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Effects of Ocean Acidification on the Behavior of Two Marine Invertebrates: A Study of Predator-prey Responses of the Molluscs Conus Marmoreus and Strombus Luhuanus at Elevated-CO2 Conditions

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Effects of Ocean Acidification on the Behavior of Two Marine Invertebrates: A study of predator-prey responses of the molluscs *Conus marmoreus* and *Strombus luhuanus* at elevated-CO_2 conditions

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Abstract

Ocean acidification has been affecting the world's oceans since the introduction of anthropogenic CO_2 into the atmosphere during the Industrial Revolution. An increase in CO_2 uptake from the atmosphere to the ocean has had a profound impact on not only the water chemistry, but marine organisms as well. Ocean acidification is known to have significant impacts on marine invertebrates in terms of calcification and reproduction; however, effects of increased CO_2 on marine invertebrate behavior are vastly unknown. Marine conch gastropods have a modified muscularized foot that allows them to escape quite rapidly when faced with a predator cone shell. Utilizing the concentration of seawater CO_2 (950 ppm) predicted at the end of the century (2100), both the prey gastropod (*Strombus luhuanus*) and its cone shell predator (*Conus marmoreus*) were examined to determine behavior changes in their predator-prey interaction. General boldness of *S. luhuanus* was heightened by elevation in CO_2 by shortening the duration of time the *S. luhuanus* took to self-right itself by almost half. Prey behavior during predator-prey interaction was not significantly changed through ocean acidification scenarios; however, there were trends to suggest that the control prey would escape faster with use of a running behavior versus a jumping behavior, which was exhibited more often by the elevated-CO_2 group. With the *C. marmoreus* predator, CO_2 highly affected the activity level of the predator, where activity was five times that of the control. However, this increased activity did not affect the predatory success of the cone shell, but could increase the possibility of the predator happening upon the prey in the wild. Alteration of behavior of predator and prey interactions of marine invertebrates could have wide-ranging implications to the whole marine food web and entire marine ecosystem.

Keywords: ocean acidification, predator-prey interaction, behavior, *Strombus luhuanus*, *Conus marmoreus*

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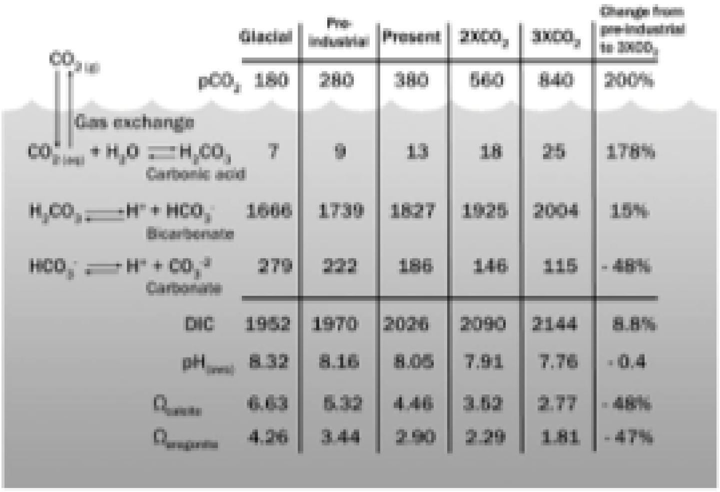
List of Figures and Tables

1.0 Introduction

1.1 Ocean Acidification

Ocean acidification has been affecting the marine system since anthropogenic carbon dioxide (CO_2) has been introduced into the atmosphere since the Industrial Revolution. Around one-third of the anthropogenic CO_2 produced within the last 200 years has been absorbed by the ocean (Sabine et al. 2004). Since the Industrial Revolution, oceans are now 0.1 units lower in pH and 30% more acidic and the rate of this change is occurring around 100 times more than in the past (Calderia and Wickett 2003; Siegenthaler et al. 2005; Royal Society 2005). Dissolved CO_2 in the oceans has been increasing linearly along with rising CO_2 in the atmosphere. Projected models of CO_2 emissions predict that ocean pH should decrease 0.3-0.4 units in response to the increased uptake of CO_2 (Calderia and Wickett 2003).

Dissolved carbon dioxide from the atmosphere has a great effect on the water chemistry of the ocean system. The inorganic carbon system is one of most important chemical equilibrium of the ocean system and is the biggest factor in controlling the pH of seawater. Dissolved inorganic carbon (DIC) has three major forms within seawater: bicarbonate (HCO_3^-), carbonate (CO_3^{2-}), and aqueous carbon dioxide, or dissolved carbon dioxide ($CO_{2(aq)}$). When CO_2 dissolves in the water column, carbonic acid (H_2CO_3) forms (Figure 1). Most H_2CO_3 dissociates quickly into a hydrogen ion (H^+) and HCO_3^-. The hydrogen ion then reacts with a carbonate ion thus producing bicarbonate. Increasing carbon dioxide into seawater increases concentrations of H_2CO_3, HCO_3^-, and H^+ and decreases the concentration of CO_3^{2-} and lowers pH (because pH depends on the concentration of H^+ in the water; $pH=-\log[H^+]$). Although the ocean uptake of CO_2 reduces the amount of CO_2 in the atmosphere, thus reducing global warming through the greenhouse effect, there is a direct effect of high CO_2 on the water chemistry, as seen in Figure 1, of the ocean that affects marine ecosystems vastly (Fabry et al. 2008).



	Glacial	Pre-industrial	Present	$2XCO_2$	$3XCO_2$	Change from pre-industrial to $3XCO_2$
pCO_2	180	280	380	560	840	200%
$CO_{2(aq)} + H_2O \rightleftharpoons H_2CO_3$ Carbonic acid	7	9	13	18	25	178%
$H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$ Bicarbonate	1666	1739	1827	1925	2004	15%
$HCO_3^- \rightleftharpoons H^+ + CO_3^{-2}$ Carbonate	279	222	186	146	115	- 48%
DIC	1952	1970	2026	2090	2144	8.8%
$pH_{(sws)}$	8.32	8.16	8.05	7.91	7.76	- 0.4
$\Omega_{calcite}$	6.63	5.32	4.46	3.52	2.77	- 48%
$\Omega_{aragonite}$	4.26	3.44	2.90	2.29	1.81	- 47%

Figure 1. Concentrations of carbon forms (in µmol/kg), pH values, and aragonite and calcite saturation states of average surface seawater of pCO_2 concentrations (ppm) during glacial, preindustrial revolution, present day, two times pre-industrial (near mid-century CO_2 predictions), and three times pre-industrial CO_2 (mid to late century CO_2 predictions). The last column shows changes from pre-industrial levels through three times atmospheric CO_2 (Fabry et al. 2008).

1.2 Effects on Marine Biota

Increased levels of CO_2 in the atmosphere directly relate to elevated levels of partial pressure of CO_2 (pCO_2), or hypercapria, and impact marine organisms by both decreased calcium carbonate ($CaCO_3$) saturation states, which directly affect calcification rates of marine biota, and through disturbance of the acid-base metabolic physiology of species. More specifically, in calcareous invertebrates, elevated pCO_2 has lead to reductions in growth, calcification and survival (Fabry et al. 2008). In marine fishes, behavior is highly affected by the increase in CO_2. The behavioral effects include altered olfactory and auditory senses, loss of lateralization, and inability to learn (Munday et al. 2009; Simpson et al. 2011; Domenici et al 2012; Ferrari et al 2012). Juvenile fish become less likely to avoid a predator cues or travel less to avoid the predator compared to control, thus increasing their mortality rate and decreasing their respective population replenishment after exposure to future ocean acidification conditions. Juvenile fish are often known to become bolder and travel further distances from their habitat, thus increasing their vulnerability to predators (Cripps et al. 2011; Munday et al. 2010; Welch 2010; Dixon et al. 2010). Predator fish as well have a lower capture success when placed under elevated CO_2 conditions suggesting the dynamics of predator-prey interactions may be altered depending on how affected the respective prey and predator are to increased levels of CO_2 (Allan et al. 2013).

1.3 Effects on Marine Invertebrates

However, marine invertebrate behavior to elevated CO_2 conditions has been even less studied than fish behavior except in very high CO_2 levels exceeding 12,000 µatm pCO_2 (equivalent to 12,000 ppm in the atmosphere). With these high levels of CO_2, there was decreased morphological defense from decreased calcification and increased avoidance to chemical cues from a predatory crab with an intertidal snail (Bibby et al. 2007). The similarity within fishes' and marine invertebrates' nervous systems suggest that the behavior of molluscs in CO_2 conditions that are predicted by the end of the century are similar to those behavioral response of fish. With ocean acidification, it is found that the GABA-A neurotransmitter is receptors are blocked due to changes in trans-membrane chloride (Cl^-) and bicarbonate (HCO_3^-) ion gradients during acid-base regulation in result of the increased bicarbonate concentrations. The behavior of marine invertebrates in more impending CO_2 conditions as projected within the end of this century have yet to be heavily researched. Watson et al. (2013) found that snail prey's escape response to a cone shell predator cue and presence was impaired by near-future CO_2 conditions (961 atm) after five days of exposure. Prey snails would exhibit less escape behavior (jumping) and escape less distance from the predator. The prey's behavior was often quite variable in which some individuals were not affected by the elevated-CO_2 exposure and still responded to the predator's presence and/or cue. However, Manriquez et al. (2013) found that near-future CO_2 levels (716 ± 12 and 1036 ± 14 atm CO_2) improved certain behaviors of a marine gastropod. They found avoidance behavior (i.e. self-righting) was improved under these conditions in a marine gastropod thus reducing vulnerability to predation. Most research on marine invertebrate behavior has been conducted with prey response to predators and very little has examined predator behavior under similar conditions

Marine invertebrates are very important to the function of all marine ecosystems and their behavior and interactions often have a profound effect in the outcome of key ecological processes, especially within the intertidal and sub-tidal zones (Stella et al. 2011). For example, marine invertebrates account for $59 billion USD of global fisheries per year (FAO 2010). Obviously, any adverse effects that the impending CO_2 levels have on the behavior of these marine invertebrates would have heavy consequences and implications not only to marine biodiversity in the intertidal zones, but global fisheries as well.

Much of the survival of species depends on the ability for organisms to avoid predation and scavenge for food. In terms of predator avoidance, most predator sensing in marine systems are completed through avoiding chemical cues that are produced by the predator. In molluscs, most of this behavior is done through moving away from the predator or the predator's chemical cue in some way (Jacobsen and Stabell 2004). Some molluscs have a very dramatic predator-escape strategy in response to a predator's presence. Within the marine family Strombidae (conchs), the predator response is to leap or jump away from predators through a fast kicking motion of their modified foot and operculum, which is usually used to right them in their shell.

If the prey loses its ability to successfully escape from its predator, the survival of individuals and possibly species may be threatened as well. The response is usually geared towards a molluscivous cone shell predator (Robertson 1961). The cone shell predatory success will also have impact on the survival of its species. If the predator is unable to find its prey, the survival of the organism may be threatened. Both predator and prey behavior under ocean acidification should be examined in order to determine the full effect changes in predator-prey interactions would make on the intertidal and sub-tidal ecosystem.

1.4 Aims

This study aims to discover the effect near-future CO_2 concentrations (950ppm) will have on both predator and prey response behavior to obtain the changes in invertebrate predator-prey interactions under future ocean acidification conditions. A series of three experiments were conducted to view prey escape response and predatory success. Two assays were conducted with the prey and one was conducted with the predator. (i) First, the fundamental exercise behavior of *Strombus luhuanus* (prey) was analyzed through a self-righting experiment. (ii) Next, the prey was placed within a test arena with its cone shell predator (*Conus marmoreus*) to test whether CO_2 affected predator-escape behavior. (iii) For the predator behavior, *Conus marmoreus* was placed within a test arena with a prey snail (*Gibberulus gibberulus gibbosus*) to test whether CO_2 affected predatory success of the cone shell.

2.0 Methods and Materials

2.1 Study Species

Strombus luhuanus

Strombus luhuanus is an herbivorous marine gastropod that belongs to the Strombidae family. It is found throughout sandy sub-tidal areas around tropical coral reefs prevalent with filamentous microalgae, in which it feeds on (Catterall and Poiner 1983). Similar to other strombid gastropods, *S. luhuanus* have well-developed eyes and an acute sense of vision, along with a highly flexible, muscularized foot that allows for a distinct "jumping" escape response (Berg 1974).

Previous CO_2 tests show that members of this conch snail family (*Gibberulus gibberulus gibbosus)* are susceptible to behavioral changes from elevated-CO_2 conditions (Watson et al. 2013). *S. luhuanus* is in much higher abundance and has a similar escape response as *G. gibberulus gibbosus*, thus it was deemed a suitable species for experimentation.

Conus marmoreus

Conus marmoreus is a molluscivorous marine invertebrate that belongs to the Conidae family (cone shells). It is found in sandy sub-tidal zones near tropical coral reefs similar to *S. luhuanus*. *C. marmoreus* feeds on molluscs including other *Conus*. It attacks prey through the use of a white proboscis that stings the prey, paralyzing it. Unlike other *Conus*, *C. marmoreus* are diurnal and are active during the day rather that strictly nocturnal (Rockel et al. 1995).

Conus marmoreus are known specialist predators of members of the Strombidae family and are known to elicit the typical escape response in strombid prey (Watson et al. 2013). However, little is known about the effects elevated levels of CO_2 would have on the ability for *C. marmoreus* to track its prey.

2.2 Systems and Water Manipulation

Prey snails and cone shell predators were collected throughout the month of November from the Lizard Island Lagoon, Great Barrier Reef, Australia (14^{o}41’S, 145^{o}28’E) and transported to Lizard Island Research Station, where they were held in a controlled aquarium facility. Both *Strombus luhuanus* and *Conus marmoreus* were randomly placed in aquarium tanks that represented current day CO_2 (400 ppm CO_2) and end of century predicted CO_2 (950 ppm CO_2) conditions. *S. luhuanus* were randomly assigned two aquarium tanks per treatment, and *C. marmoreus* were randomly assigned to three separate tanks per treatment. Eighteen snails and three cone shells were placed within each 32L (38L x 28W x 30H cm) aquarium. The strombs fed on the algal film that grew within each surface of the aquarium and from rocks collected from the Lagoon. Strombs were kept for 10-13 days in each treatment group and were tested afterwards. Cone shells were feed *Gibberulus gibberulus gibbosus*, another member of the Strombidae family, semi-regularly. The cone shells were placed in captivity for 14-15 days and tested afterwards. Each aquarium was supplied with control or elevated-CO_2 seawater at a rate greater than 780 mL/min. Seawater was pumped into the aquarium from the Lizard Island Lagoon into 2 x 60L header tanks where it was diffused with ambient air (control treatment) or 100% CO_2 to achieve desired pH (elevated-CO_2 treatment). Control systems maintained a desired pH of 8.182 (mean ± SE; 8.160 ± 0.005). Dosing the seawater with CO2 to a desired pH of 7.861 (mean ± SE; 7.828 ± 0.006) created the elevated-CO2 seawater (Table 1). A pH-controller (see Appendix A) was attached to the elevated-CO_2 header tank to maintain the pH to its desired pH level. The pH and temperature of the seawater were measured daily using both a pH and temperature probe (see Appendix B and Appendix C). Seawater CO_2 concentrations was confirmed with a portable CO_2 equilibrator and manifold.

Table 1. Seawater chemistry for each treatment on 10/11/2013 and 13/11/2013 (mean ± SE).

Treatment	**Temperature (^oC)**	**pH**
Control	28.0 ± 0.005	8.16 ± 0.005
Elevated-CO_2	28.1 ± 0.094	7.83 ± 0.006

2.3 Data Collection

Behavioral changes from exposure to elevated-CO_2 levels of *S. luhuanus* and *C. marmoreus* were measured through two separate experiments for *S. luhuanus* and one experiment for the *C. marmoreus*. All trials were videotaped with a Canon Powershot G15 or a Nikon Coolpix waterproof digital camera. All behavioral trials were conducted in seawater at the same CO_2 level as the experimental treatment of the stromb or cone shell tested (i.e. control or elevated-CO_2). Mean stromb shell length (±SE) was 54.01 ± 0.45mm and total animal wet mass was 31.28 ± 0.65g (power regression; $p<0.40001$, $r^2=0.6858$). Shell mass compromised 58.3% of the whole animal wet mass.

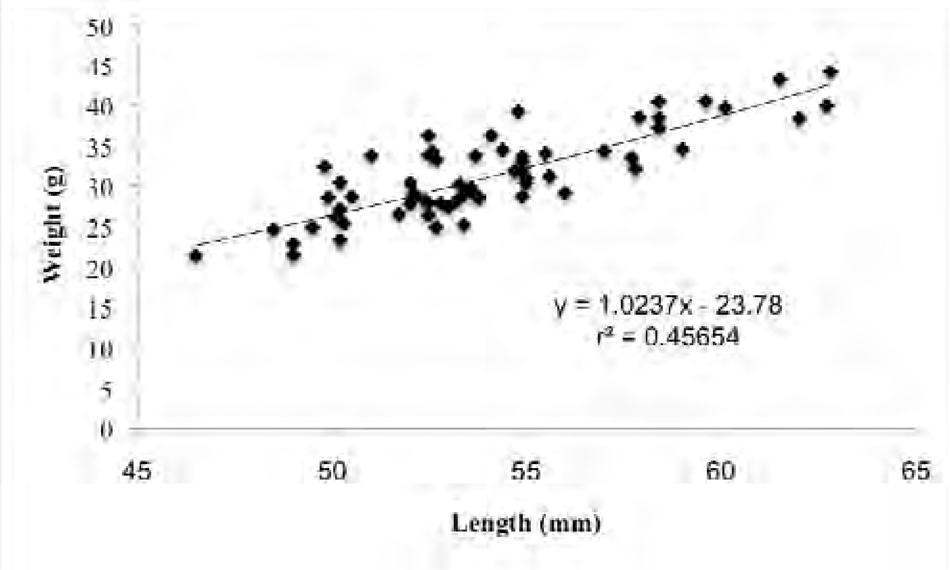


Figure 2. The relationship between length and wet weight of *S. luhuanus* used in trials (n=64).

Mean cone shell length (± SE) was 59.73 ± 2.97 mm and total animal wet mass was 54.8772 ± 7.08 g (power regression; $p<0.0001$, $r^2=0.9799$).

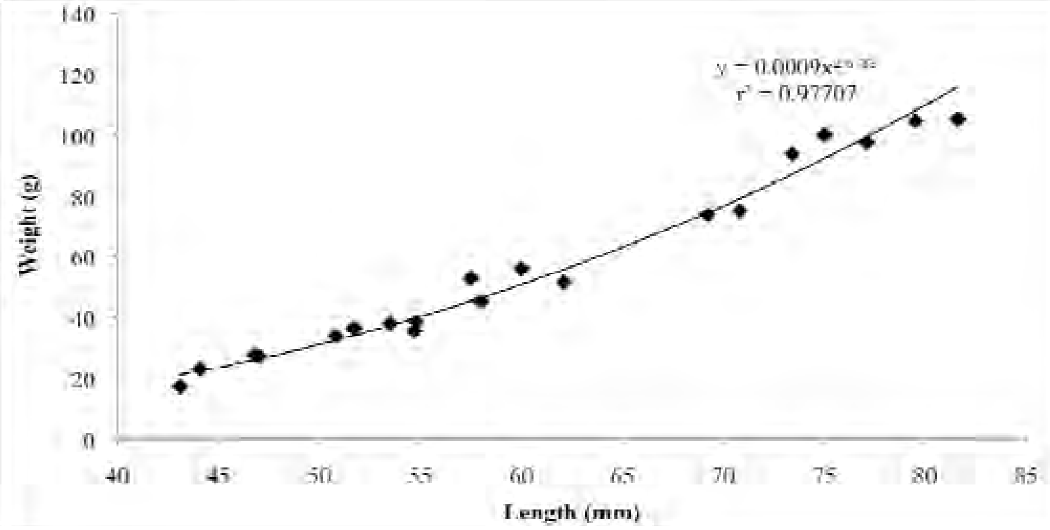


Figure 3. The relationship between length and wet animal mass of *C. marmoreus* used in trials (n=9).

2.3.1 ***Effect of Elevated-CO$_2$ on*** **S. luhuanus** ***Behavior***

After 10-13 days in control or elevated-CO$_2$ treatment aquaria, stromb behavior was tested in two separate experiments measure exercise ability and escape behavior from its cone shell predator.

i. Experiment 1: effect of elevated-CO$_2$ on exercise ability

The ability for an animal to self-right itself when placed upside-down is known to be an accurate test of the animal’s exercise ability and a measure of boldness (Manriquez et al. 2013; Watson et al. 2013). In order to examine the effect elevated-CO$_2$ exposure has on the exercise behavior *S. luhuanus*, control and CO$_2$-exposed strombs were placed upside down and recorded the time taken and number of foot twitches and flicks were necessary for the animal to self-right. Twitches were distinguished by slight movement of the operculum outside the stromb shell, where as a flick was measured as the full movement of the operculum towards the substrate. Time was recorded by use of a stopwatch and then verified through video analysis. Those strombs that exceeding three minutes to right themselves were excluded from the data set due to previous testing that deemed the average righting time was around 1.5 minutes. The test arena for the trial was within a large circular tank (104 cm in diameter) filled with 5 cm of sand and seawater with a depth of 20 cm above the sand (See Appendix D). A total of 23 control and 20 CO_2 strombs were tested individually for self-righting.

ii. Experiment 2: effect of elevated-CO_2 on escape behavior

To test predator-escape response behavior of control and CO_2 strombs, a single stromb was placed in the center of the same test arena as Experiment 1 after recording its self-righting behavior. Each stromb was allotted at least 10 minutes in between experiment types. The stromb was placed 2 cm away from the *C. marmoreus* cone shell predator without a barrier. Predator and prey anterior ends faced each other and the trial was recorded for 5 minutes or until the stromb would hit the wall of the arena. In order to account for variability in predator, the cone shell was removed from the tank after every trial and residual mucus produced from the cone shell was also removed. A total of 23 control and 20 CO_2 strombs were tested. Through video analysis, the latency of the first response (jump or run), percentage of behavioral response per treatment, total distance traveled (before the animal hit the wall), time to hit wall, and the speed (cm/s) at which the animal traveled were measured. Latency of first response and time to was timed via a stopwatch and then verified with the video analysis. The rate was calculated by dividing the total distance traveled by the difference of time taken for the animal to start its primary behavior to when the animal hit the wall. The distance and time to the wall and rate were calculated with 6 control and 10 CO_2 strombs, but first response time and first behavior was conducted with all 23 control and 20 CO_2.

2.3.2 *Effect of Elevated-CO_2 on* C. marmoreus *Behavior*

In order to observe behavioral changes of cone shell behavior from elevated CO_2 levels, a similar test was conducted as described in stromb Experiment 2 above. After 14-15 days of exposure to either control or elevated-CO_2 treatments, *C. marmoreus*'s predator-prey interaction with a control prey snail (*G. gibberulus gibbosus*) was conducted. A different prey snail was used because *G. gibberulus gibbosus* is known to illicit a strong predatory response when in the presence of this snail and this part of the study builds on known responses of the prey snail *G. gibberulus gibbosus* to *C. marmoreus* at control and elevated-CO_2 (Watson et al. 2013). The experiment consisted of two time periods: the acclimation period and the experimental period. For the acclimation period, the cone shell was placed in the center of the arena and allotted 15 minutes to acclimate itself to the environment. After the 15 minutes expired, the cone shell was placed back in the center of the arena and a prey snail was placed 2 cm in front of the predator. Predator and prey anterior ends faced each other and the interaction was recorded for 15 minutes. A total of nine cone shells from each treatment were examined. Two major aspects were measured via video analysis: distance traveled and final distance from prey as measurement of both activity behavior and predatory success.

2.4 Data Analysis

Video analysis of both the *S. luhuanus* and *C. marmoreus* was conducted through the use of Apple QuickTime. In order to more accurately measure the distance aspects of both experiments, the video analytical program ImageJ was used. One-way analysis of variance (ANOVA) was used to deem significance of behaviors measured throughout the trials by the different treatment groups.

3.0 Results

3.1 Effects of Elevated-CO_2 on S. luhuanus

Exercise Behavior

Elevated-CO_2 did not seem to affect *S. luhuanus*'s ability to self-right. However, the findings suggest that the strombs boldness increased under elevated levels of CO_2. Strombs placed under elevated-CO_2 conditions seemed to right almost twice as fast as the control strombs (Figure 3). This difference in time taken self-right was deemed significant (ANOVA; p=0.0048, F=8.95, df=1).

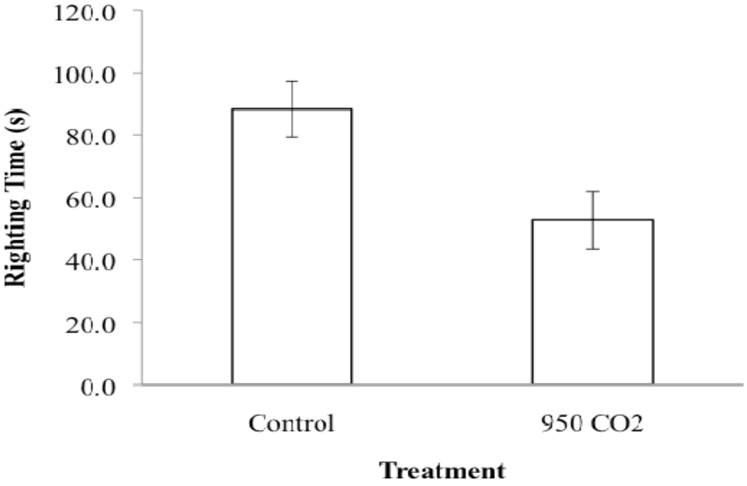


Figure 4. Time elapsed to self-right by both control and elevated-CO_2 strombs after 10-13 days of exposure to each respective treatment (mean ± SE; n=23,20).

Strombs in the control group righted with a mean of 89.5 ± 9.01 s, whereas the elevated CO_2 righted with a mean of 52.8 ± 9.30 s. There was not, however, difference between the numbers of twitches (mean ± SE; control 0.682 ± 0.290, elevated CO_2 0.444 ± 0.145; p=0.497) or flicks (mean ± SE; control 2.55 ± 0.205, elevated CO_2 2.11 ± 0.290; p= 0.217) between groups.

Escape Behavior

In terms of the escape response to the presence of a cone shell predator, there did not seem to be any difference between control and CO_2-exposed groups of *S. luhuanus* but there were some trends. Control strombs seemed to take longer to react to the predator cone shell (Figure 1). However, this difference was deemed insignificant (ANOVA; p=0.338, F=0.94, df=1).

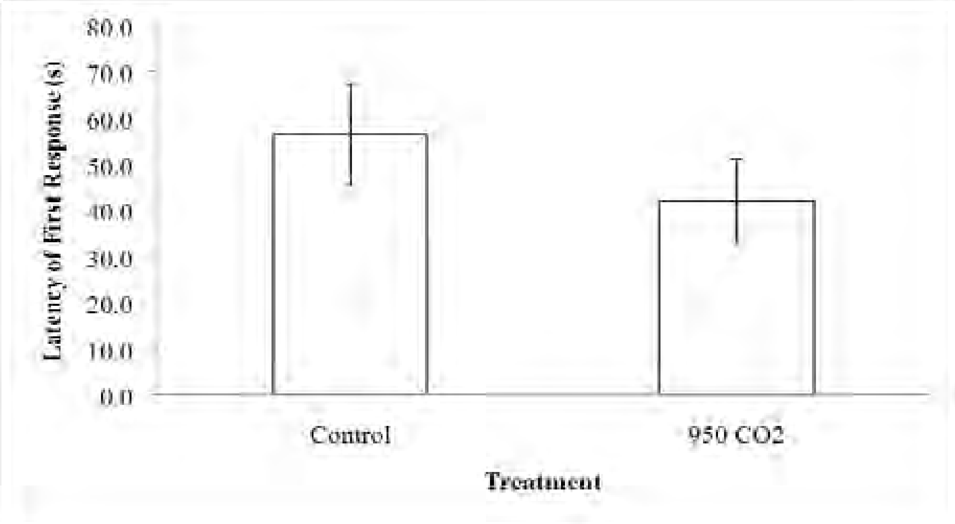


Figure 5. Time taken by *S. luhuanus* to each jump or run in response to the *C. marmoreus* predator during a 5-minute interaction after 10-13 exposure to either control or elevated-CO_2 scenarios (mean ± SE; n=23, n=20).

The control group would respond on average within 56.5 ± 10.9 s, whereas the elevated-CO_2 group responded with their first response within 42.1 ± 9.04 s. In terms of first response behavior, the strombs responded quite similarly between control and CO_2 treatments. However, two strombs within the CO_2 did not respond at all to the predator. Elevated-CO_2 strombs also seemed to favor jumping versus running as a primary response to the predator (Figure 5). However, these differences were not significant.

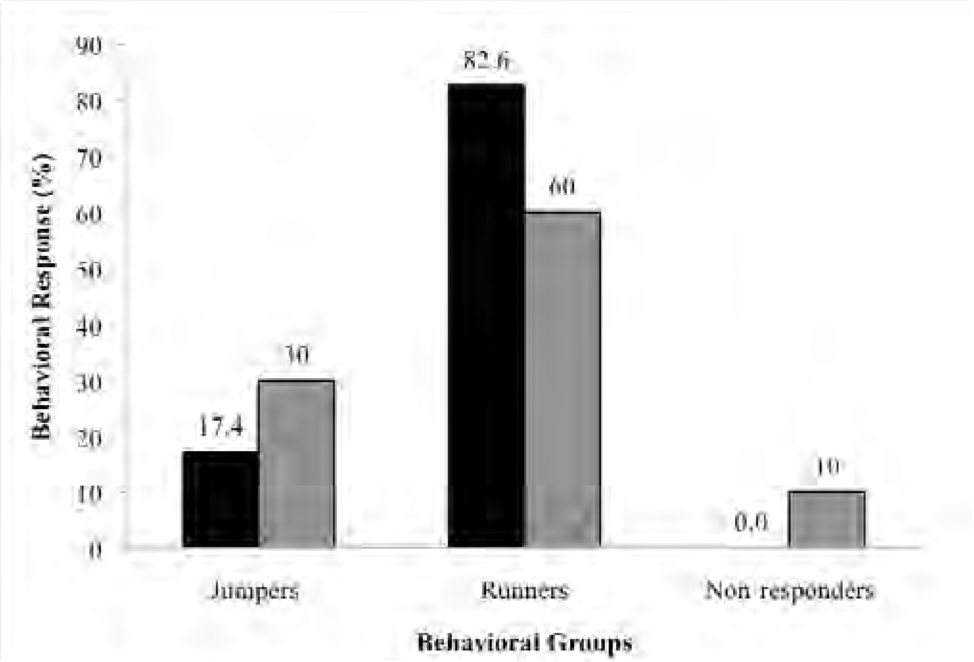


Figure 6. Primary behavioral response of control (black) and elevated-CO_2 (gray) *S. luhuanus* after 10-13 days of exposure to each respective treatment during a five-minute predator-prey interaction (n=23, n=20).

Elevated CO_2 seemed to affect the time and rate at which the animal would hit or sense the wall. However, there was no difference in the distance traveled to get to the wall. Control strombs reached the wall around 50 s before strombs within the elevated CO_2 group (Figure 6). This difference was slightly significant (ANOVA; p=0.041, F=5.18, df=1).

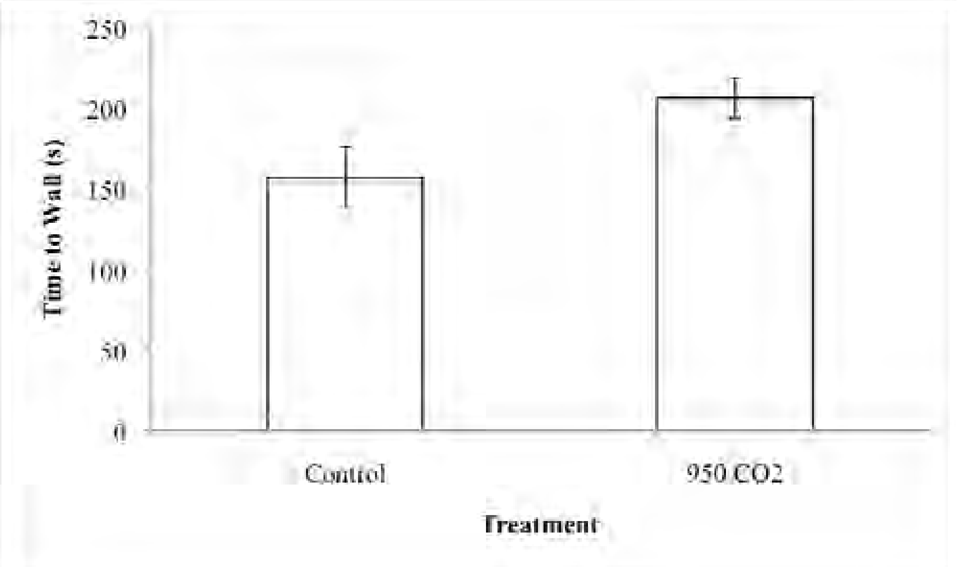


Figure 7. Time elapsed for *S. luhuanus* to reach or sense the arena wall during the 5-minute predator-prey interaction after 10-13 captivity in control or elevated-CO_2 aquarium (mean ± SE; n=6, n=10).

The mean (± SE) time for control strombs was 157 ± 18.8 s in comparison to the elevated-CO_2 strombs that took 206 ± 12.4 s. The distance to the wall, however, was insignificant (mean ± SE; control 78.0 ± 10.7 cm, elevated CO_2 74.7 ± 12.5 cm, p=0.844) between groups. There seemed to be a trend with the rate at which the animal traveled to the wall. Control strombs traveled slightly faster than the CO_2 strombs in terms of cm/s towards the wall to escape from the predator (Figure 7). However, this difference was deemed insignificant (mean ± SE; control 0.494 ± 0.020 cm/s, elevated CO_2 0.413 ± 0.038 cm/s, p=0.098).

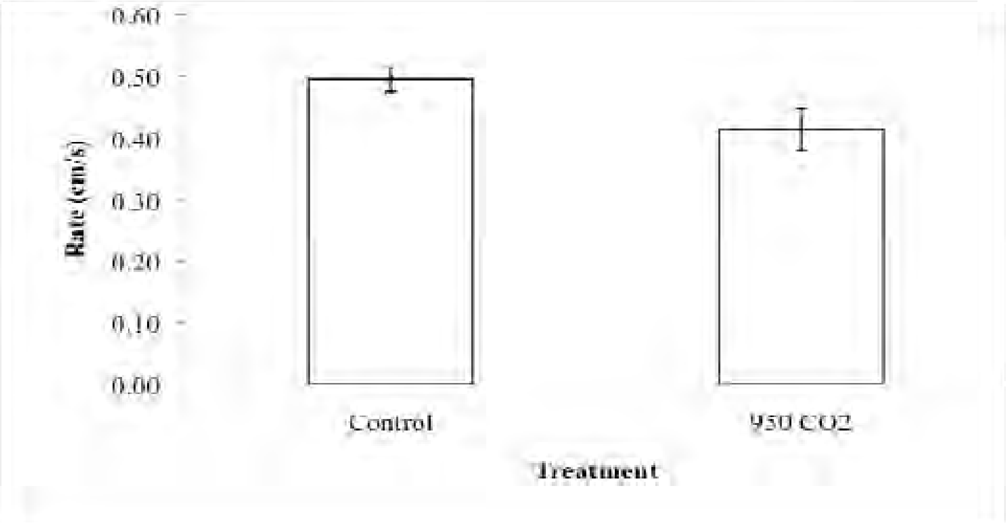


Figure 8. Rate at which *S. luhuanus* traveled during a five minute predator-prey interaction after 10-13 captivity in control and elevated CO_2 conditions (mean ± SE; n=6, n=10).

3.2 Effect of Elevated-CO_2 on C. marmoreus

Elevated-CO_2 had an effect on the distance traveled by each cone shell predator during the 15-minute interaction, but not the distance from the prey at the end of the interaction. The distance traveled is also attributed to the general activity of the predator. The distance traveled by the elevated-CO_2 animals was approximately six times that of the control animals. The variability of the group around eight times that of the control group (Figure 8). These differences were significant (ANOVA; p=0.034, F=5.34, df=1).

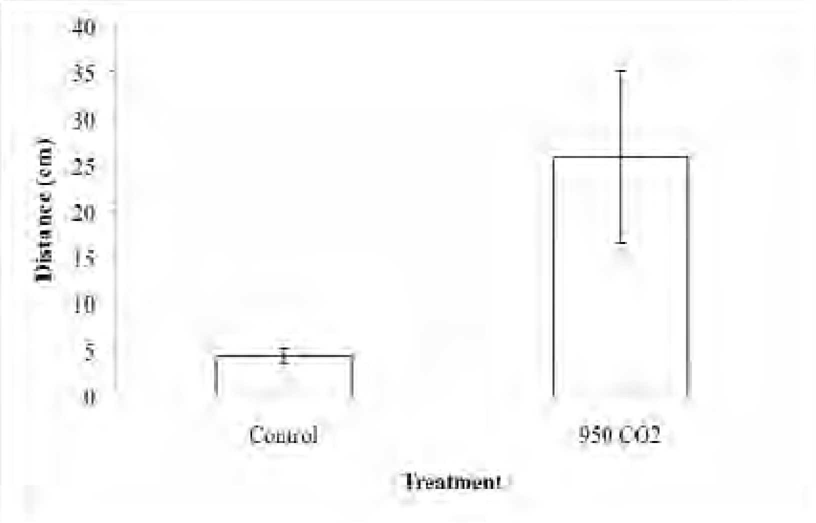


Figure 9. Total distance traveled by the *C. marmoreus* predator during the 15-minute interaction with prey *G. gibberulus gibbosus* after 14-15 days of either control or elevated-CO_2 treatment (mean ± SE, n=9).

The control cone shell predator had an average of 4.25 ± 0.770 cm compared the greater distance traveled by the elevated-CO_2 predators (mean ± SE, 25.7 ± 9.26 cm). The control cone shells were approximately twice the distance away from the prey as the elevated-CO_2 cone shells were (Figure 9). This distance was insignificant (mean ± SE, control 26.5 ± 3.79 cm, elevated CO_2 38.0 ± 8.83 cm, p=0.247).

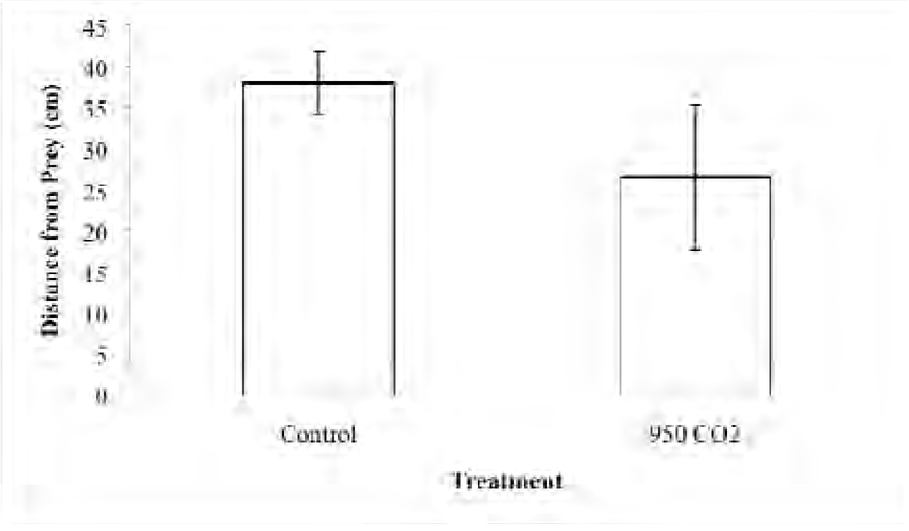


Figure 10. Final distance of *C. marmoreus* from *G. gibberulus gibbosus* prey after a 15-minute interaction after held 14-15 days in control and elevated-CO_2 conditions (mean ±SE; n=9).

4.0 Discussion

4.1 Effect of Ocean Acidification on Prey Behavior

Near-future CO_2 concentrations (950 ppm CO_2) affected some important behavioral traits of *Strombus luhuanus* that impact its interaction with predators. *S. luhuanus* placed in the elevated-CO_2 treatments self-righted itself significantly faster than those placed in control (ANOVA; p=0.004852). Measurement of righting time provides good indication whether or not an individual is exhibiting a stress-related response to the changes in pH caused by ocean acidification. A faster self-righting is suggested to be an adaptive benefit in behavior by previous studies (Manriquez et al. 2013). Righting at a faster rate could also indicate that boldness will increase with elevated-CO_2 with marine molluscs. When self-righting, the stromb becomes briefly more vulnerable to predators due to the exposure of their operculum foot. This increase in boldness was also observed in fish that were placed under similar elevated-CO_2 scenarios (Dixon et al. 2010). Although self-righting exposes the stromb to predators, overall faster self-righting may be a potential advantage because it lessens the duration of time the prey is vulnerable to predation. This could mean that faster self-righting could be a positive effect of increased CO_2 in the oceans on mollusc behavior. This possible advantage to marine gastropods under ocean acidification was also found by Manriquez et al. (2013) with Chilean snails.

During the predator-prey interaction, there were comparable lesser effects of elevated-CO_2 levels on prey escape response compared to exercise ability and boldness. There was no significant difference between latency of first response and type of behavioral response (ANOVA; p=0.338). However, there was a trend with the type of behavioral response *S. luhuanus* exhibited between the two treatments. Within the elevated-CO_2 treatment, more *S. luhuanus* chose to jump as a first escape response versus run like most of the control. This could suggest that there will be a behavioral shift from running to jumping as predator escape strategy. However, the jump response could have resulted from the stressful conditions are suggested to be induced by the chemical changes associated with ocean acidification (Manriquez et al. 2013). Jumping as an escape strategy may allow for the stromb to escape at a faster rate than running would, which may also be an advantageous change in behavior due to increased CO_2. However, this shift in behavior may actually be less efficient for the stromb. The jumping behavior requires possibly more energy per behavior than the running behavior. This could disallow the strombs from escaping farer distances in the wild from predator because jumping would tire the stromb out more quickly than the running behavior. However, the metabolism of *S. luhuanus* is still unknown so it is unsafe to say whether or not the running behavior is more energy efficient compared to the jumping behavior. Two *S. luhuanus* within the elevated-CO_2 treatment chose not to respond to the predator, which suggests that the animal did not sense the predator or did not view the predator as a threat, therefore did not see the urgency to escape. Perhaps with a larger sample size, the number of non-responders would increase because more individuals would be affected by the ocean acidification. The inability for the prey to sense the predator has been also seen with other studies on marine gastropods (Watson et al. 2013). The reduced risk assessment of *S. luhuanus* to its cone shell predator is similar to the lowered risk avoidance in juvenile fish (Ferrari et al. 2011).

Although there was no significant difference with first response time and behavior, there was difference in the ability for the prey to escape their predator. Control *S. luhuanus* generally traveled in a more direct path to the wall of the arena and were able hit or sense the wall faster than those animals in elevated-CO_2 levels. The rate at which control animals reached the wall also seemed slightly faster than those under ocean acidification conditions, although this difference was deemed insignificant (ANOVA; p=0.0979). This trend implies that control strombs may be more successful at escaping their cone shell predator in the field because they are able to travel in a more direct way to escape their predator and at a faster rate. This difference in prey escape success supports what was found in other snails in the Strombidae family, where prey snails under elevated-CO_2 levels escaped less distance from the predator, which suggests that those individuals would be less successful in escaping predation in the wild (Watson et al. 2013). Overall, the results indicate that there will be behavioral changes within marine gastropods like *S. luhuanus* within near future predictions of CO_2 concentrations in the ocean. These changes can be advantageous to the gastropod in terms of risk avoidance (i.e. faster self-righting) and changes in behavioral responses (running to jumping behavior) to avoid predation. However, ocean acidification could also have negative side effects through lowering risk assessment to predator presence and ability to escape efficiently.

4.2 Effect of Ocean Acidification on Predator Behavior

Elevated-CO_2 affected predator cone shell *C. marmoreus*' behavior mainly in terms of activity level and not necessarily predatory success. Individuals under ocean acidification conditions traveled further distances during the predator-prey interactions than under control conditions. The increased distance traveled is related to the general activity level of the animal, which suggests the cone shell predator will become more active under future CO_2 conditions. However, this increase in activity level did not necessarily mean the predator under elevated-CO_2 was more successful at capturing prey, where no prey was captured within the observation period of 15 minutes. There was no significance between control and elevated-CO_2 strombs distance from their prey as indication of predatory success (ANOVA; p=0.247). However, increased activity of predator could increase the probability of the predator coming across the prey and possible prey capture as well. Two *C. marmoreus* within the elevated-CO_2 treatment that traveled the furthest distance actually went past their prey because the prey buried itself. This could suggest that the predator either did not sense the prey or that burying is a successful anti-predator response for the prey snail. However, more research is needed to distinguish the true cause for this inability to sense the prey.

The results support similar findings found in terms of increased activity level and boldness. Similar in fish, the boldness in the cone shell predator increased with elevated levels of CO_2 as the predator was more active throughout the interaction and moved further distances from the starting point than the control predators (Dixon et al. 2010). However, this increased activity did not have an effect on the predatory capture success. It seemed like both groups of predators would have been equally likely to capture prey if the interaction was continued for a longer period of time, which does not support findings found by Allen et al. (2013) that indicated that predators under higher CO_2 conditions have less prey capture success. The high variability within the individuals of the elevated-CO_2 treatment was also denoted by Watson et al. (2013), where individual variation in behavioral changes was heightened by near-future ocean acidification projections because some individuals are more tolerant to changes in their chemical environment than others.

5.0 Conclusion

5.1 Limitations of Study

Although this study did produce some significant findings, there was variation within the experiment that could have been avoided. Throughout the experiment, temperature and pH of the tank systems would fluctuate. In all the treatments, the seawater ranged in temperature from around $27^{o}C$ in the morning to almost $30^{o}C$ in the late afternoon/evening due to the fluctuations in storage water temperatures. Increased temperature lowers CO_2 solubility and therefore increases CO_2 concentrations in the water. Therefore, if the temperature were maintained at a more constant level, the experiment would have been more controlled. Every individual has a different threshold to changes in pH and temperature. Although it is hard to account for all this individual variation, it is best to control as many outside variables such as pH and temperature to account for this variability within individuals.

Another way to account for individual variation and strengthen the significance of this study, more replications of each experiment should be conducted. With both prey and predator species, there were limited numbers of individuals that had been in elevated-CO_2 treatments for a long enough time period to test. More sample sizes with equal numbers per treatment would have strengthened the data and could have allowed for more significance within the different behavioral assays. Particularly with the predator-prey interaction with *S. luhuanus*, it may have been advantageous to have a longer exposure time without tank contamination from its cone shell predator. At the beginning of the captivity period (up to day four), the predator was housed within the same aquarium facility's room. This could have exposed the prey to the predator's scent and possibly affected their response when they were placed in the presence of their predator differently than if they were not exposed to that predator odor. Also, the predator-prey arena was too small to capture the full escape response of the *S. luhuanus*. Most all the *S. luhuanus* hit or sensed the wall during the trials, and would continue to move once they hit the wall, which suggests that the prey would have continued to travel away from the predator if the arena was bigger in size. This is why the duration of time it took to sense or hit the wall was taken to account for this error. However, to determine whether or not the prey strombs escape success was impaired under elevated-CO_2 condition, a larger test arena would be necessary to determine full distance traveled away from the predator. Finally, a control group of prey strombs without the predator presence would have further clarified differences in activity level between control and elevated-CO_2 animals as well as verified whether or not the elevated-CO_2 strombs were responded to the predator or just moving around due to increases in activity level due to ocean acidification.

5.2 Future Studies

In order to fully determine the changes in predator-prey interactions with cone shells and their respective prey snail, a complete cross-examination of the different experimental treatments must be conducted. This is necessary to further knowledge of predator-prey interactions' change in future ocean acidification changes. A complete cross-examination required elevated-CO_2 individuals to be placed with both control and other elevated-CO_2 individuals. Both of the predator-prey experiments conducted in this study used both a control predator and control prey, respectively, during the trial. To address the interaction to changes in prey escape and ultimate predatory success, both prey and their predators must be simultaneously exposed to the same changes in their physical environment that will exist with ocean acidification.

Ocean acidification is not the only factor threatening the world’s oceans in the near future. Sea Surface Temperatures (SST) are estimated to increase at least 1-3^{o}C by the end of this century (Hansen et al. 2006). The combination of both ocean acidification and increased SST will most definitely have coupled effects on not only marine invertebrate behavior but their environment as well. Future studies to study the effect of both increased temperatures and decreases in pH have on marine invertebrates and their predator-prey interaction are necessary to completely examine the possible behavioral changes these organisms will face in the future.

5.3 Ocean Acidification and its Implications

Ocean acidification will most certainly affect marine invertebrate behavior in the near future. Some of these behavioral changes may be slightly advantageous, while others may make the species, and in some cases individuals, less likely to survive. Ocean acidification will increase the rate in which species have to evolve to their respective advantage and disadvantages. Predators will face pressure to account for increased prey avoidance behavior (through faster self-righting). Increased CO_2 affects marine invertebrates’ behavioral strategies for both predator-avoidance and predatory success. Prey will be less likely to escape from the predator due to the reduction in wariness and proper decision-making; whereas, predators may become more active, but yet possibly less likely to track down prey. Overall, this alters trophic level interactions that affect the whole marine ecosystem dynamics and marine fisheries. Marine invertebrates are an essential part of the lower trophic level interactions that marine fisheries are greatly dependent upon. With the near-future ocean acidification scenario, it is possible that changes predator-prey interactions will have wide-ranging negative impacts on the marine food web and the fisheries that depend on the stability of that delicate dynamic.

6.0 References

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7.0 Appendices

Appendix A: Aqua Medic pH controller

The device used to input and control the amount of CO_2 into the CO_2 water systems.

Appendix B: Mettler Toledo pH meter

Appendix C: C-22 Temperature Probe

Appendix D: Predator-prey Arena
Used for both *S. luhuanus* and *C. marmoreus* trials

S. luhuanus and *C. marmoreus* predator-prey interaction