Biomass Effects on Carbonate Chemistry in both a Flow-through and Static Experimental Laboratory Set-up

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Experimental Approached to understanding Ocean Acidification
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Abstract

There are many things to consider when setting up experimental laboratories, especially in the context of ocean acidification. We studied the impact of biomass load and flow-through rate on the carbonate chemistry of aquaria at two different levels of pH. We found that all of these factors play a large role in how consistent you can expect to maintain the chemistry of your system. In the flow-through system we observed decreased levels of pH relative to the control tanks for all levels of biomass. Our static experimental system experienced very large fluctuations in chemistry over short time periods. Our results indicate that it is necessary for pilot studies of this nature to be performed before ocean acidification laboratory studies are undertaken. Factors like biological load and flow-through rate are likely to influence the chemistry of any experiment.

Keywords

ocean acidification, carbonate chemistry, methods, biomass, pH, alkalinity, flow-through, static, *Myatilus, M. trossulus*

Introduction

The field of ocean acidification is expanding rapidly. A significant portion of the research in the field focuses on the response of specific organisms to environments they are likely to experience in the future. All over the world experimental laboratory set-ups are being designed which attempt to control the carbonate chemistry so that expected future conditions may be studied. There can be many problems associated with experimental lab set-ups; temperature fluctuations, decreases in alkalinity, and bacterial growth are just a couple examples. We are interested in how biomass can alter the carefully controlled carbonate chemistry in these experimental set-ups. Our expectation is that there is a limit to the amount of biomass you can put in a given volume tank before the biological effects start to swamp your carbonate chemistry control over the system. Although our questions in particular relate to the study of ocean acidification, many of our results will be applicable to anyone who is raising organisms in an experimental laboratory setting.

Methods

Organism collection and handling

We chose to use local mussels as our test of biomass effects because they are robust and large numbers are available locally. Additionally they can go several days without being fed, which has the potential to complicate the carbonate chemistry.

We collected 56 mussels during low tide from the boat dock at the end of Jackson Beach Road, early in the morning on Saturday July 9, 2011. Jackson Beach is located at the north end of North Bay, on the East side of San Juan Island. After collection the mussels were stored in a flowing water table until introduction to the treatment tanks six days after collection. The mussels are a mix of *Myatilus* spp., probably *M. trossulus*.

Prior to starting the experiment, we arranged the mussels into four groups of similar size distribution. We tagged each mussel with paint of four different colors and a label of numbers 1-14 for the four different pH and flow treatments. Mussels were measured with calipers for length, width and thickness. Mussel length ranged from 41-53mm with an average of 47.3. Additionally we weighed each mussel using the buoyant weight technique. At the end of the experiment mussels were re-measured for size and buoyant weight and additionally a wet weight measurement was taken. The average wet weight for the individual mussels was 11.2 ± 2.2g.

Experimental Set-Up

We completed our experiment in the new ocean acidification experiment lab at Friday Harbor Laboratory, at the University of Washington. Our design consisted of two treatments of target pH 7.5 and 8, corresponding to an expected pCO_2 of approximately 400 and 1200µatm. Each pH treatment had two different flow rates and two sets of four different numbers of mussels per tank: 0, 2, 4, and 8.

Each treatment consisted of a cooler acting as a pre-equilibration tank. This was held at a relatively constant pH using addition of pure carbon dioxide gas to CO_2 stripped air, controlled by an automated PID controller. Additionally, seawater temperature was controlled to a target of 12° Celsius using a chiller and heating combination. In each cooler eight small plastic containers holding approximately 3.25L seawater act as individual tanks. The pre-equilibrated seawater is introduced to the tanks by "drippers"

providing a consistent flow rate of either 12 (low flow-through rate) or 24 mL/min (high flow-through rate). Temperature is controlled in the tanks using the pre-equilibration, temperature controlled seawater as a bath which the tanks sit in. A small pump within each tank keeps the water circulating and well mixed. The system was initially set up as a flow through; once water had been introduced to one of the small treatment tanks it flushed to waste. After the 30 hours we turned off the flow-through water to the tanks, establishing a static state. The small pumps continued to mix the water within each small tank, but there was no exchange which allowed us to examine static state systems.

We randomly distributed the number of mussels in each tank as well as the flow rate of each tank around the treatment cooler to eliminate any tank effect. See Figure 1. Mussels from each color group were haphazardly distributed within a treatment.

Carbonate Chemistry Monitoring

Discrete water samples were taken directly from each of the 16 tanks at time zero, and approximately 3, 6, 12, and 30 hours after experiment start. At the time of discrete sampling we also recorded the salinity, and the temperature and pH using a Honeywell Durafet pH probe. Additional measurements were taken with the probes between discrete sampling times to increase resolution of the data set. For spectrophotometric pH measurements discrete samples were collected directly into 30mL cells. For alkalinity analysis using an open cell titration, 130mL samples were taken in 0.5L borosilicate glass bottles. Both spectrophotometric pH and alkalinity were analyzed according to the *Guide to best practices for ocean CO_2 measurements*, SOP 7 and SOP3b respectively (Dickson et al. 2007). Spectrophotometric pH values are reported at the sample temperature as calculated by CO2calc (Robbins et al. 2010) using pH at measurement temperature and alkalinity. Durafet pH measurements correlated well with the discrete spectrophotometric pH measurements.

Results

Flow-through System

We saw variability in the carbonate chemistry throughout our experiment, both through space and time. The starting pH of the tanks for the low (approximately 7.5) pH

treatment ranged between 7.56 and 7.67 for the eight individual tanks. The starting pH of the high (approximately 8.0) pH treatment ranged between 7.79-7.90. The variation in pH over the course of the experiment compared to before introduction of the mussels is shown in Figure 2.

The data shows trends dependent on the biomass in the tank. Tanks that had eight mussels in them have the largest deviation (negative) in pH relative to both the start of the experiment and to the control tanks with no mussels. Tanks with two or four mussels generally had pH values that were more similar to the control tanks, although variation in pH is observed for all levels of biomass.

Additionally, trends in pH are apparent depending on whether the treatment was high flow-through (4.2 hour turnover rate) or low flow-through (2.1 hour turnover rate). pH in the low flow tanks decreased by up to 0.2 pH units relative to the control tank, while in the high flow tanks the maximum decrease was 0.1 pH units.

Total alkalinity values for the different treatments are given in Figure 3. Most noticeable is that for all eight low pH treatments the alkalinity increased over the course of the experiment, while the high pH treatments either stayed the same (low flow-through) or decreased (high flow-through).

Static Experiment

pH and alkalinity values for the static tank experiment are given in Figure 4. We see large changes between the starting and ending values for both pH and alkalinity. Most notable in the pH graphs is the change of the high pH treatment from beginning to end. See Table 1 for average values.

Static System	Average pH at start of experiment	St. Dev	Average pH at end of experiment	St. Dev
High pH treatment	7.72	0.09	7.47	0.12
Low pH treatment	7.55	0.07	7.57	0.10

Table 1. Average pH of eight tanks for each treatment measured spectrophotometrically at the start and end of the static system experiment.

Over the course of less than 24 hours the pH of the high treatment decreased by 0.25 pH units on average. The low pH treatment saw no significant change to the average pH.

Alkalinity shows a remarkable trend as well. At the beginning of the static experiment alkalinity is very consistent. All values were within 25 µmol/kgSW. By the end of the experiment alkalinity has scattered significantly, ranging from 2000-2150 µmol/kgSW. The changes in alkalinity are representative of both the variation in biomass in the tanks and the pH treatment. Three out of the four high biomass treatments increased in alkalinity by approximately 65 µmol/kgSW, while the fourth did not change. Treatments with four mussels saw a slight increase in alkalinity (15-40 µmol/kgSW) with the exception of one tank, which saw a large decrease. In contrast, the low biomass treatments (two mussels) decreased slightly. Alkalinity of the control tanks stayed fairly constant as would be expected.

Discussion

Flow-through System

The differences in pH of the high biomass treatments relative to the other treatments probably indicates that the biomass associated with eight mussels, approximately 85g wet weight, is too large to expect consistent chemical conditions for the given volume of water (3.25L). Treatments with two and four mussels are likely okay biomass loads, but flow-through rate plays an important role in keeping the carbonate chemistry consistent, even at these lower biomass levels. The high flow treatments of various biomasses match the control better than the low flow treatments. These affects on carbonate chemistry appear to be exacerbated in the low pH treatment; a potentially important finding in the context of ocean acidification experiments, which often examine treatments at or around pH 7.5.

The trends in alkalinity of the flow-through system over time could indicate many things. The decrease in alkalinity seen in the high pH treatments could be attributed to calcification, which reduces alkalinity by two µmoles for every µmol of $CaCO_3$ precipitated. This trend is not observed for the low pH treatment tanks. One possible explanation for this difference is that less calcification took place in the low pH

treatment, giving less modification of the alkalinity of the water. This is supported by the fact that the starting and ending alkalinity values are very tightly clustered for 7 out of 8 of the low pH treatments.

Alkalinity does not appear to be dependent on mussel biomass for the low pH treatment and is not modified compared to the control. However, the low flow-through, high biomass treatment does not fit this trend, but instead increases over the control. It may be that this increase in alkalinity is being caused by an accumulation of ammonia from waste due to high biomass, low flushing, and lack of a bacterial community. Ammonia contributes to total alkalinity by one mole per mole ammonia. (Wolf-Gladrow et al. 2007).

Static Experiment

The difference of 0.25 in average pH of the high pH treatment from the beginning to the end of the static experiment is unexpected and unexplained. It is unlikely that it is related to biomass, since the low and high pH treatments have the same distribution of mussels. Also, the similar standard deviation of the values from beginning to end give additional confidence that it is not a biomass effect. Possible explanations range from increased metabolic activity of the mussels at high pH relative to the mussels at low pH, to experimental design problems like growth of a bacterial colony in the high treatment tanks only. Additional investigation into this result is necessary to see if results are repeatable and to determine cause.

There was a similar trend of large variation in alkalinity in the high pH treatment, although it is not as straightforward because there is a biomass effect in the alkalinity data. Interestingly though, for all of the alkalinity changes, the magnitude of change was greater for the high pH treatments for the low pH treatments. This could support the argument that the mussels at low pH were much less metabolically active (and therefore having a smaller impact on the carbonate chemistry) than the mussels in the high pH treatment. Although generally having carbonate chemistry that does not change is a good thing, we wonder if in this scenario the lack of change in the parameters for the low pH treatments indicates a lack of fitness or distress in the mussels.

In the static system, since there are no inputs to the system, we expect that the two main influences on alkalinity would be calcification (decreases alkalinity) and excretion of ammonia (increases alkalinity) in the absence of bacterial activity. Using this model, it appears that in the high biomass treatments the excretion signal generally outweighs any calcification, potentially another indicator that eight mussels per 3.25L is too heavy of a biomass load. This signal is dampened for the four mussel treatments and the calcification signal in alkalinity actually outweighs the excretion signal when there are only two mussels per tank. This suggests that for static systems even lower biomasses are necessary than when dealing with flow-through systems. Additionally, allowing for a bacterial colony to seed the tank will further change the carbonate chemistry response. Although this will potentially mitigate the increase in alkalinity, bacterial oxidation of ammonia to nitrate causes a decrease of alkalinity by two moles per mole nitrogen (Wolf-Gladrow et al. 2007), and a net effect of decreasing alkalinity by one mole. Likely this balance between calcification, ammonia release and ammonia oxidation are driving the changes in alkalinity we are seeing.

We speculate that the extremely low alkalinity (and pH) exhibited at the end of the experiment by one of the high pH treatment tanks with four mussels, is the result of a sick or dead mussel in the tank.

Conclusion

Carbonate chemistry is difficult to control in the presence of biological activity. There will be fluctuations within almost any system, even without the presence of biology. We found that eight mussels with accumulated wet weight of ~85g is too much for a 3.25L tank if you want to maintain the carbonate chemistry within reasonable bounds. Additionally and unsurprisingly, the amount of new seawater you are adding to the system in a flow-through setting can greatly decrease the impact that the biology has on the consistency of the carbonate chemistry in your tank.

Our results for the static tank system potentially have big implications for systems that do not have flow-through capabilities and must instead do water changes. Even over time periods as short as 20 hours there can be significant changes in the carbonate

chemistry, even with low biomass loads. This study indicates that caution should be taken when using static systems.

The variations in carbonate chemistry we have documented in our results suggest that biomass and rate of flow-through studies are essential components of any pilot study conducted prior to an experimental laboratory study.

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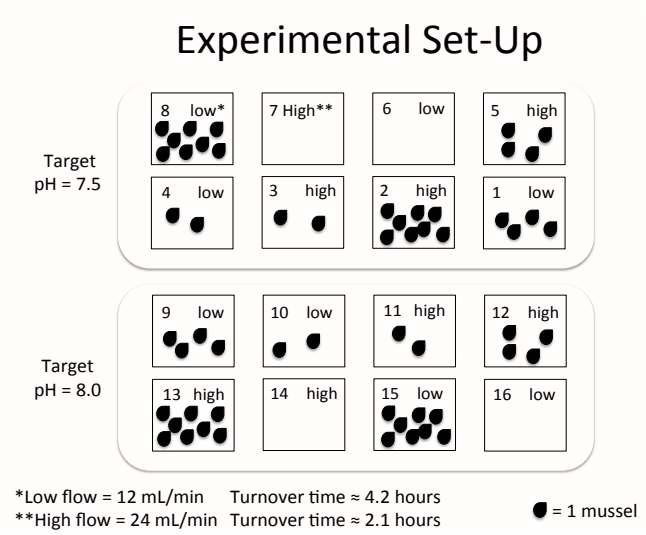
Figure 1. Diagram of experimental set-up. Large rounded rectangles represent the pre-equilibration coolers. The smaller squares represent individual treatment tanks with tank number, high or low flow-through rate and number of mussels.



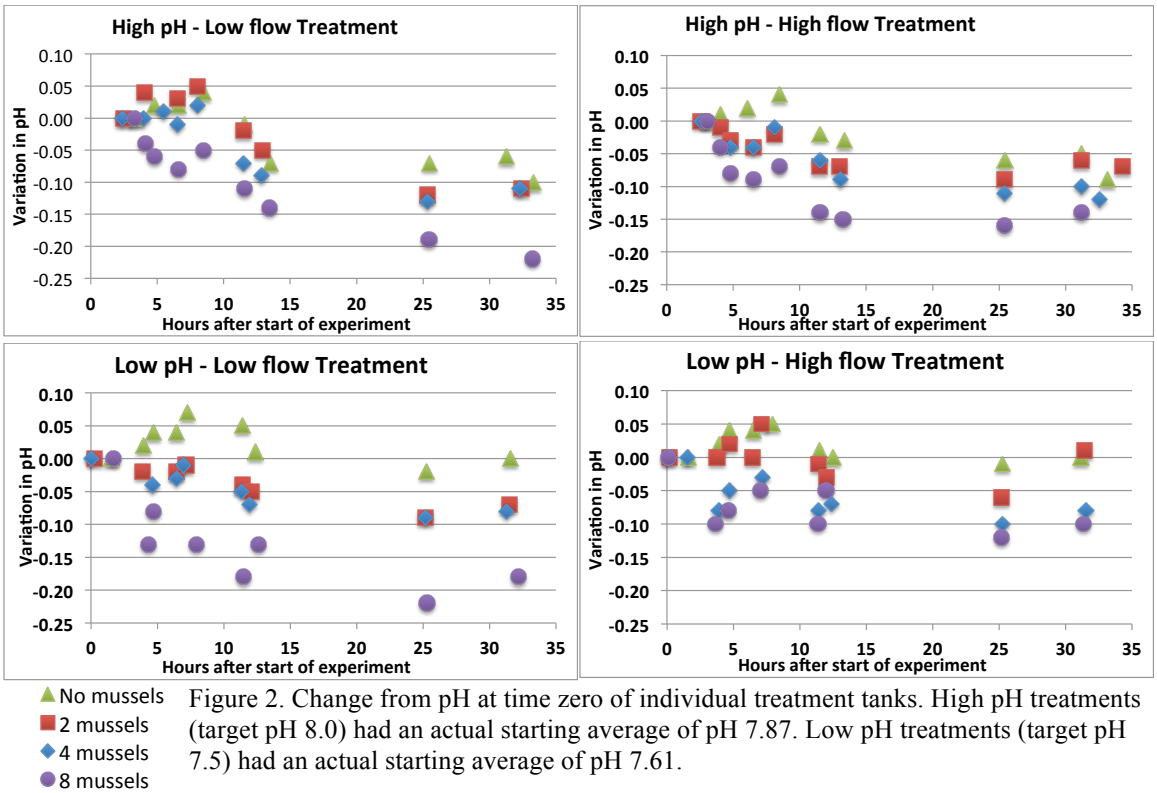


Figure 2. Change from pH at time zero of individual treatment tanks. High pH treatments (target pH 8.0) had an actual starting average of pH 7.87. Low pH treatments (target pH 7.5) had an actual starting average of pH 7.61.

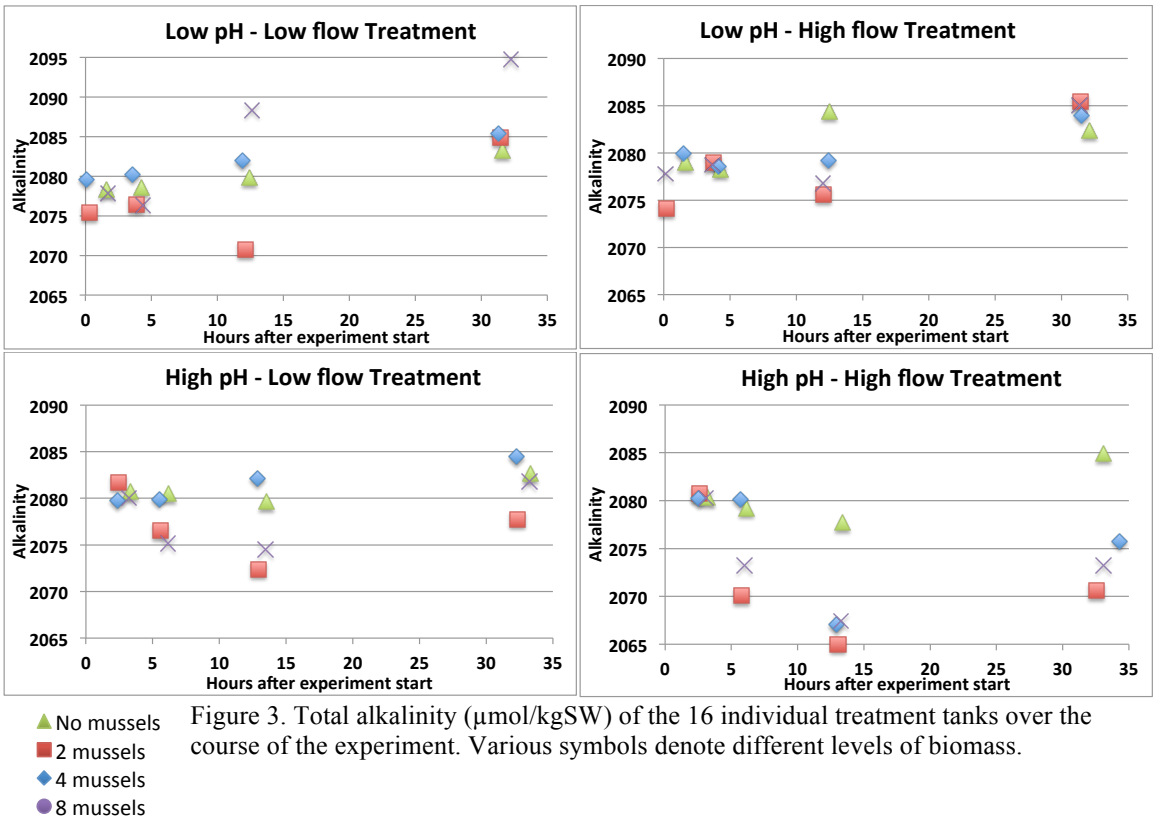


Figure 3. Total alkalinity (µmol/kgSW) of the 16 individual treatment tanks over the course of the experiment. Various symbols denote different levels of biomass.

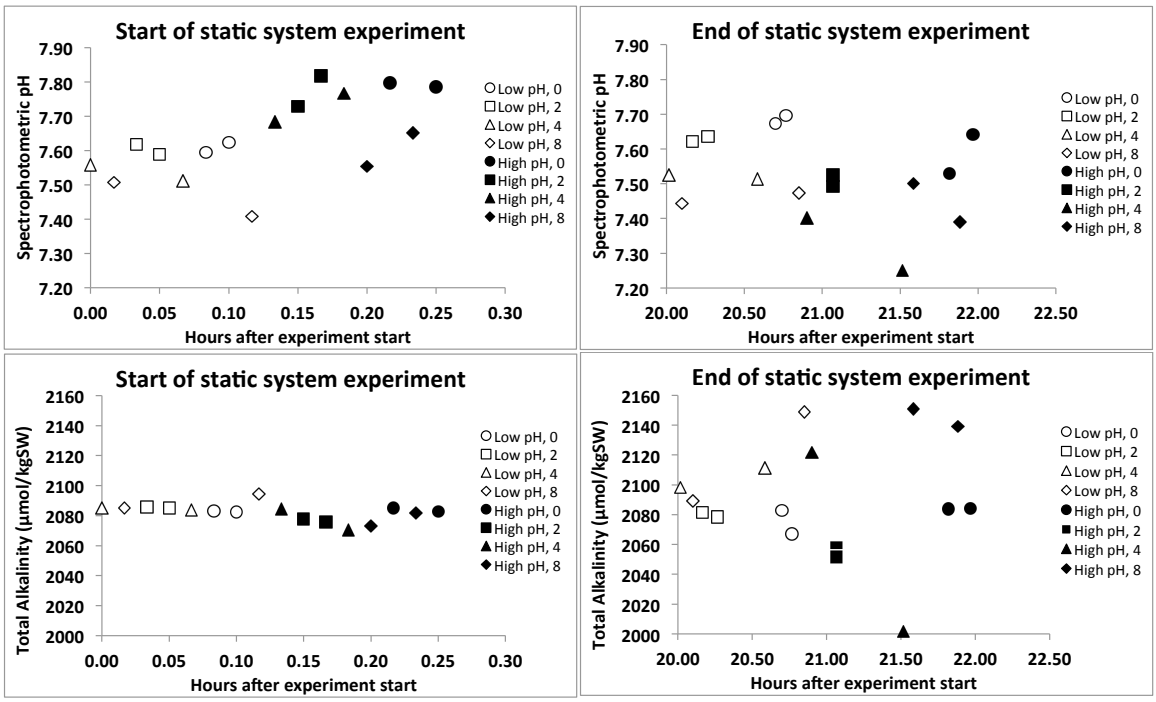


Figure 4. Static tank experiment results. Spectrophotometric pH and total alkalinity at beginning and end of experiment for all 16 tanks. All pH values are reported at the sample temperature. The eight treatments in duplicate are given by high or low pH and the number of mussels (0-8) per tank.