The Effect of Ocean Acidification on Feeding Rate of Hydrozoan Jellyfish *Mitrochoma cellularia*

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Abstract

Global ocean acidification caused by an increase in absorbed CO_2 is changing oceanic ecology world wide, however little is known on how this change will effect medusozoan Cnidarians (jellyfish). Many studies have predicted that lower pH may be causing an increase in the abundance of jellyfish, however many more have refuted these results. Jellyfish are predators of epipelagic crustaceans and compete with fish for prey. A change in jellyfish population or feeding behavior may affect the entire pelagic ecosystem. This project aims to investigate the relationship between feeding rate of jellyfish *Mitrocoma cellularia* on crustacean prey *Artemia* spp., under variable seawater CO_2 concentrations: ambient local seawater (about 700ppm), 400 ppm and 950 ppm. Feeding rates of *M. cellularia* were calculated and compared with those of *Aequorea victoria* under local seawater conditions and the feeding rate of *A. victoria* under CO2 treated saltwater conditions was calculated. It was found that M. cellularia had a faster feeding rate than A. victoria in ambient conditions ($p=0.042$). No significant difference was found among the feeding rates under the three water treatments ($p= 0.297$) however a slight trend of increasing feeding rate was observed as pH decreased. To understand how Ocean acidification affects medusozoan Cnidarians more refined methods are needed as well as an increase in trials.

Introduction

Increased burning of fossil fuels through the past 150 years has contributed to increasing anthropogenic CO_2 in the atmosphere. This secondarily causes a relative increase in the amount of CO_2 dissolved in the oceans, which in turn lowers the pH of sea water, in a phenomenon termed ocean acidification (Caldeira & Wickett 2003). An increase in oceanic CO_2 reduces the abundance of the carbonite minerals argonite and calcite (Guinette & Fabry 2008). These minerals are important components of many marine invertebrate calcified structures (Kleypas and Yates 2009). The increase of hydrogen (H^+) ions (i.e. lower pH) may also affect metabolic rates and can lead to the increased acidity in body fluids and tissues (Purcell & Winans 2010). The effects of ocean acidification on gelatinous zooplankton, such as our study organism the hydromedusae *Mitrocoma cellularia*, is poorly studied, consistently showing mixed results.

Ocean acidification may be causing an increase in jellyfish blooms world wide (Purcell et al. 2007). Yet the effects of ocean acidification on jellyfish blooms is relatively unknown and results are inconsistent (Condon et al. 2012). The term jellyfish in this paper refers to any medusozoan species. Attrill et al (2007) reported a significant correlation of jellyfish frequency in the North Sea from 1971 to 1995 accompanied by a decrease in pH from 8.3 to 8.1 and suggested that continued ocean acidification will increase the frequency of jellyfish in the North Sea in the near future. However another study surveying a larger area of the North Sea found no correlation between pH and jellyfish bloom frequency (Purcell & Winans 2010).

If jellyfish blooms are in fact increasing in frequency, the ecosystem and human economy will be greatly impacted. Jellyfish are predators of epipelagic crustaceans and competitors of fish for planktonic prey. A change in this dynamic may affect the entire pelagic ecosystem (Purcell & Arai 2001; Purcell 2005; Guinette & Fabry 2008). Jellyfish blooms, such as those of the hydromedusae A*urelia aurita* off the coast of Japan, can cause fishing nets to be clogged and may also compete for prey items and indirectly reduce populations of economically important fish (Attrill et al. 2007; Purcell et al.

2007). Poisonous jellyfish blooms of cubomedusae can cause aquatic human activities to be interrupted due to their dangerous nature(Purcell et al. 2007; Condon et al. 2012). Understanding the physical impact of ocean acidification on jellyfish will help us to make more accurate predictions of jellyfish populations, so we can prepare for the future.

Few previous studies have examined ocean acidification impacts on jellyfish morphology, physiology, and behavior. Feeding and activity rate decreased in the hydromedusae *Rhopilema esculenta* below pH 7.4 (Purcell et al. 2007). The decrease in feeding rate contrasts with the hypothesis that higher ocean acidity may be influencing higher frequency of jellyfish blooms. Juvenile scyphozoan *Aurelia labiata* form smaller statoliths in low pH conditions (Purcell & Winans, 2010). Statoliths are contained in the balance organ (the statocyst) of many medusozoan species (Collins et al., 2006). *Mitrochoma cellularia* has multiple balance organs, statocysts, along the rim of its bell. Although nothing is known about the effects of reduced statoliths on medusozoans, it may negatively impact jellyfish.

Ocean acidification may have a larger effect on the crustcean prey of jellyfish. The respiration rate and feeding rate of *Centropages tenuiremis*, a planktonic copepod, increases under an elevated CO_2 level of 1,000μatm and associated water acidity of pH 7.83, (Li & Gao 2012),suggesting a physiological stress caused on planktonic copepods by ocean acidification. This may cause jelly fish frequency to increase because the jellyfish will be able to feed more easily.

This project aims to investigate the relationship between feeding rate of jellyfish *Mitrocoma cellularia* on crustacean prey *Artemia sp.*, under ocean acidification. Calculating feeding rates is a useful way to determine if organisms are responding to their conditions in a positive or negative way. Artemia are related to crust copepods and have similar morphology. We expect that Artemia will react similarly to the copepod *Centropages tenuiremis*, when placed in water treated with CO_2. The feeding rate of *M. cellularia* may increase under ocean acidification conditions. Through this investigation, we hope to gain more insight into the possible change in feeding behavior, and the accompanying physiological stress, that ocean acidification may cause on epipelagic medusozoans and their crustacean prey.

Methods

The study was conducted at the University of Washington Friday Harbor Laboratories (FHL), on San Juan Island, Washington. An epipelagic leptomedusa, *Mitrocoma cellularia*, abundant at FHL during the summer, was used as the experimental predator species for this study as well as another *Leptomedusae*: *Aequorea victoria*. Both leptomedusas were collected from the FHL docks. Adult *Artemia* (6-7 days old), a planktonic crustacean, hatched from commercial eggs as the prey species. Ocean acidification conditions were simulated using seawater acidified to 400ppm and 950ppm CO_2 concentration, provided by the Ocean Acidification Lab, FHL. To represent local present-day seawater acidity, estimated to be about 700ppm, 1 micron filtered on-site seawater was used. Individual experimental trials were conducted in a 1 gallon (3.7 l) jar.

The first trial was conducted in 12 jars (3.7 l) of local sea water, three as controls containing only *Artemia*, and nine as experimentals, containing both *Mitrocoma cellularia* and *Artemia*. Temperature was maintained in a sea-table water bath at 14°C. *Mitrocoma cellularia* of similar size (bell diameter of 40.6-52.9 mm) were collected before the trial between 8 and 10 am and one was placed in each of the experimental containers. The *M. Cellularia* were acclimated for seven hours after which *Artemia* (n=50) were slowly added to each of the 12 jars. After one hour, the *M. cellularia* were removed and sized by placing them in a shallow dish and measuring the diameter of its bell with a caliper. Next, Artemia were filtered out using a fine mesh and counted.

Two water treatments were then tested: 400ppm CO_2 acidified seawater (pH 8.00) and 950 ppm CO_2 acidified seawater (pH 7.65). The treatments were separated between the 12 jars; five experimental containers plus one control of each seawater treatment. Temperature was maintained in a sea-table waterbath at 14°C. To few *Mitrocoma cellularia* were found so 10 *Aequorea victoria* were collected between 8 and 11 am and used instead (bell diameter of 36.5-52.0). The *A. Victoria* were then placed into the experimental jars and allowed to acclimate for four hours. *Artemia* (n=50) were then added to all 12 containers and left to incubate for one hour. The *A. Victoria* were removed and measured and the *artemia* were counted as described above.

A third trial of 12 jars (3.7 l) was conducted using local filtered seawater and nine *Aequorea victoria* (bell diameter ranging from 36.4-51.1). The trial was run using the same methods as the first, however the *A. victoria* were acclimated for four hours, before the artemia (n=50) were added.

Quantification and analysis

Feeding rate was calculated using the methods developed by Titelman and Hansson in 2006. Individual clearance rate is used as an index of feeding rate of individual jellyfish. Individual clearance rate of one experimental trial (F, l ind^{-1} h^{-1}) was calculated as follows:

$F = (V/t)*\ln(C_{start}/C_{end})$, where V is the volume of seawater in the container (l), t is the incubation time (h) and C_{start} is prey concentration (l^{-1}) at the beginning of incubation and C_{end} is prey concentration at end of incubation.

Data analyses were run using SigmaPlot^{tm} 12. A Mann-Whitney rank sum analysis was run comparing median clearance rates of *Aequorea victoria* and *Mitrocoma cellularia* in local seawater conditions. A Kruskal-Wallis one way analysis of variance was used to test for a significant difference in medians between the clearance rates of *Aequorea victoria* in the three water treatments. A Mann-Whitney rank sum test was run to determine the statistical significance in the difference of the median clearance rates of *A. victoria* in two seawater treatments (400 ppm and 950 ppm) due to a high P value. Histograms were created of the median clearance rates of *A. victoria* under all test conditions and of the median clearance rates of *A. victoria* and *M. cellularia* in ambient water conditions. Two more histograms were made showing the mean final *artemia sp.* count for the conditions described above.

Results

After comparing the median clearance rates between *Mitrocoma cellularia* and *Aequorea victoria* a significant difference was found. The median clearance rate of *Mitrocoma cellularia* was found to be 3.58, with a minimum and maximum deviation ranging from 0.73 to 8.52 (fig. 1). The median clearance rate of *Aequorea victoria* was found to be 1.25, with a minimum and maximum deviation ranging from 0.31 to 5.60 (fig. 1). A Mann-Whitney rank sum analysis of the two median clearance rates determined a Mann-Whitney U statistic to equal 17.00 and a P value of 0.042 (n=9).

Comparing the median clearance rates of *Aequorea victoria* in the three water treatments resulted in a slight upward trend of rates as CO_2 concentration increases. The Kruskal-Wallis one way analysis of variance between the clearance rates of *Aequorea victoria* under the three water treatments reported H= 2.426 with 2 degrees of freedom and a P value of 0.297. The median clearance rate under filtered ambient water was 1.215 (n=9) and the median clearance rate in 400ppm CO_2 and 950 ppm CO_2 of *A. victoria* (n=5) were 0.645 and 1.427 respectively (fig. 2). The maximum and minimum deviation from the median clearance rate of A. victoria in the two water treatments were 0.47-0.83 (in 400ppm) and 0.00-2.56 (in 950 ppm) (fig. 2). The Mann-Whitney rank sum analysis of the median clearance rates of *A. victoria* in the two CO_2 treated water conditions resulted in a Mann-Whitney U statistic of 10.00 and a P value of 0.690.

Discussion

This study aimed to test the effect of Ocean acidification on the feeding rate of the medusozoan *Mitrocoma cellularia*. Due to several unforeseen factors, we were unable to complete the investigation as we had originally planned. We were able to test the feeding rates (clearance rates) of *M. cellularia* under ambient seawater. However, due to a negative population change, we were unable to test the effects of seawater treated with CO_2. We changed our experiment to compare the feeding rates between two Medusozoa species (*M. cellularia* and *Aequorea victoria*) in ambient water as well as the effects of ocean acidified water on the hydromedusa *A. victoria*.

Our trials of M. cellularia and Aequorea victoria in ambient water demonstrated that M. cellularia had a faster clearance rate than Aequorea victoria (fig. 1). The p value determined by the Mann-Whitney rank sum analysis is below 0.05 indicating that the difference is significant and may not be due to chance. This result indicates a key difference between the metabolism of the two species with similar bell diameter. It was found in a study comparing dietary niches of Hydromedusae species that there is an overlap between *A. victoria* and *M. cellularia* (Costello and Colin 2002). The faster feeding rate of *M. cellularia* may be advantageous when competing with *A. victoria* for food. However the difference between clearance rates may be due to the fact that the acclimation periods for *M. cellularia* and *A. victoria* were different, 7 and 4 hours respectively. The *Mitrocoma cellularia* may have required more food then *A. victoria*, after being in the ambient conditions for a longer time. The controls of *A victoria* have a large maximum and minimum error for the final *artemia* count (fig. 3) indicating that the value for the experimental condition may not be accurate. What we may draw from the ambient trials, is that conclusive results are not possible with variable acclimation time and failed controls.

During work with *A. victoria* under the three water conditions (ambient, 400 ppm and 950 ppm), it was found that the Ocean acidification level had next to no significant effect on feeding rates. There was a slight upward trend as pH decreased, assuming that local seawater CO_2 levels are about 700 ppm, however with a small n value and the p value exceeding 0.05 we cannot exclude the null hypothesis that the results are due to chance. If there was a significant p value we might have attributed the increase in feeding rate to the increase respiration of the artemia. We could also argue that the metabolism of the hydromedusaes increased causing a need to increase feeding rate in higher acidification. If more trials of the experiment were completed (increasing n) we may have been able to determine if a correlation between ocean acidification and feeding rates exists. As of now we cannot accept or reject our hypothesis that feeding rate of Aequorea victoria will increase as pH decreases.

During our study we witnessed a change in population dynamics of hydromedusan jellyfish in Friday Harbor, Washington. In the beginning of a four week period, we noticed an abundance of small (1-2 cm in diameter) Mitrocoma cellularia specimens. A week later fewer Mitrocoma cellularia were observed of larger size (2-4 cm) although 12 individuals were easily caught in less than half an hour. By the end of the third week only 9 individual M. cellularia were seen in a two hour period, with bell diameters ranging from 4-6 cm. An increase in frequency of Aequorea victoria was also observed throughout this time period. This trend in population dynamics is a common occurrence (Mills 1981).

Although our results indicate no significant difference between the feeding rates of Aequorea victoria in CO2 treated water, we were able to see a slight upward trend. The increase in feeding rate under lower pH indicates that these hydromedusas have a negative response to ocean acidification. The increase in feeding rate may mean that metabolism is sped up. Because global oceans are absorbing more CO2 from the atmosphere it is important research how these changes will affect global ecology. Our methods need to be further refined and used in more trials before we can make reliable conclusions. The feeding rate between species were different. This may be due to different morphological characteristics, such as bell shape and tentacle length. We were unable to quantify the physical differences between Aequorea victoria and Mitrocoma cellularia, however with further research we may be able to find an characteristics that enable a faster feeding rate. In conclusion more refined methods and a greater number of trials are needed to enable gain significant.

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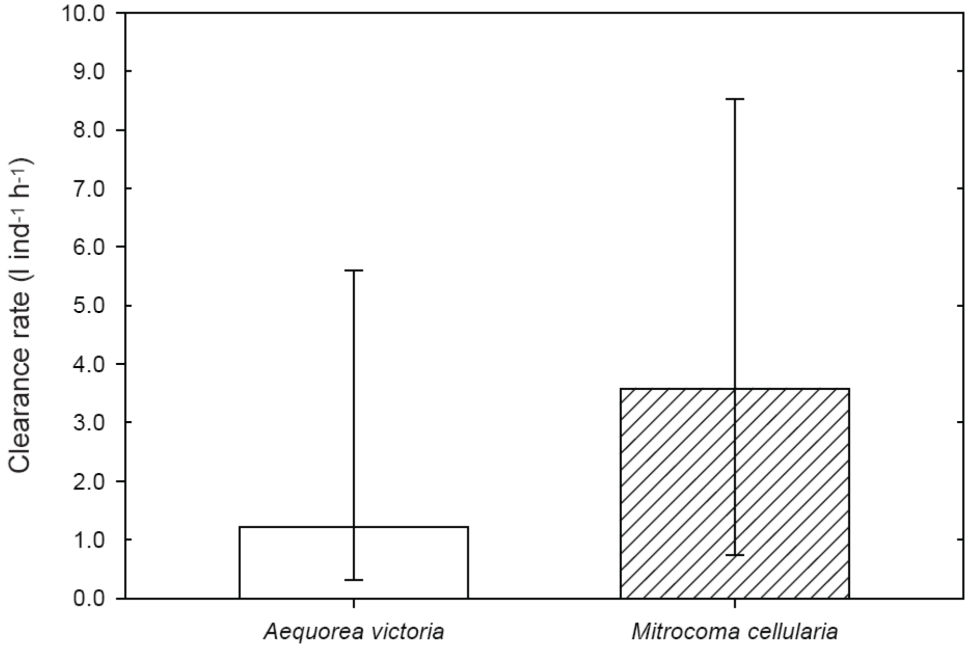

Figure 1: Histogram of median clearance rates of *Aequorea victoria* and *Mitrocoma cellularia* under ambient filtered water conditions. The error bars indicate the maximum and minimum deviation from the median clearance rates. *Aequorea victoria* had a slower feeding rate (1.215) compared with *Mitrocoma cellularia* (3.580). A Mann-whitney statistical analysis resulted in a p value of 0.042 where T= 62.000 and n=9.

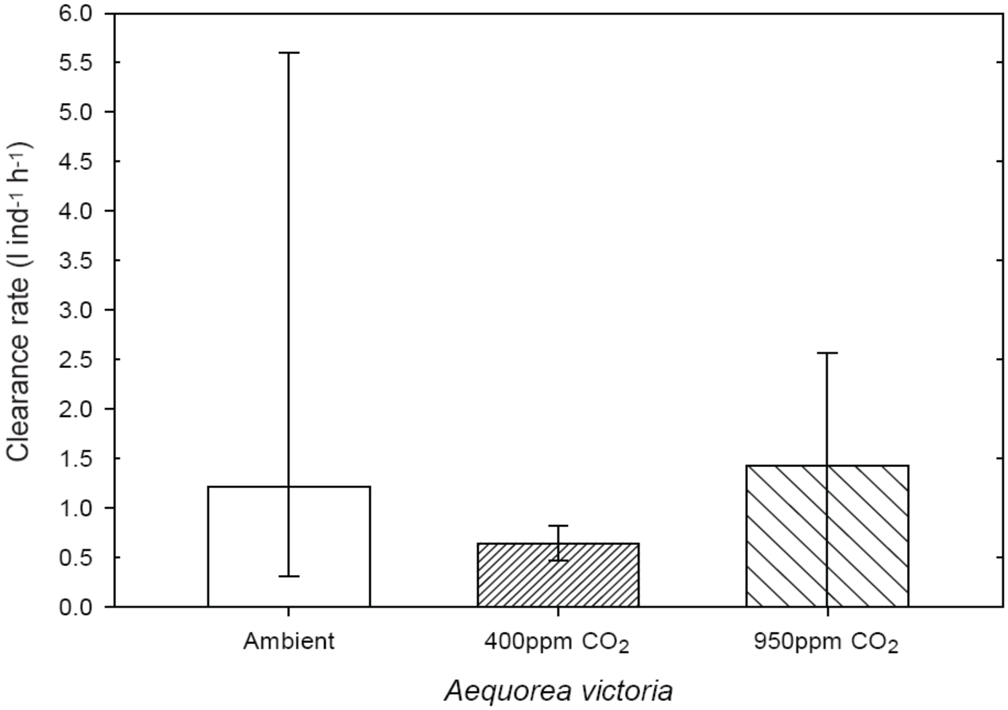

Figure 2: Histogram of median clearance rates of *Aequorea victoria* under three seawater treatments, ambient filtered water, 400 ppm CO_2 and 950 ppm CO_2. Error bars indicate the maximum and minimum deviation from the median clearance rate under each condition. The median clearance rate in ambient, 400ppm CO_2 and 950ppm CO_2 are 1.215, 0.645 and 1.427 respectively. A Kruskal-Wallis One Way Analysis of Variance on Ranks was used to test for a significant difference, resulting in a H= 2.426 with 2 degrees of freedom (N= 9, 5 and 5). A p value of 0.297 indicates no significant difference.

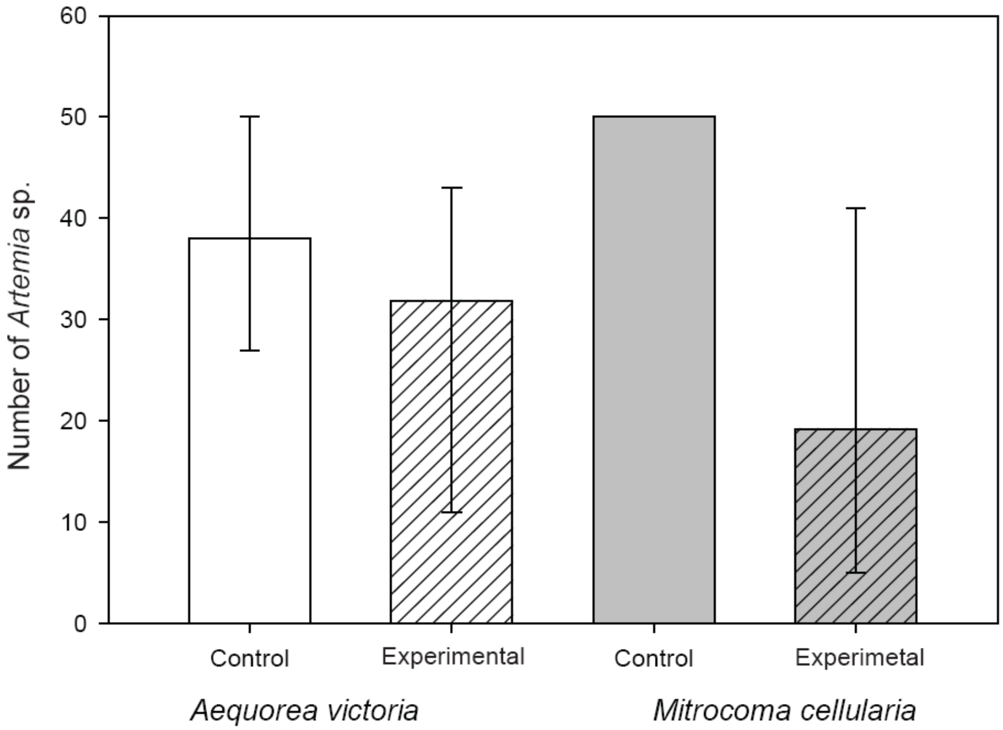

Figure 3: Histogram of the mean final *Artemia* sp. count in the ambient water trials of *Aequorea victoria* and *Mitrocoma cellularia* after a one hour incubation period. Error bars indicate the maximum and minimum deviation from the mean. The error bar in the control indicates that a significant margin of error may be influencing the experimental results.

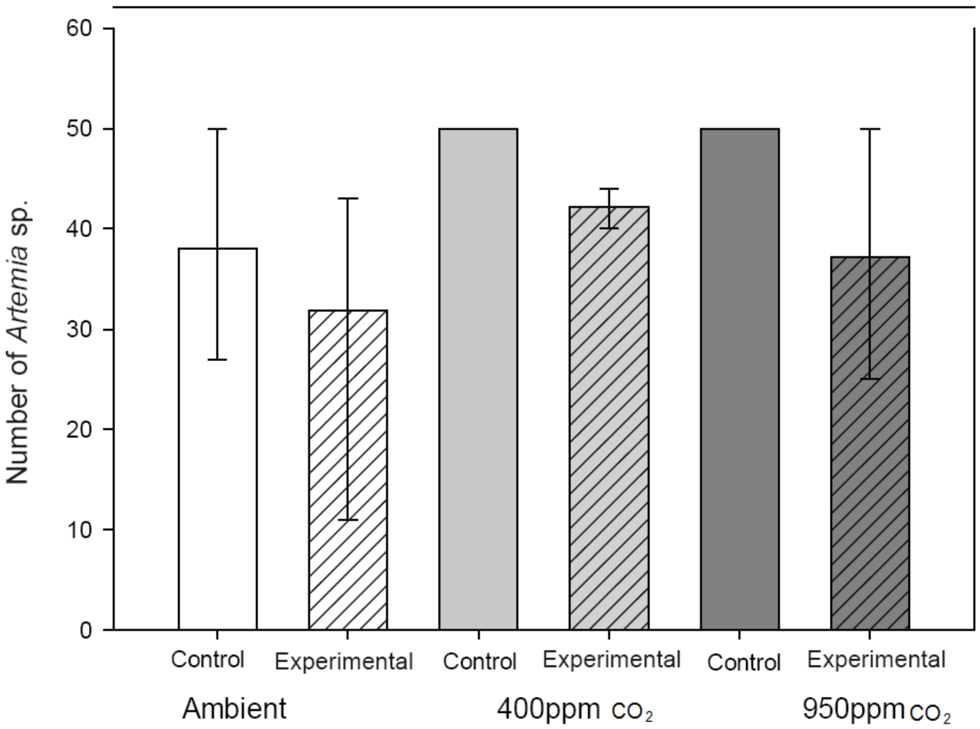

Figure 4: Histogram of the mean final *Artemia* sp. count in the three water treatments (ambient, 400 ppm CO_2 and 950 ppm CO_2) of *Aequorea victoria* after a one hour incubation period. Error bars indicate the maximum and minimum deviation from the mean. The large error bar in the control of *A. victoria* feeding rates under ambient conditions, indicates that a significant margin of error may be influencing the experimental results.