceeded for 24 days, from the 24th of February to the 19th of March, but the ALCOAST was to a different room in the ARC. The tanks were kept on a 12-hour light: 12-hour dark cycle, with light from 7am to 7pm, and the crabs were fed frozen and peeled CenSea shrimp 1-2 times a week to minimize waste in the tanks and help maintain water-quality. The tanks were kept at room temperature (Table 6). To prevent crabs from escaping the system during the second trial, rubber bands were used to hold the lids shut. This method proved to be successful, and all 20 animals were still alive at the end of the 24-day trial.

Seawater-quality

Trial 1

Temperature, pH, and salinity were measured during Trial 1, but not other parameters of water-quality.

Trial 2

Alkalinity, calcium hardness, nitrate, nitrite, ammonia, and phosphate levels of the seawater were measured weekly throughout the duration of Trial 2. LaMotte was the water-quality company from which the supplies were used. Alkalinity was measured an additional 2-3 times a week on Tuesdays and Thursdays.

Hemolymph extraction

Following the 3-week acclimation period, the animals were removed from the experimental tanks, labeled, and transported to the lab within 1 hour. In the lab they were each individually bled using a 1mL syringe and needle. Enough hemolymph was collected to perform the assay, which was roughly 100 micro-Liters. The hemolymph spun at 13,300 x g for a total of 5 minutes. The supernatant was stored at -80 ºC for future assays. This was performed for both Trial 1 and Trial 2.

Performing the Estradiol Enzyme-Linked Immunosorbent Assay (ELISA)

An estradiol ELISA kit (Cayman Chemical) will be used to measure estradiol in the hemolymph extracted from animals in Trial 1 and Trial 2 and from a pool of crabs taken as a positive control.

RESULTS

Construction of the ALCOAST

The construction of this system generated a low-cost and accessible ocean-acidification simulator costing ~$600.

Figure 2. Completed ALCOAST during Trial 1. The acidified system is on the right and the control system is on the left.

Figure 3a,b. Individual tanks attached to the ALCOAST containing *C. maenas*

Seawater-quality data

Overall seawater-quality data, including nitrate, nitrite, ammonia, calcium hardness, and phosphate was gathered once a week during the second trial, as well as alkalinity 2-3 times a week. This was done to maintain a stable environment for the animals and to gauge the potential for certain water-quality factors to play a role in the disruption of the endocrine system of green crabs. In doing so, it was discovered that there were several intervening factors in the seawater-quality, such as excessive levels of nitrite and nitrate that made it impossible to isolate data on pH. While overall water-quality data was only gathered during Trial 2, temperature, salinity, and pH data were gathered for both trials (Table 1).

Table 1. Trial 1 mean pH, salinity, and temperature over the 24-day period

	Control	Experimental	Natural Seawater (1 sample)
pH	7.87	7.45	7.95
Salinity (ppt)	36	36.2	35
Temperature (˚C)	23.2	23.1	

Table 2. Trial 2 mean pH, salinity, and temperature average over the 24-day period

	Control	Experimental
pH	7.99	7.54
Salinity (ppt)	35.54	35.38
Temperature (˚C)	15.7	15.6

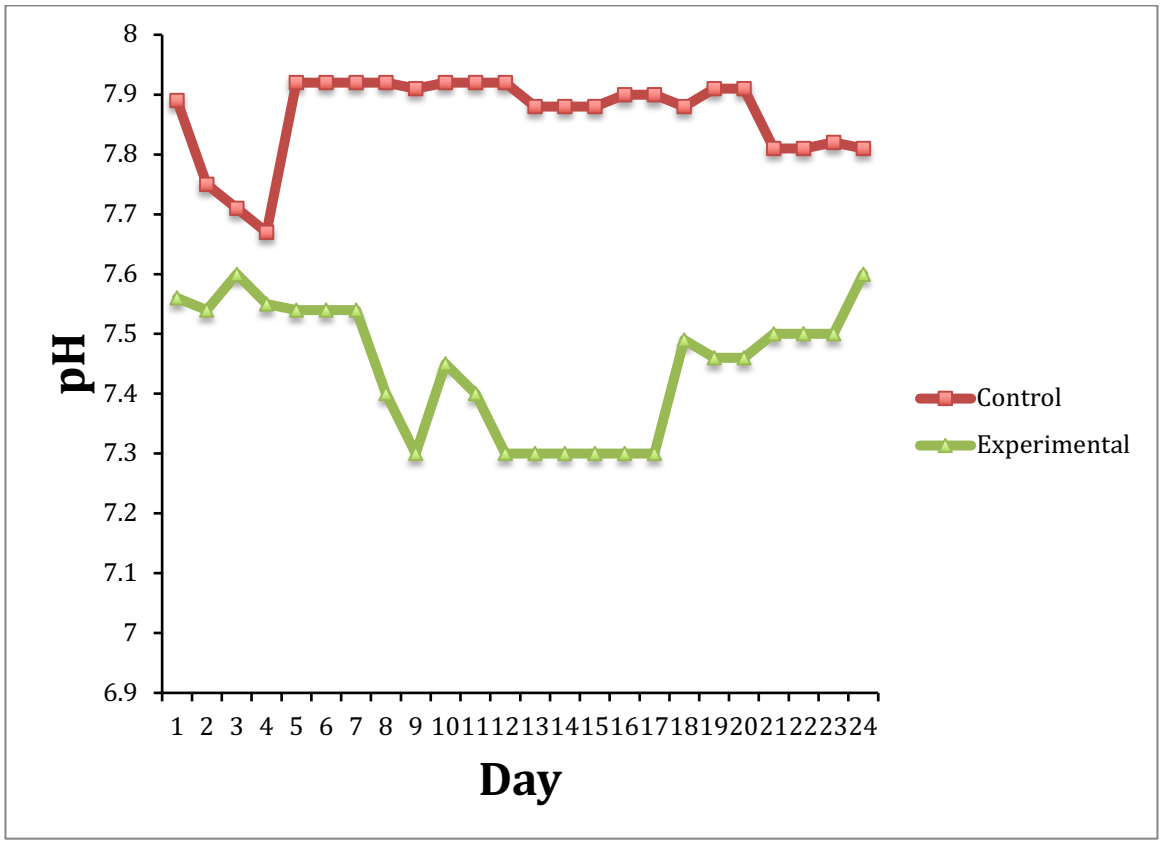

Figure 4. Linear comparison of experimental and control pH for Trial 1

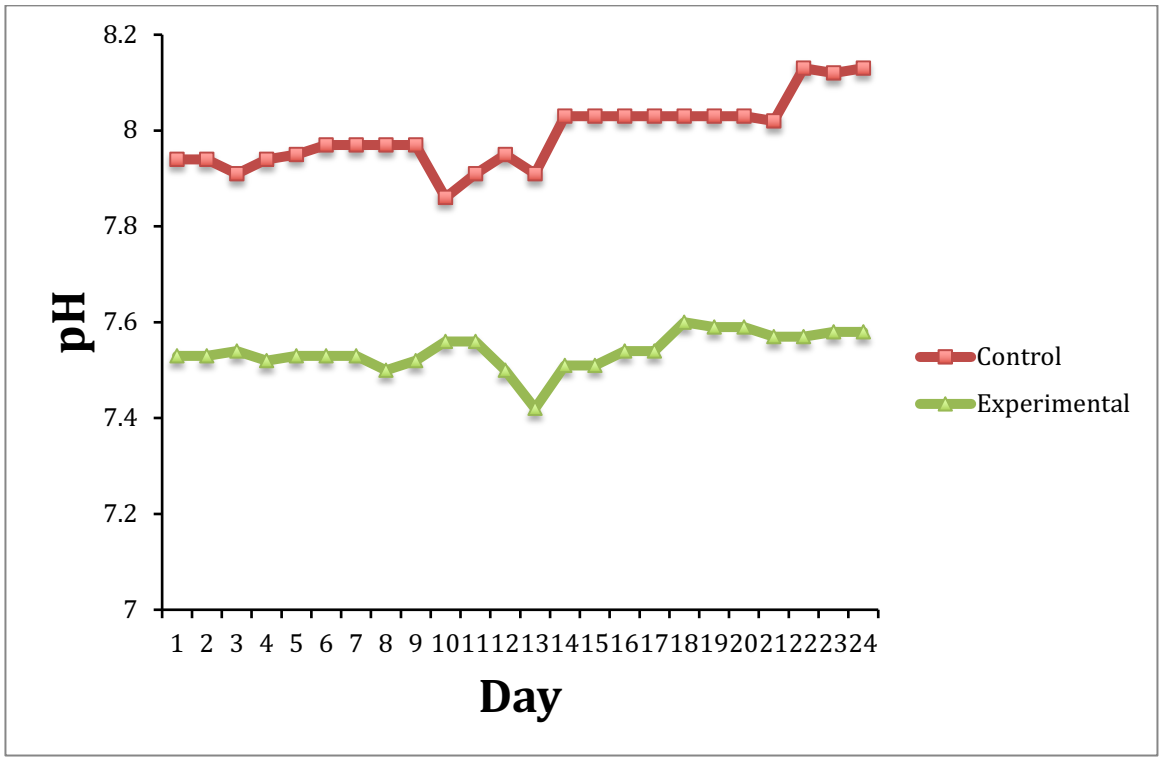

Figure 5. Linear comparison of experimental and control pH for Trial 2

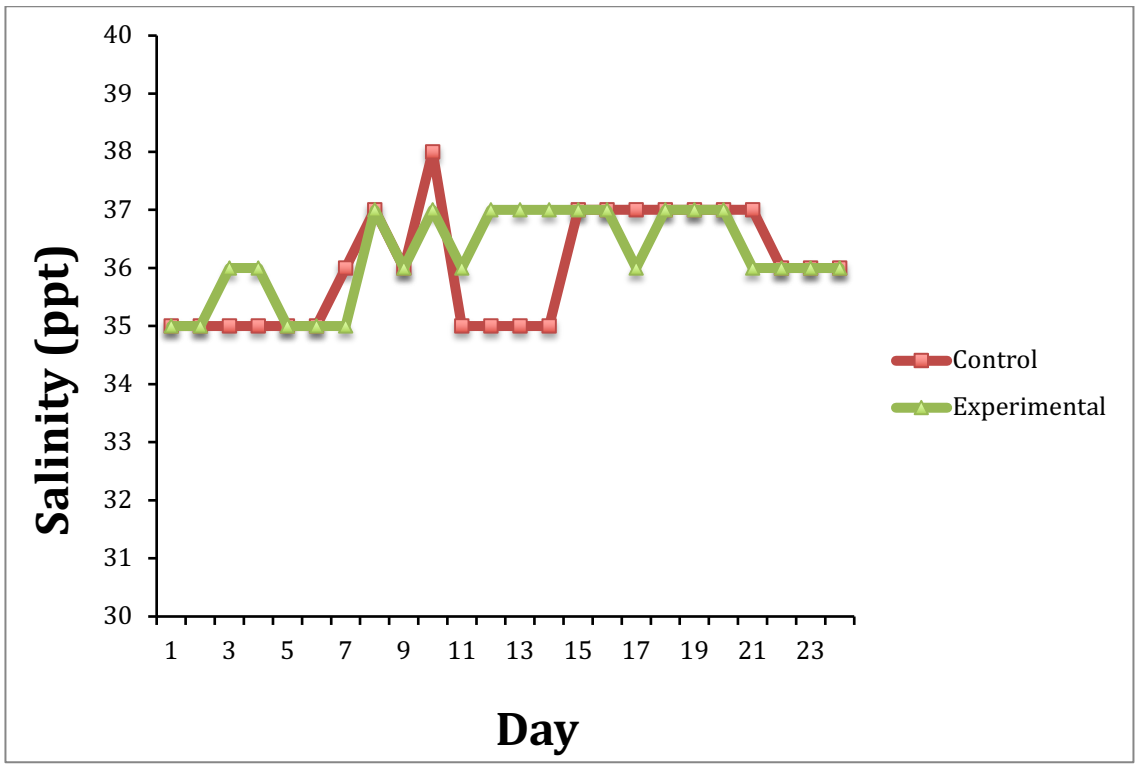

Figure 6. Linear comparison of experimental and control salinity for Trial 1

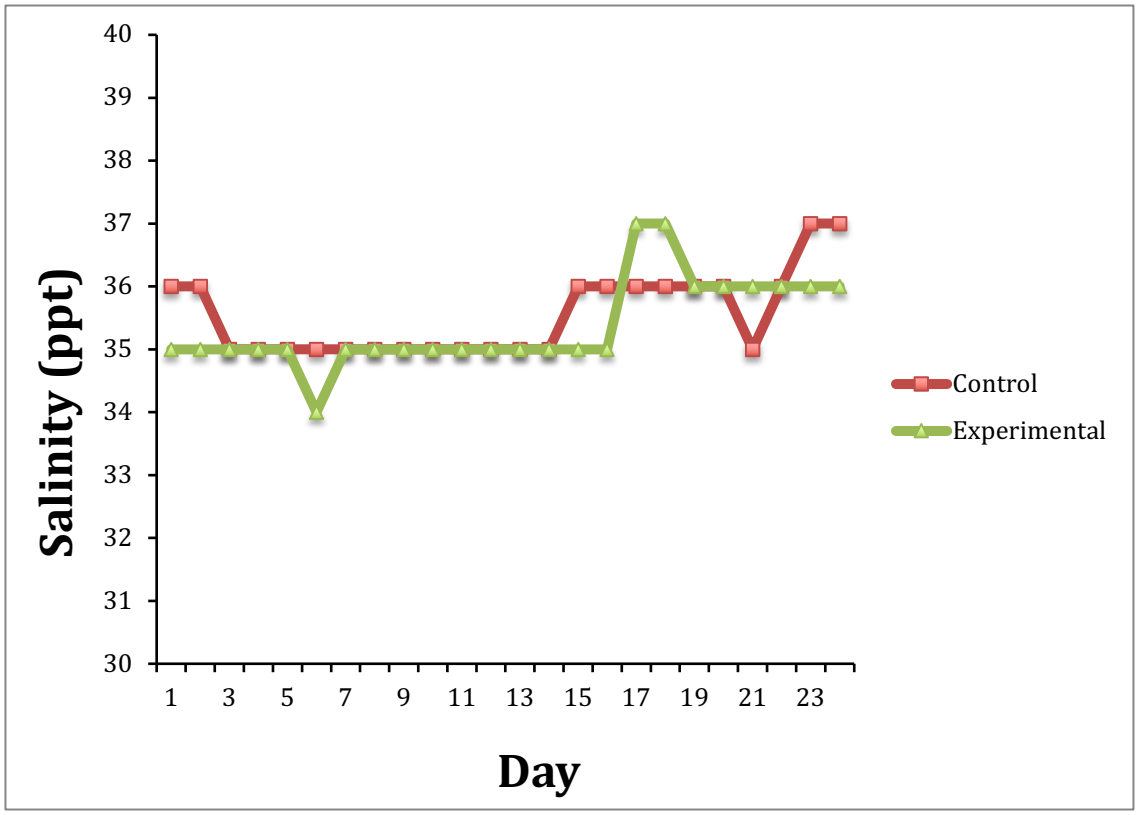

Figure 7. Linear comparison of experimental and control salinity for Trial 2

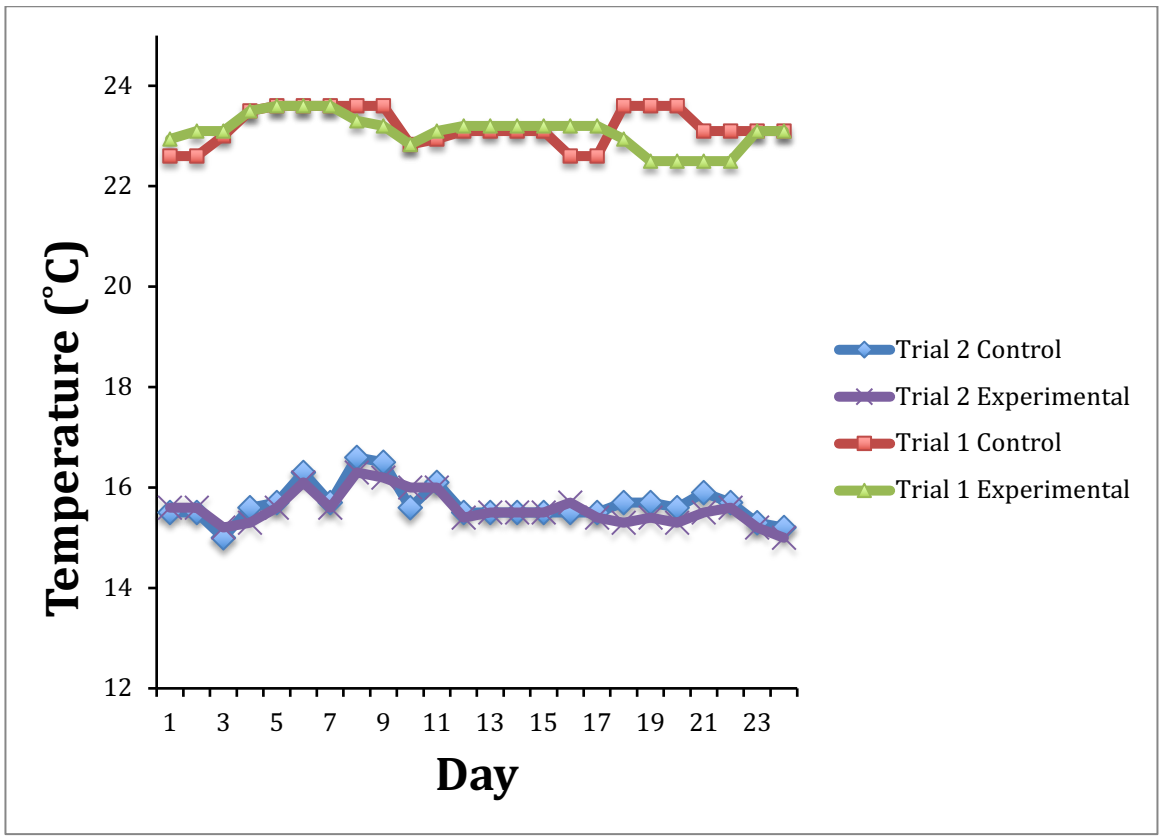

Figure 8. Linear comparison of experimental and control temperature for Trial 1 and Trial 2.

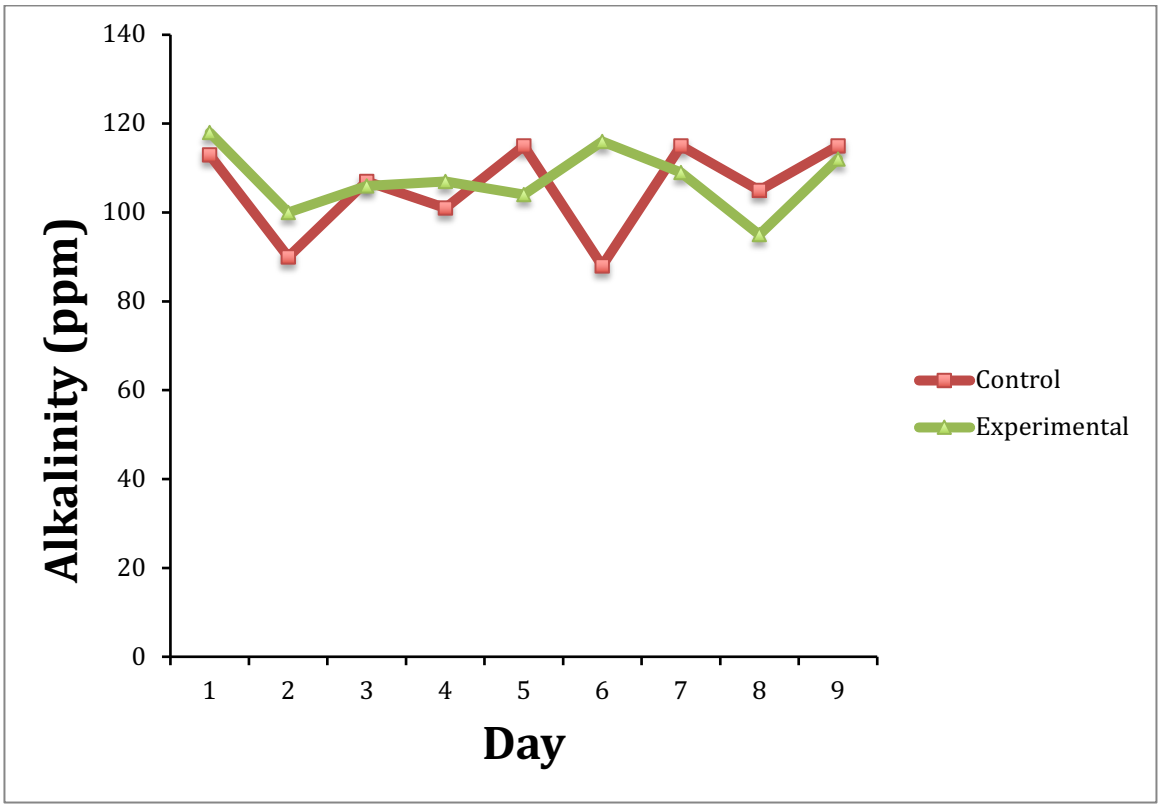

Figure 9. Linear comparison of experimental and control alkalinity for Trial 2

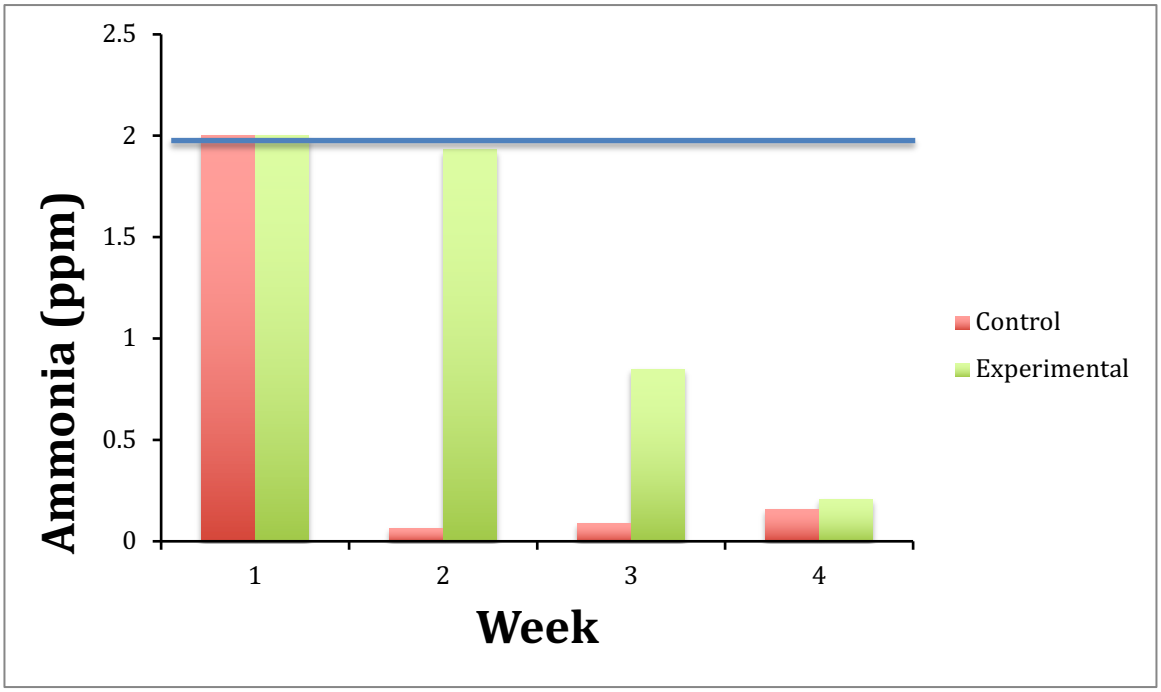

Figure 10. Bar comparison of experimental and control ammonia for Trial 2. The bars marked at 2ppm represent over-range/unreadable data, which is anything greater than 2.0 ppm.

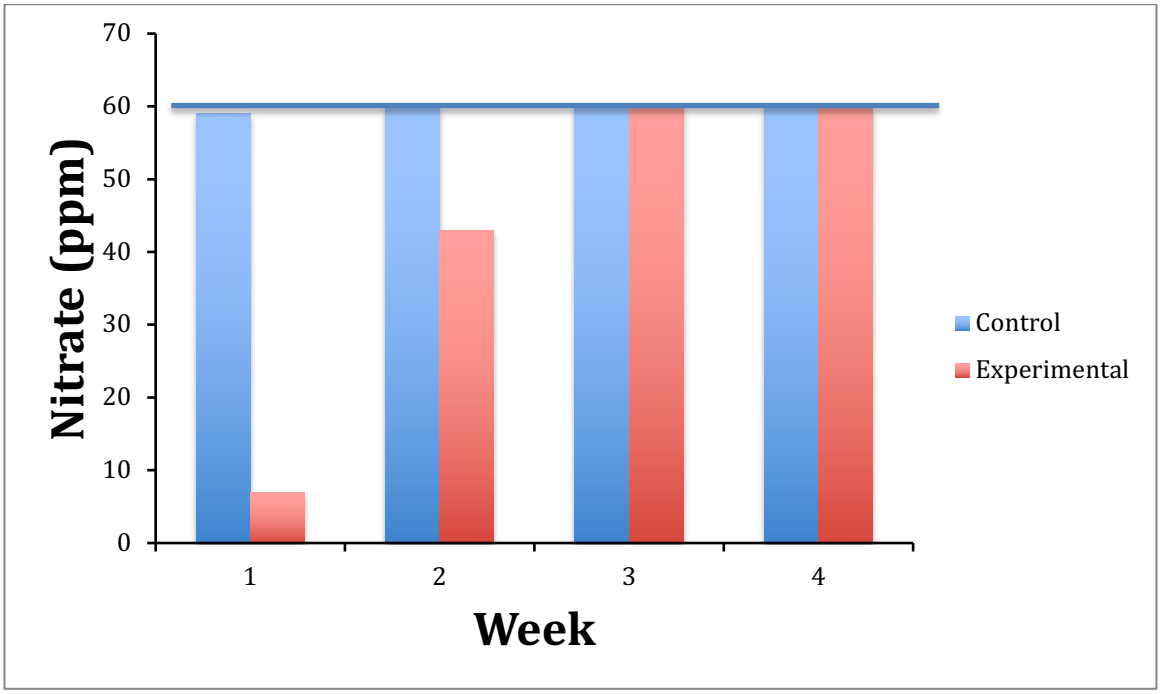

Figure 11. Bar comparison of experimental and control nitrate for Trial 2. The bars marked at 60 ppm represent over-range/unreadable data, which is anything greater than 60 ppm.

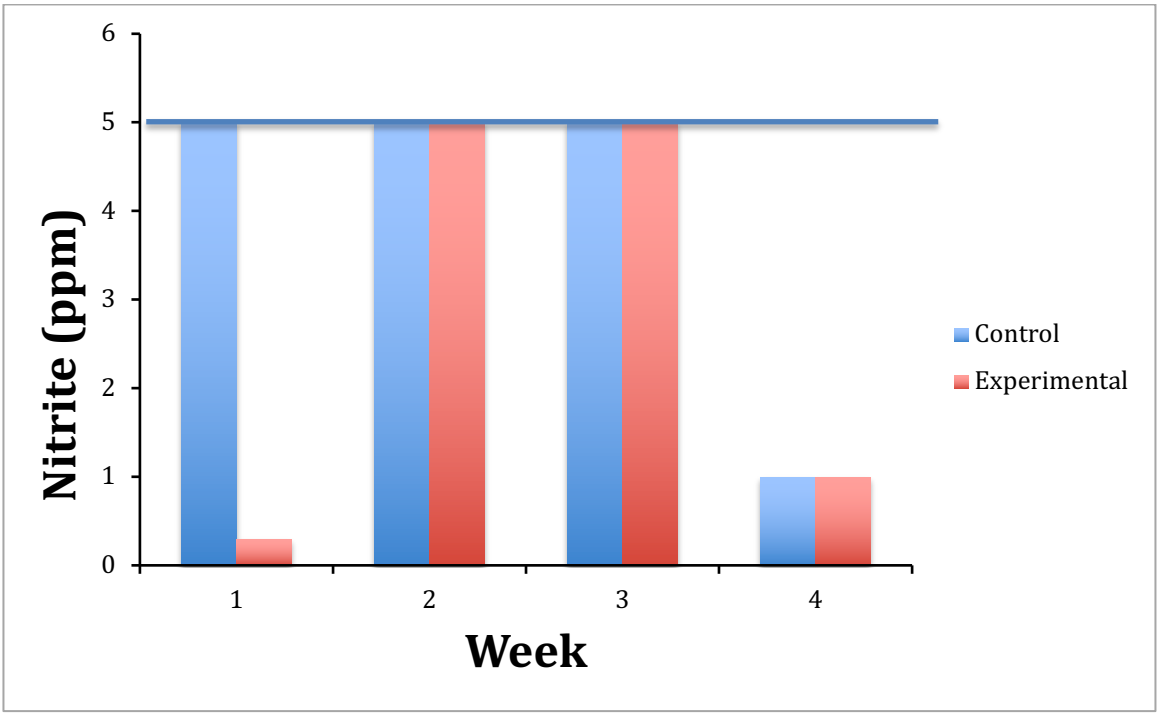

Figure 12. Bar comparison of experimental and control nitrite for Trial 2. The bars marked at 5ppm represent over-range/unreadable data, which is anything greater than 5.0 ppm

DISCUSSION

To accurately predict the effects of climate change on aquatic organisms, it is important to study interactions between environmental variables and physiological function of the animal (Bozinovic 2011). To do so with accuracy, the system used to gather data must be reliable and able to support a great range of aquatic life in a predictable and reproducible manner. While the ALCOAST proved to be an excellent preliminary model for ocean-acidification research to be conducted by students wanting to overcome financial barriers to research, there are aspects of the design that require modifications to ensure that the seawater-quality data is readable, accurate, and sustainable. Once these modifications have been made, STEM accessibility should continue to grow, and students of all ages should be able to conduct ocean-acidification research using this system as a model.

There were three overarching goals of this experiment: to construct, maintain and implement a low-cost, user-friendly system to explore the physiological boundaries of *C. maenas* to acidified conditions, to monitor seawater-quality parameters within the system and the condition of crabs to evaluate system performance, and to investigate the significance of acidified conditions on endocrine disruption in *C. maenas*. To achieve these goals, seawater-quality measurements were taken and hemolymph samples were collected from the animals to ensure there would be sufficient analysis of experimental factors that could have played a role in manipulating the data gathered.

pH measurements

Ideally, the pH of the control system would have averaged out to be higher than 7.87 for Trial 1 (Table 1) and 7.99 for Trial 2 (Table 2)**.** Natural seawater generally has a pH closer to 8.3, but exposing the animals to a system with a slight variation in pH was not a concern, as green crabs are extremely adaptable organisms, which contributes greatly to their success as an invasive species. Furthermore, autonomous sensors have recorded natural pH variability in coastal ecosystems that often does not align with predicted global ocean pH, proving that slight variability in the seawater is natural and something that animals are exposed to in their natural habitats (Hofmann 2011). As with the measurements taken in the control system of the ALCOAST, the experimental system demonstrated variation, but this was less significant than the variation in the control system. The observed variation in the pH of the control system could be the result of environmental CO2 in the area where the system was situated.

For Trial 1, the average experimental pH over the 24-day period was 7.45 (Table 1). For Trial 2, the average experimental pH over the 24-day period was 7.54 (Table 2). The pH was more stable in Trial 2 than in Trial 1, as is demonstrated by comparing Figure 4 to Figure 5. Additionally, it can be noted that the pH of natural seawater where green crabs reside was 7.95, which is lower than the 8.2-8.3 that open ocean water is typically measured at. This can be attributed to the intertidal zones in which green crabs inhabit as compared to open water depths (Beal 2015).

Several observations suggest that crabs in acidified water are stressed. First, the molting rate was higher in acidified tanks, as two animals molted in the acidified system during Trial 1 while none molted in the control system, and two animals molted in the

acidified system during Trial 2, while only one molted in the control system. In addition to this, when extracting the hemolymph from the Trial 2 animals, anecdotal observations suggested that crabs in acidified tanks bled less. Observations were too rare to be numerically significant, but literature has suggested that ocean-acidification has significant negative effects on crustaceans, such as increasing their mortality rate, decreasing their growth rate, and causing morphological changes (Long 2013). Longer trial periods would be necessary to obtain meaningful data, which could provide more information about the period of acclimation to acidified conditions that a species undergoes in a rapidly changing environment.

Seawater-quality measurements

Maintaining seawater-quality in the ALCOAST did not prove to be successful, which could be attributed to insufficient water filtration. As is demonstrated in Figures 11 and 12, the green crabs held in the ALCOAST during Trial 2 in both the experimental and control systems were exposed to high levels of nitrate and nitrite. Any measurement above 60 ppm is unreadable for nitrate, and any measurement above 5 ppm is unreadable for nitrite. Additionally, the ammonia levels in the system were unreadable during the first week of measurements, but became manageable during weeks 2-4 after water changes were implemented multiple times weekly (Figure 10). Any measurement above 2 ppm is unreadable for ammonia.

As has been previously noted in literature, the survival rates of crabs when exposed to excessive amounts of ammonia and nitrite has been seen to decrease linearly with the exposure time and concentration (Koo 2005). Thus, it would come as no surprise to observe decreasing survival throughout the trial periods, but the experimental period of

24 days did not prove to be long enough to make such observations. While several of the animals died during Trial 1, this was likely due to their multiple escapes from the system. This issue was addressed for the Trial 2 period by utilizing rubber bands to hold down the lids of the tanks. Had there been longer trial periods, it is likely that there would have been a correlation observed between seawater toxicity and animal survival rate.

While certain seawater parameters were not manageable due to a lack of proper filtration in the ALCOAST, salinity, temperature, and alkalinity remained somewhat consistent throughout both trials (Figures 6-9). The salinity of the system remained constant by utilizing the pre-made seawater in the ARC, and when alkalinity was low, a dry sodium bicarbonate buffer (Kent Marine super-buffer) was measured out according to the bulk reef supply reef calculator on bulkreefsupply.com and added to the sump tank of each system. The aim was to keep the alkalinity between 100 and 200 ppm, but the control system ranged between 88 ppm and 115 ppm while the experimental system ranged between 95 ppm and 118 ppm, which sometimes proved to be a little too low. The temperature was never manually manipulated because the ALCOAST was kept at room temperature of the basement in the ARC throughout both trials.

Furthermore, calcium and phosphate levels of the seawater were not measured until the beginning in the second week of Trial 2. The acceptable level of phosphate in seawater is less than 4.0 ppm, and that of calcium is between 380 and 450 ppm. Both parameters fell within the acceptable range in the trials: between 1 and 2 ppm for phosphate and 365-413 ppm for calcium.

Estradiol Enzyme-Linked Immunosorbent Assay (ELISA) Measurements

Estradiol plays a critical role in the development and behavior of marine organisms, including invertebrates, but it is expected that these factors will be significantly affected by ocean-acidification (Keay 2009). With ocean-acidification comes greater disruption of hormone signaling by pollutants in the environment, thus estradiol was chosen as the hormone to test (Keay 2009). Unfortunately, logistics and timing of this project did not allow for final ELISA analysis. The hemolymph was extracted from the animals, frozen and stored, but the assay has not yet been completed. It was assumed that it would have been difficult to analyze the data provided by the ELISA, considering the pH data was not properly controlled. Without proper differentiation of these parameters, determining the cause-and-effect relationship between what had been changed (pH) and what was going to be measured (estradiol) would not be feasible. For this reason, the attention of the project turned away from performing the ELISA and more towards fine-tuning the ALCOAST. Despite these complications, it is our hope to perform the ELISA during the summer of 2018.

Conclusions: Biological filtration and ideas for reconstruction

To counter erratic conditions within the system, the ALCOAST should include a biological filter. I attempted to maintain the seawater-quality of the system through mechanical filtration because a biological filter might affect the water chemistry too drastically in a system so small. Additionally, I assumed that the natural biofilms in the system would be able to complete the nitrogen cycle in the ALCOAST, but this did not happen, thus suggesting that biological filtration may be the greatest contributor to maintaining seawater-quality.

During biological filtration, bacteria in the water break down harmful concentrations of ammonia, convert them to nitrites, and then break them down into nitrates, the least toxic form (Camargo 2005). While the pH, salinity, alkalinity, and calcium were maintained at appropriate levels required to achieve my research goals (Figures 4-9), the system was not able to control the dangerous levels of nitrite and nitrate that were being recorded (Figures 11,12), and it was only possible to control ammonia levels by conducting excessive water changes (Figure 10). It is well documented that nitrite exposure can be very harmful to animals, and while freshwater animals appear to be more sensitive to nitrate than marine animals, precautions still need to be taken to minimize their adverse effects (Camargo 2005). In my experiment, I could not be certain whether the crabs were responding physiologically to the pH change or as stress responses to the sub-optimal increased levels of ammonia, nitrate, and nitrite. Without controlling these conditions, it will be impossible to assess the physiological responses of animals to acidification conditions.

In addition to adding a biological filter, including a more reliable mechanical filter, crab-proof lids, and plugs at the head tanks that could turn off the main water flow would be necessary (Figure 3). Furthermore, larger sample sizes would be tested, more trial periods would be conducted, and water-quality would be measured more consistently throughout every trial period. Should this point be reached, an ELISA assay of hormone levels would be more meaningful to analyze. Fortunately, the system is modular, meaning it can be broken down and stored for future students to use.

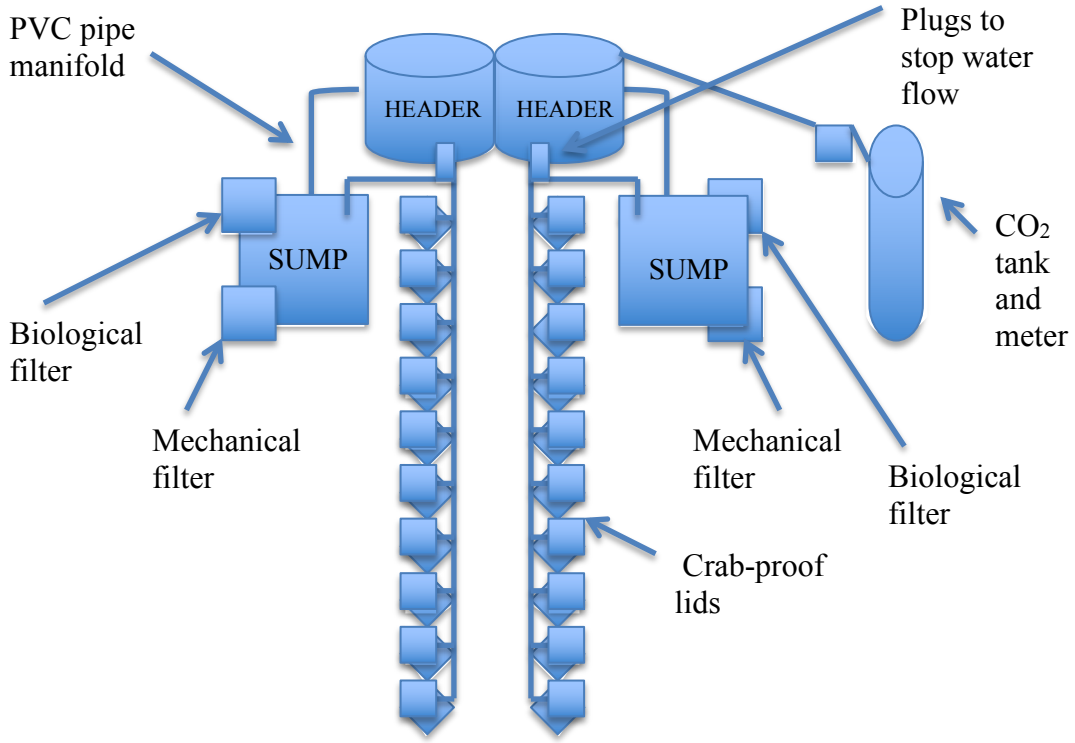

Figure 13. Schematic diagram of what the modified ALCOAST would look like

While unable to make definitive conclusions concerning an animal's physiological response to manipulated environmental conditions, the significance of these findings is still relevant for future use in the development of a low-cost system for student use. I constructed a low-cost system and successfully manipulated and maintained pH to explore the physiological boundaries of aquatic organisms. In doing so, I could demonstrate the strong ability of the green crabs to adverse conditions. Green crabs proved to be highly adaptive animals that are well-suited to facing climate change. Still, there is a major lack in data that is specific to the natural habitat of green crabs, as well as movement of bulk water and spatio-temporal variability, making the study of organismal pH tolerance only quasi-predictable (Kapsenberg 2016). While specific understanding of a green crab's environment is lacking, their ability to endure imperfect water conditions

became clear through this experiment. The ALCOAST and similar systems should continue to provide critical information concerning physiological responses and boundaries of aquatic animals under acidified conditions.

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