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The Effect of Ocean Acidification on Feeding Rate of Hydromedusa *Aequorea Victoria*

Abstract

Global ocean acidification may be causing an increase in abundance of medusoid hydrozoans. Many medusoid hydrozoans are predators of epipelagic crustaceans and ecological competitors of fish for epipelagic prey. A change in this predator-prey dynamic may affect the entire epipelagic ecosystem. Ocean acidification exerts a physiological stress on planktonic crustaceans, which possess calcareous exoskeleton. It is therefore hypothesized that ocean acidification increases the feeding rate of medusoid hydrozoan on crustacean prey. This project aims to investigate the relationship between feeding rates of hydromedusa *Aequorea Victoria* on crustacean prey *Artemia sp.*, under variable seawater CO_2 concentrations: 450ppm CO_2 and 950ppm CO_2 . Each individual of *A. Victoria* was given 50 individual *Artemia sp.* and let feed for 1h under ambient temperature. Clearance rate was then calculated from the raw count of prey remaining after 1h. There is no statistically significant difference between the clearance rates of *A. Victoria* among the two treatment conditions and ambient seawater ($n=5\sim9$, $p>0.05$). However, median of clearance rate under 950ppm CO_2 treatment condition was higher than that under 450ppm CO_2 treatment condition, suggesting a possible general trend.

Introduction

Increased burning of fossil fuels through the past 150 years has contributed to increasing anthropogenic CO_2 in the atmosphere(Caldeira and Wickett 2003). 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 and Wickett 2003). An increase in oceanic CO_2 reduces the abundance of carbonite materials, carbonite and calcite, which are important components of calcareous structures in many marine invertebrates (Guinette and Fabry

2008). This in turn causes an increase of hydrogen ions in the ocean and lowers the pH of seawater, which may affect metabolic rate and body fluid acidity in gelatinous cnidarians such as medusoid hydrozoans (Purcell and Winans 2010).

Ocean acidification may be causing an increase in the abundance of medusoid hydrozoans worldwide (Purcell et al. 2007). However, studies have shown mixed results and the mechanisms of cause and effect are unknown (Condon et al. 2010). Attril et al. (2007) reported a significant correlation between medusoid hydrozoan frequency in the North Sea from 1971 to 1995, accompanied by a decrease in pH from 8.3 to 8.1. They then suggested that continued ocean acidification will increase the frequency of medusoid hydrozoan 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 medusoid hydrozoan bloom frequency (Purcell and Winans 2010).

Medusoid hydrozoans are predators of planktonic crustaceans and competitors of fish for epipelagic prey. A change in this predator-prey dynamic may affect the entire epipelagic ecosystem (Purcell and Arai 2001; Purcell 2005; Cuinette and Fabry 2008) and may indirectly reduce populations of economically important fish (Attril et al. 2007; Purcell et al. 2007).

Calcareous statoliths are contained in the balancing organ (statocyst) of many medusoid hydrozoans. *Mitrocoma cellularia* and *Aequorea Victoria* have multiple statocysts along their ring canals. The effects of ocean acidification on adult hydromedusae statoliths are currently unknown. Ocean acidification may have a greater effect on crustacean prey of medusoid hydrozoans given their calcareous exoskeleton and small body size of planktonic crustaceans. The respiration rate and feeding rate of *Centropages tenuiremis*, a planktonic copepod, increases under an elevated CO_2 levels of 1,000µatm and associated pH of 7.83, suggesting a physiological stress caused on planktonic copepods by ocean acidification (Li and Gao 2012).

This study aims aims to investigate the relationship between feeding rate of hydromedusae *Mitrocoma cellularia* and *Aequorea Victoria* on crustacean prey *Artemia sp*. under ocean acidification conditions. It is hypothesized that ocean acidification increases the feeding rate of *M. cellularia* and *A.Victoria.* Through this study, we hope to gain more insight into the possible change in feeding behavior, and accompanying physiological stress that ocean acidification may cause on epipelagic hydromedusae and their planktonic crustacean prey.

Materials and Methods

Experimental site and materials

This study was conducted at the University of Washington Friday Harbor Laboratories (FHL) on San Juan Island, Washington. Two species of epipelagic hydromedusae, *Mitrocoma cellularia* and *Aequorea victoria* were used as experimental predator species for this study. *M. cellularia* and *A. Victoria* were collected from the FHL docks. *Artemia sp*., a planktonic crustacean, were hatched from commercial eggs as the prey species. Ocean acidification conditions were stimulated using seawater acidified to 450ppm CO_2 and 950ppm CO_2 concentration, provided by the Ocean Acidification Lab, FHL. Filtered on-site seawater was used as ambient treatment. Individual experimental trial was conducted in a 1 gallon (3.7l) jar.

Experimental procedures

We tested three water treatments: 450ppm CO_2 seawater, 950ppm CO_2 seawater and on-site seawater. Trials were conducted in 'batches' of 12. An initial trial with *Mitrocoma cellularia* was conducted with three replicates for each of the three treatment conditions, and one control for each treatment condition. Control container contains prey but no predator. One batch with on-site water was conducted for each of the two species *M. cellularia* and *Aequorea victoria* with nine replicates and three controls. One batch with five replicates and one control for each of the two CO_2 treatments and one control for each CO_2 treatment was conducted with *A. victoria.* Trials were conducted in individual 3.7l closed containers with one predator per container in a sea table water bath with consistently flowing on-site seawater to maintain constant temperature range of ~11℃ for initial trial and ~14℃ for subsequent batches. Temperature of on-site seawater in water table was measured by a suspended thermometer.

For each experimental batch, the same species of hydromedusa, either *Mitrocoma cellularia* or *Aequorea victoria* of relatively similar size, bell diameter 28.0mm to 52.9mm for *M. cellularia* and bell diameter 31.2mm to 52.0mm for *A. Victoria*, were collected between 8:30 and 10:30am on the day of experiment. Individual medusae were immediately placed in each 3.7l treatment container without prey item. Hydromedusa were allowed to acclimate to the experimental conditions for >4h.

Artemia sp. were hatched prior to experiment. *Artemia sp*. (n=50) were counted and slowly added to each of the 12 jars and gently mixed to approach even distribution of prey. Experimental batches were incubated for approximately 1h and ended by removal of hydromedusae. Remaining *Artemia sp*. were sieved out using a 75µm mesh and counted. At the end of the experiment, the size of each medusa was estimated by placing medusa in a shallow dish and measuring the bell diameter with a caliper.

Quantification of feeding rate

Feeding rate was quantified using an equation developed by Titelman and Hansson in 2005. Individual clearance rate was used as an index of feeding rate of individual hydromedusa. Individual clearance rate of one experimental trial (F, l ind-1 h-1) is calculated as follows:

F = (V/t)*ln(Cstart/Cend), where V is the volume of seawater in the container (l), t is the incubation time (h) and Cstart is prey concentration (l-1) at the beginning of incubation and Cend is prey concentration at end of incubation.

Results

Data for initial trial on was not analyzed due to poor recovery rate of *Artemia sp*. 16~41 out of 50 *Artemia sp*. were recovered from control jars without predator.

Significant difference was found between individual clearance rate of *Mitrocoma cellularia* and *Aequorea Victoria* under ambient conditions (Fig. 1). Mann-Whitney Rank Sum test was performed, with n=9, U=17.00, p=0.042. For clearance rate of *M. cellularia*, max=8.52, min=0.73, median=3.580. For clearance rate of *A. Victoria*, max=5.60, min=0.31, median=1.215.

No significant difference was found among individual clearance rates of *Aequorea Victoria* under ambient conditions, 400ppm CO_2 treatment condition, and 950ppm CO_2 treatment condition (Fig. 2). Kruskal-Wallis test was performed, with n=5, H=2.426, p=0.297. For clearance rate under 400ppm CO_2 treatment condition, max=0.826, min=0.472, median=0.645. For clearance rate under 950ppm CO_2 treatment condition, max=2.565, min=0, median=1.427.

Fig.1 Individual clearance rate of *Mitrocoma cellularia* and *Aequorea victoria.* Bar height represents median. Error bar represents maximum and minimum value. Mann-Whitney Rank Sum test (n=9), T=62.00, p=0.042. Significant difference found in clearance rate between the two species.

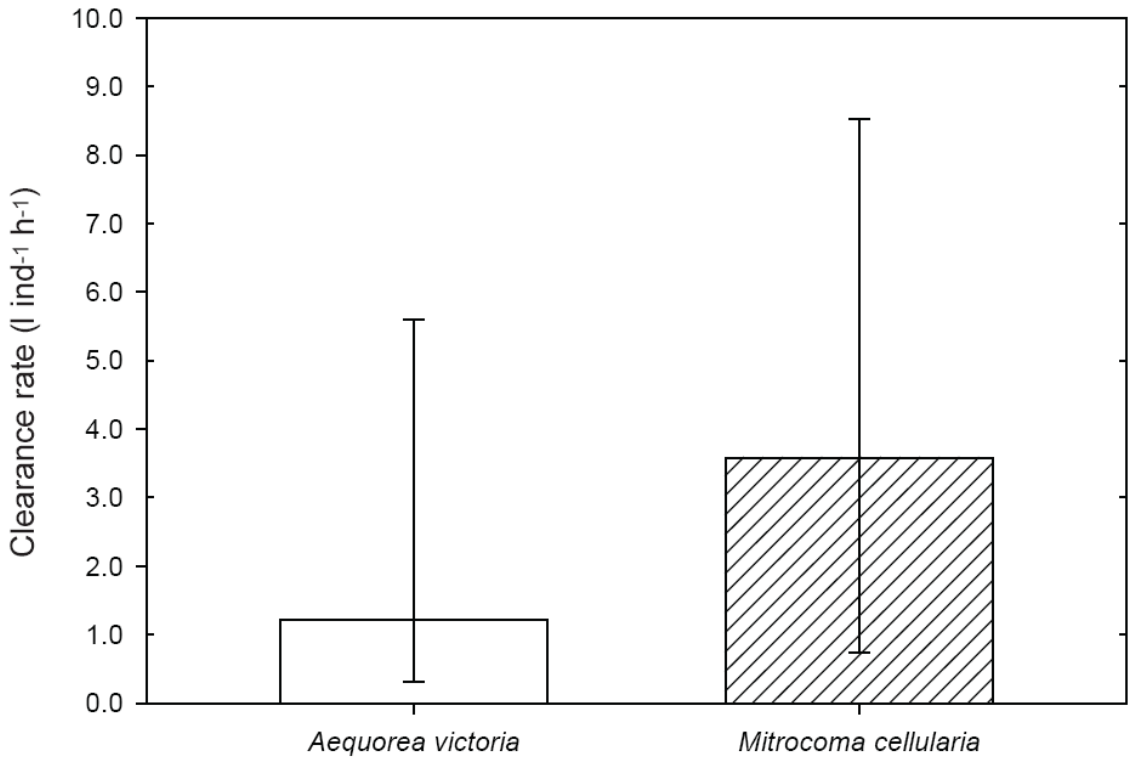
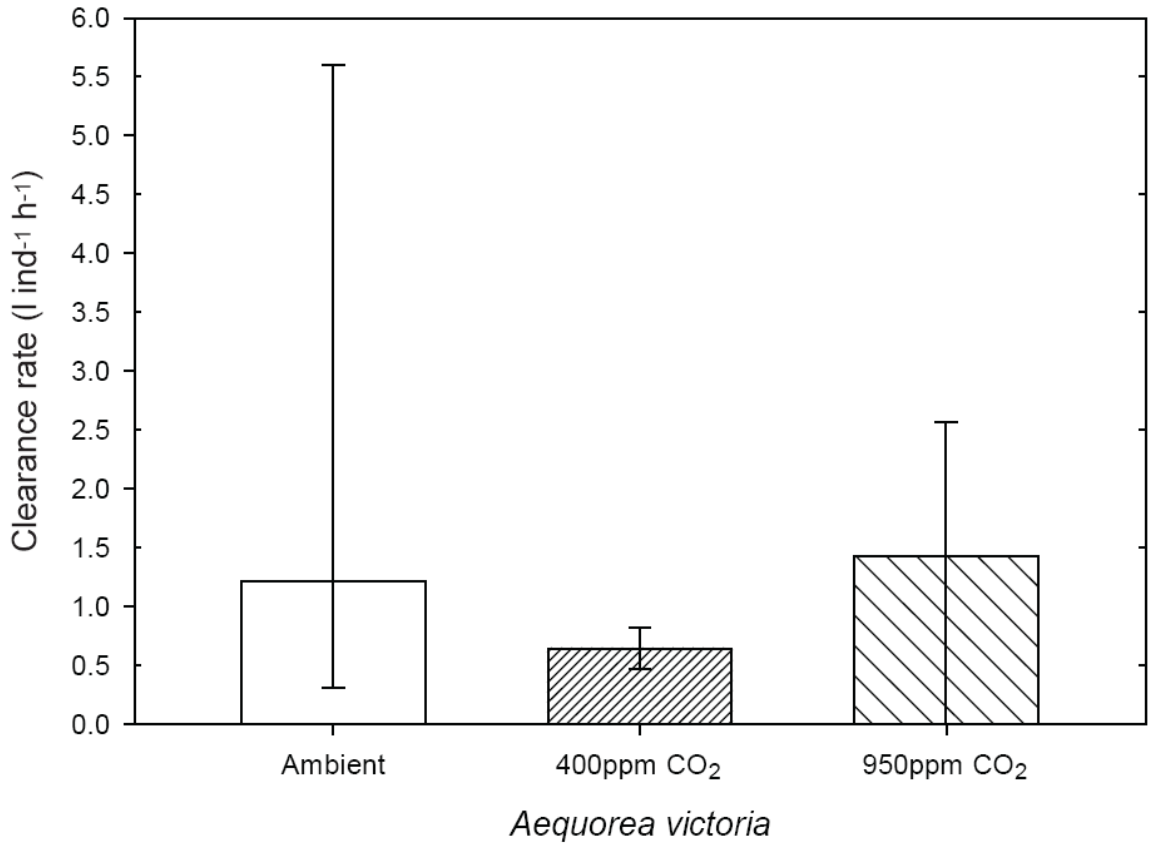


Fig.2 Individual clearance rate of *Aequorea Victoria* under ambient conditions, 400ppm CO_2 treatment condition, and 950ppm CO_2 treatment condition. Bar height represents median. Error bar represents maximum and minimum value. Kruskal-Wallis test (n=5), H=2.426, p=0.297. No significant difference found in clearance rate of *A. Victoria* under the above treatment conditions.



Discussion

Although a statistically significant difference was found between individual clearance rates of *Aequorea Victoria* and *Mitrocoma cellularia* ($p<0.05$), this result is confounded due to poor recovery of *Artemia sp*. from the controls. 27~50 out of 50 *Artemia sp*. were recovered from control jars without predator (Fig.3). Mann-Whitney rank sum test was performed to test for significant difference between clearance rate in control containers and clearance rate in experimental containers. No significant difference was found, with $p=0.711$. This implies that the clearance rate under ambient conditions may not be valid as they are not significantly different from clearance rate in control containers. C containers without predator accounts for the manual recovery of $n=50$ prey originally placed in all containers. Recovery of $n<50$ in control jars may be due to technical inconsistencies when performing the manual task of filtering *Artemia sp*. from the containers. Larger size of predator, such as *Aurelia aurita,* and prey species, such as fish or brachyuran larvae would reduce technical error due to easier visual recognition of both species.

No statistically significant difference was found among individual clearance rates of *Aequorea Victoria* under ambient conditions, 400ppm CO_2 treatment condition, and 950ppm CO_2 treatment condition ($p=0.297$). Raw count for 950ppm CO_2 treatment condition has a broad range of values (Fig.4), and median clearance rate under 950ppm CO_2 treatment condition (median=1.427) is higher than that under 400ppm CO_2 treatment condition (median=0.645), suggesting a possible general trend . A larger sample size is necessary to further investigate this possible trend. Furthermore, the CO_2 level in on-site seawater has not been quantified, therefore it is not certain if under 400ppm CO_2 treatment condition is lower or higher than ambient seawater CO_2 levels. Quantification of CO_2 level in on-site seawater would better establish this possible trend.

Titelman and Hansson (2006) have discovered that ingestion rate in *Aurelia aurita* increases proportionately with prey concentration, but clearance rate remains relatively constant. An increase in clearance rate therefore implies an increase in the proportion of prey consumed in a given time. A second hypothesis can be formed, that ocean acidification may increase the relative abundance of medusoid hydrozoans, and decrease the relative abundance of planktonic crustaceans due to an increase in clearance rate of medusoid hydrozoans. Long-term study of

similar nature to this study, and population monitoring in the wild should provide further insight into the effect of ocean acidification on medusoid hydrozoans and planktonic crustaceans.

Fig. 3 Average number of *Artemia sp.* after 1h of feeding. Error bar represents maximum and minimum values of raw count. Control for *A. Victoria* had large margin of error.

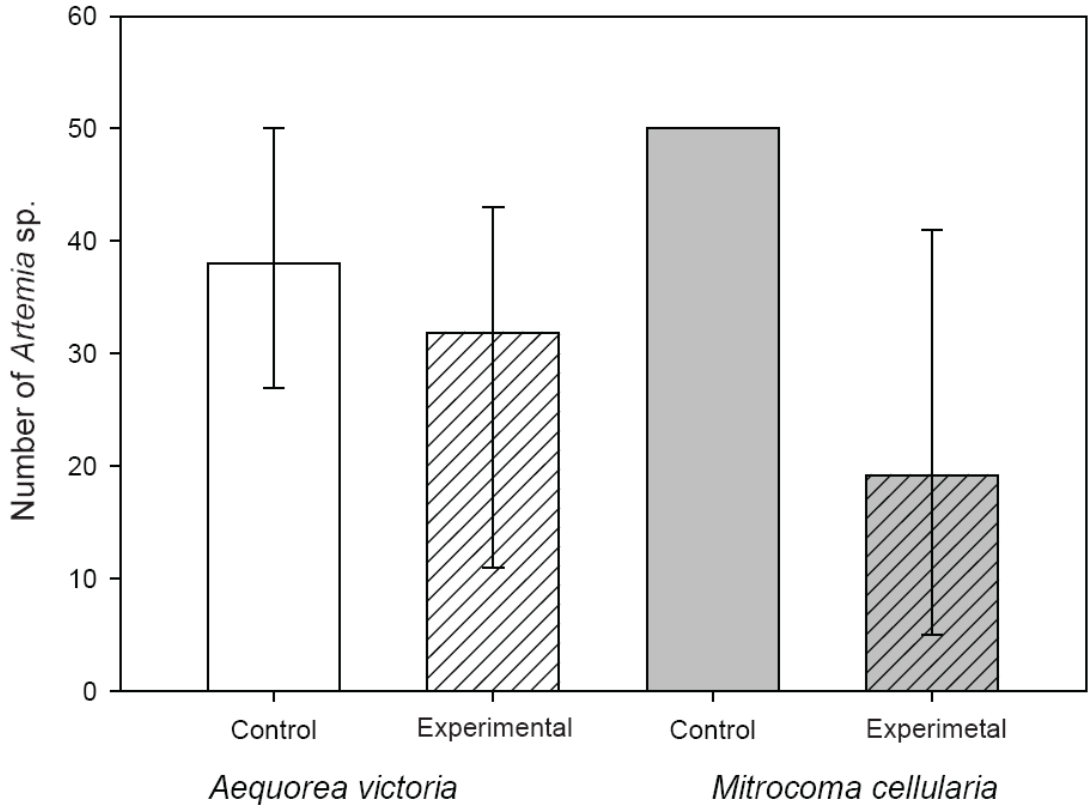
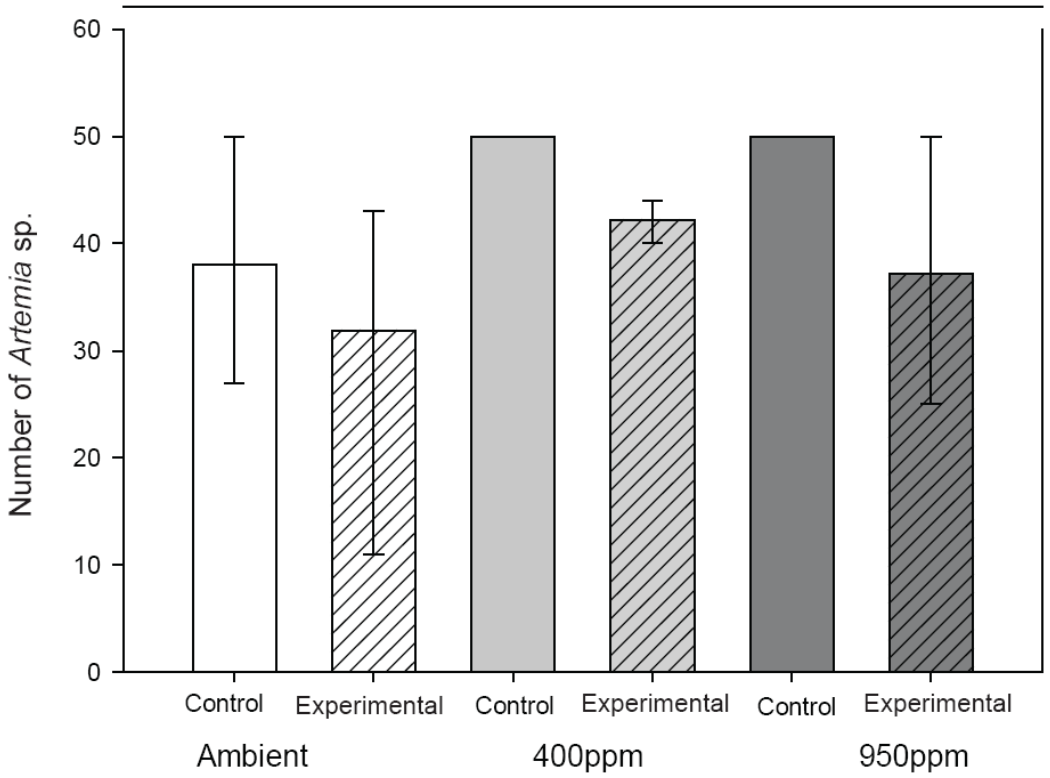


Fig. 4 Average number of *Artemia sp.* after 1h of feeding of *Aequorea victoria* under ambient conditions, 400ppm CO_2 treatment condition, and 950ppm CO_2 treatment conditions. Error bar represents maximum and minimum value of raw count. Raw count for 950ppm CO_2 has a broad range of values.



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