Sources of Error in Carbonate Chemistry: What happens when Biology and Chemistry meet?

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Abstract:

Two different experimental systems were used to evaluate sources of error in carbonate chemistry measurements at Friday Harbor Labs, WA. One system, set up in Laboratory 6, examined the effects of different filters and a UV sterilizer. The second system explored the carbon input from feeding live versus dead algae in the presence of mussels, *Mytilus trossulus*. The use of an activated charcoal filter and UV sterilizer increased the pH of incoming seawater more than that of a pleated sediment filter and UV sterilizer. There were no discernable changes in the Dissolved Inorganic Carbon (DIC), and no trends could be seen in Total Alkalinity. Feeding live or dead algae produced similar results over most carbonate measurements. All treatments decreased treatment tank pH, increased tank pCO_2, and increased tank DIC. However, feeding dead algae notably decreased treatment tank Total Alkalinity, while mussels that were fed live food did not show a difference in this parameter when compared to non-treated water.

Keywords: Carbonate chemistry, measurement error, feeding, filtration, UV sterilizer

Introduction:

It seems that virtually anything found in a seawater system can affect carbonate chemistry. Photosynthesis and respiration of microorganisms and plants can alter pCO_2, Total Alkalinity (T_A) can be altered by the presence of calcifying organisms, and photosynthesis, respiration and calcification combined can affect readings of pH and Dissolved Inorganic Carbon (DIC). With ocean acidification research on the rise, minimizing the error of carbonate chemistry measurements is becoming more crucial. As biologists, we rely on items such as biological filters to minimize organismal waste products and keep our water clean, as well as phytoplankton/algae as food sources for our organisms to keep them acclimated for experimental research. However, there can be no doubt that utilizing these tools can alter the carbonate chemistry of an experimental system. While it is a reasonable assumption that experimental organisms are fed during ocean acidification projects, finding ocean acidification literature that speaks to using biological filtration is a bit more difficult. Most studies will note they utilized a filter in an experimental system, however the type of filter is not specified. Only two studies were found, Gutowska et al., (2008), and Spicer et al. (2007), which specifically state activated charcoal or biological filtration was used to minimize ammonia build-up in tank waters. Interestingly, there is no current literature that speaks to how these sources of carbon can affect and alter experimental carbonate chemistry measurements.

The purpose of my experiments were to: 1) determine how algae (both live and dead forms) would affect carbonate chemistry measurements when fed to blue mussel, *Mytilus trossulus*, and 2) how the addition of an activated charcoal filter and a UV sterilizer into a flow-through system would affect carbonate chemistry measurements.

Materials and Methods:

Filtration Experimental Set-up

A flow-through system in Laboratory 6 was used in order to test the effects of pleated sediment filters, activated carbon filters, and UV sterilizers on carbonate chemistry. Seawater in the system is fed through six-pleated sediment filters (pore size of: 20µ, 10µ, 5µ, 1µ, 0.35µ, and 0.2µ), aligned in series. After being fed through the 0.2µ filter, seawater was then run through a UV sterilizer (normally turned off), and fed into mixing reservoirs (Figure 1). This set-up was considered the control of the experiment. The system was run for 15 minutes, after which, a water sample was collected for analysis of Total Alkalinity and pH. The UV sterilizer was turned on, and the system left to run an additional 15 minutes. At the end of this time point, another water sample was collected for analysis.

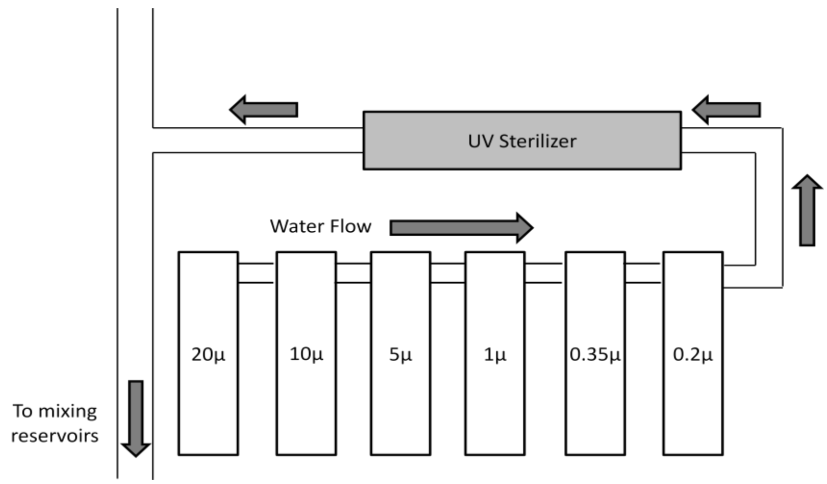

Figure 1. Laboratory 6 sediment filter set-up

To test the effects of a carbon filter, an activated charcoal canister was added to the system, along with an additional sediment filter to remove large charcoal particles. Water was first fed through the 20µ sediment filter, bypassing the 10µ, 5µ, 1µ and 0.35µ filters, run through the charcoal filter and sediment filter, and then fed through the 0.2µ filter before entering the UV sterilizer (Figure 2). Again, the system was run for 15 minutes with the UV sterilizer on, and an additional 15 minutes with the UV sterilizer off, with water samples collected at the end of each time point. Both experimental set ups were run three times, on three different days.

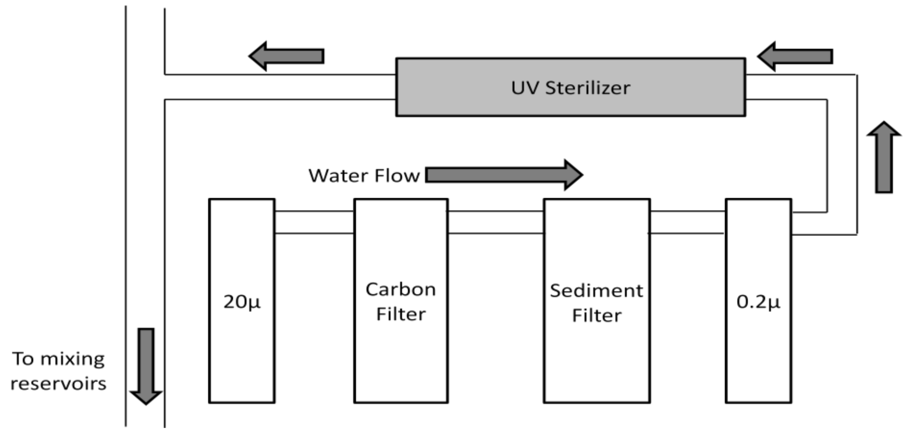

Figure 2. Laboratory 6 carbon filter set-up

Feeding Experimental Set-up

To examine the effect of feeding on carbonate chemistry, an experimental cooler (114 L) was set up in Laboratory 7. The cooler held eight- 3.25 L treatment tanks, each with a small pump to circulate water. Water flow into the tank was controlled with sprinkler heads, which provided a flow rate of approximately 1.17 L/hour. Cooler temperature was controlled with a Honeywell UDA 2182 Universal Dual Analyzer, which also controlled, via rotometer, the amount of CO_2 and air fed into the cooler and hence, the treatment tanks. Water was fed from an outside header tank into the experimental cooler, and the inside water level controlled by a float. Additionally, chilled water was pumped into the cooler via a Venturri injector (Figure 3).

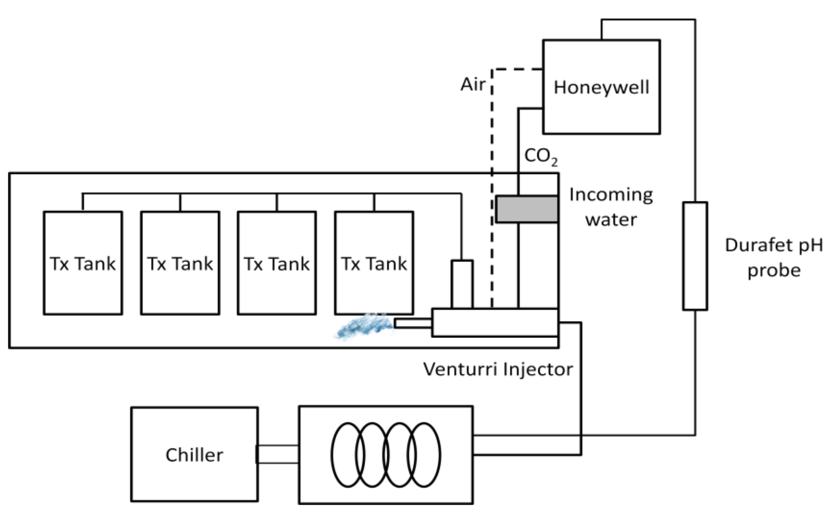

Figure 3. Laboratory 7 experimental set-up

Tanks were randomly assigned (two each) to one of four treatment groups. Treatments included tanks that were fed live algae, *Dunaliella tertiolecta*, or Shellfish meal 1800 (i.e. dead algae; a mixture of *Isochrysis spp, Pavlova spp, Thalossiosira weissflogii*, and *Tetraselmis spp*), and contained either four or zero blue mussels, *Mytilus trossulus*. Tanks were fed the same density of algal cells, whether they received live or dead algae, for three days, with water samples collected on each day.

Mussels were collected from the boat ramp at Argyle Lagoon at low tide, and immediately transported back to Friday Harbor Labs in five gallon buckets. Organisms were placed in a water table with flowing seawater for 24 hours. After this brief acclimation period, 16 similarly-sized mussels, (mean weight 13.58 ±2.38 g) were placed into experimental treatment tanks. Tanks were fed early in the morning, at which point they were switched over to static flow in order to allow mussels time to feed on the algae. A water sample was also collected from the experimental cooler at this time for Total Alkalinity and pH analysis. After approximately six hours, water samples were collected from treatment tanks, salinity and temperature recorded, and water flow was continued.

For all trials, water samples were evaluated for T_A and pH (Dickson et al., 2007 SOP 3b and 6b, respectively). During feeding trials, a Durafet pH probe was used to measure this parameter. However, water samples were randomly taken from feeding treatment tanks and measured with a spectrophotometer to ensure probe readings were accurate. CO_2 Calc was used to calculate pCO_2 and DIC measurements from all T_A and pH values. The results from CO_2 Calc should be interpreted with caution, as these values are based upon calculations, versus direct measurement.

Results:

Laboratory 6

On all three sampling days, an increase in pH was noted across all treatments. Day 1 samples increased from 7.5 to 7.72, Day 2 samples increased from 7.06 to 7.27, and Day 3 samples increased from 7.18 to 7.50. Water samples that were treated with UV sterilization had a higher pH than those that were not treated with UV sterilization, and the carbon filter samples had a higher pH than the water samples from the sediment filters (Figure 4).

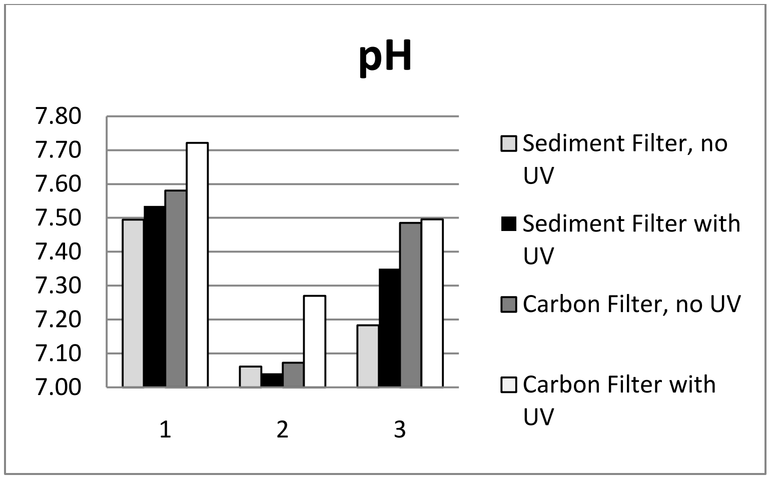

Figure 4. Lab 6 pH results

As pCO_2 and pH have an inverse relationship, it therefore follows that a decline in pCO_2 was found across all treatments. Day 1 samples decreased from 1323.76 to 743.38 µatm, Day 2 samples declined from 4110.43 to 2320.96 µatm, and Day 3 samples dropped from 2938.38 to 1116.91 µatm (Figure 5).

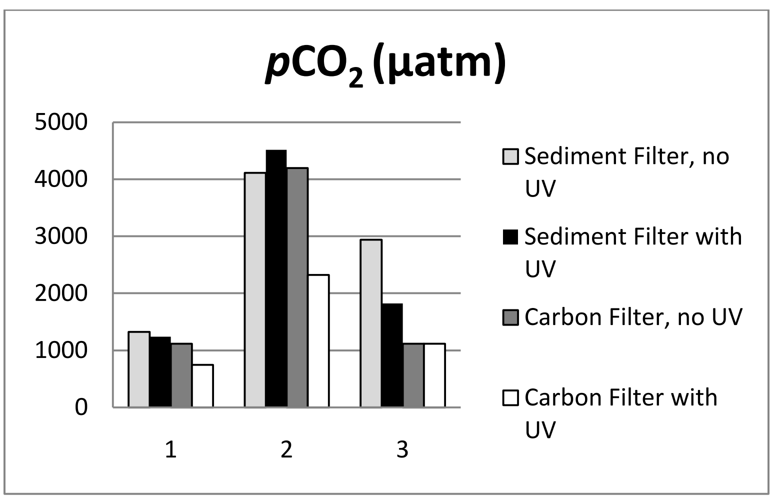

Figure 5. Lab 6 pCO_2 results

DIC measurements did not seem to change much across treatments, or across sampling dates. The largest difference found was between the sediment and carbon filter water treated with a UV sterilizer on Day 2, at 147.02 µmol/kg. All other differences between treatments were well below 100 µmol/kg (Figure 6).

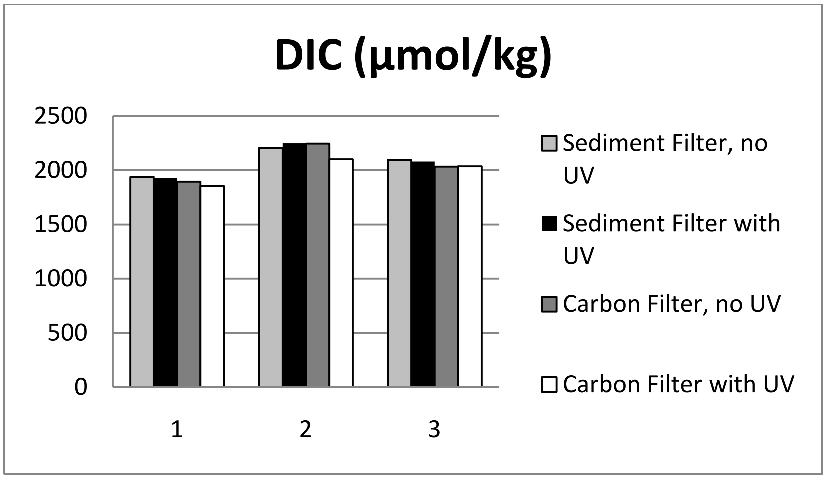

Figure 6. Lab 6 DIC results

No clear trends can be found for Total Alkalinity measurements. It appears that on all three sampling days the sediment filter treated with UV water was slightly higher than the water that was not treated with a UV sterilizer. On sampling Days 1 and 3 it appears the carbon filter treatments were almost identical, but on Day 2, the water that was filtered without UV sterilization was significantly higher than water that was treated with UV sterilization (2140.63 and 2071.46µmol/kg, respectively; Figure 7).

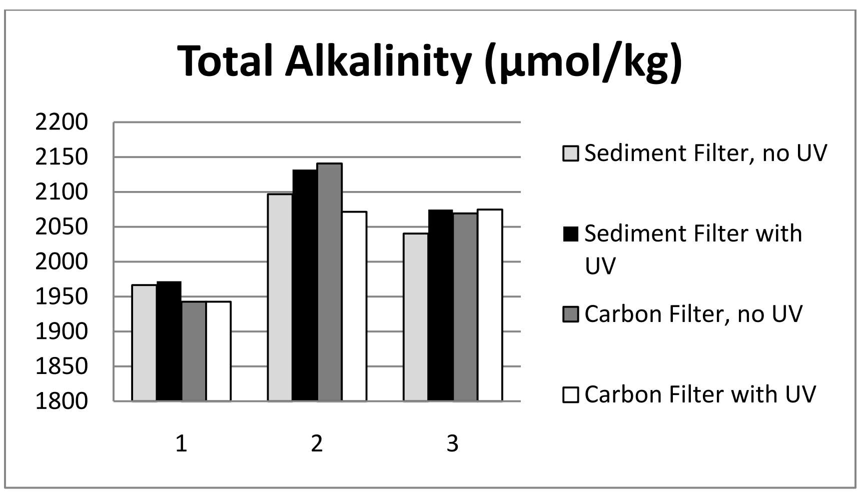

Figure 7. Lab 6 Total Alkalinity results

Laboratory 7

The pH of treatment tanks with mussels in them decreased over the course of the experiment, with no apparent difference seen between tanks treated with live or dead food (pH of approximately 7.6, compared with a cooler pH of 8.0). The tanks that were fed with no mussels in them also exhibited a decline in pH, approximately 0.15 to 0.21 pH units, but this decrease was less drastic than in the treatment tanks with live mussels (Figure 8).

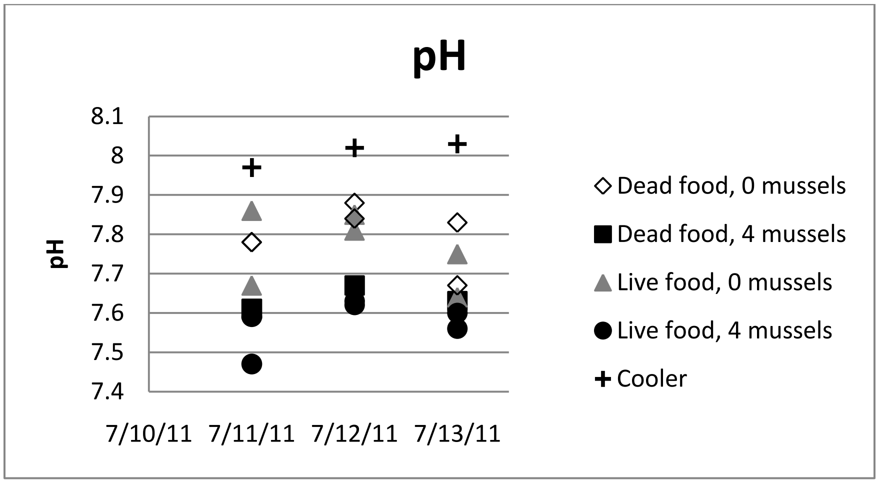

Figure 8. Lab 7 pH results

The pCO_2 of treatment tanks increased the most with either live or dead food in the presence of mussels, approximately 415 µatm, (Cooler pCO_2), to approximately 1216 and 1028 µatm, respectively. As with pH, the treatment tanks that were fed in the absence of mussels showed a similar response (Figure 9).

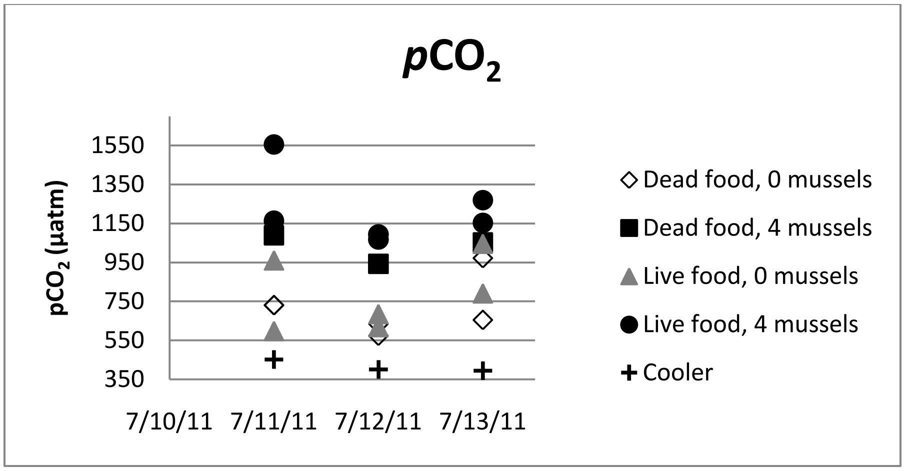

Figure 9. Lab 7 pCO_2 results

DIC increased the most with live food versus dead algae, particularly in treatment tanks with mussels (average increase of 122 µmol/kg). Again, the tanks which were fed live or dead food with no mussels showed similar trends, and the tanks which were fed food with mussels in them displayed similar responses (Figure 10).

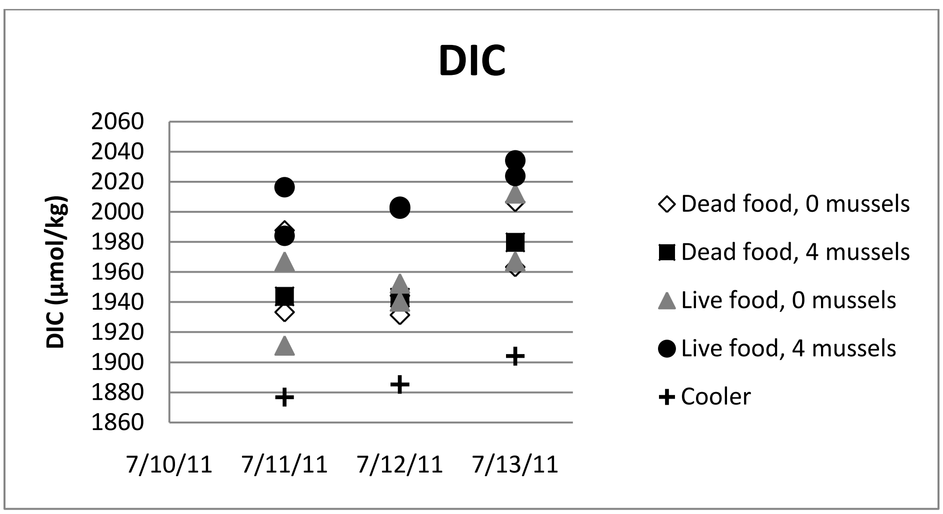

Figure 10. Lab 7 DIC results

Interestingly, the only treatment which deviated from the cooler in terms of Total Alkalinity was the tank which contained mussels and fed dead algae. Another trend that was noted was the steady increase in T_A that occurred over the course of the experiment (Figure 11).

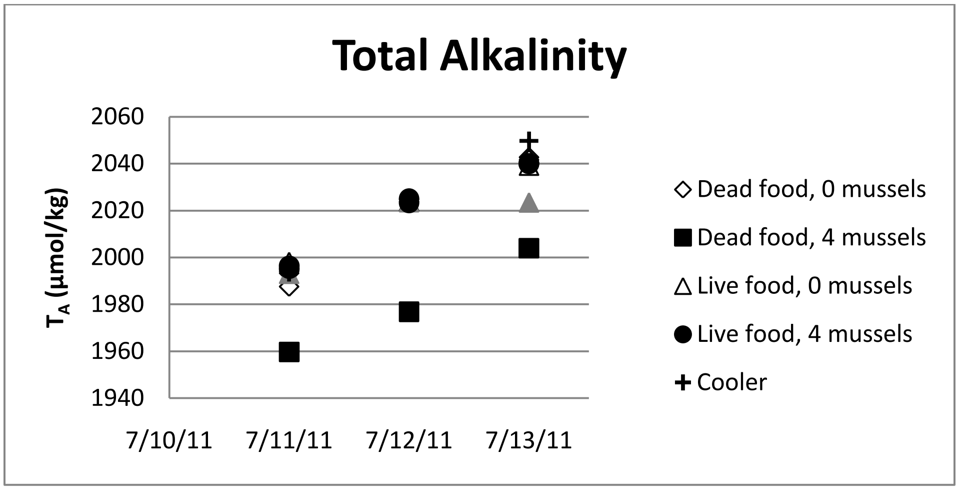

Figure 11. Lab 7 Total Alkalinity results

Discussion:

Laboratory 6

The increase noted in pH as well as the decrease noted in pCO_2 seems to be related to two factors; one, the use of a UV sterilizer and two, the use of a carbon filter. It is possible that the activated charcoal in the filter was stripping out some of the CO_2, which would account for an increase in pH. Additionally, using a UV sterilizer can produce free hydroxyl (OH^-) radicals in the water (Wateen solutions, 2011). These radicals could also affect the pH and pCO_2 measurements. Altering pH by using a carbon filter could also have implications for photosynthesis, respiration, and calcification of organisms (Hurd et al., 2009). It seems clear that using a carbon filter does introduce error into measurement of carbonate chemistry, on the order of approximately 0.2 pH units.

The DIC measurements are interesting as the addition of a carbon filter should affect the amount of total carbon in the system. It does appear from the data that the use of a UV sterilizer decreased the amount of total carbon from the filter alone on Days 1 and 3 in both sediment and carbon filter treatments. However, as this parameter was calculated instead of directly measured, further investigation would be required to answer this question.

It seems that a UV sterilizer increases the Total Alkalinity when used with a sediment filter, but this pattern does not hold true when using a carbon filter. Although there are no obvious trends in Total Alkalinity, the samples were taken on different days at different times, which points to the fact that incoming water has a large affect on carbonate chemistry, which can overshadow treatment effects. Further investigation is needed to determine how to compensate for this issue.

Laboratory 7

Feeding live or dead algae seems to have more of an impact on carbonate chemistry than different filters and UV sterilizers. The trends seen in pH, pCO_2 and DIC measurements indicate there is no difference in affect whether you feed live or dead algae. pH decreased in treatment tanks with mussels approximately 0.4 pH units. This would indicate the algae were respiring, adding CO_2 into the treatment tanks. The experimental cooler was placed in such a way that little ambient light was able to reach the treatment tanks. Over the course of the experiment, it appears that DIC increased in all treatment tanks with mussels, which could also indicate respiration.

The possibility of photosynthesis and respiration affecting carbonate chemistry results has been well documented, and it follows that the parameter to measure, which cannot be altered by either process, is Total Alkalinity (Chrisholm and Gattuso, 1991). Given the small changes of T_A seen in the feeding experiment, it would follow that to minimize error in chemistry due to photosynthesis and respiration, to feed live food in a low-light environment.

In research areas that are not near the coast, flow-through experimental systems are not practical; water filtration is necessary to keep experimental organisms alive. While organisms in flow- through systems can survive without biological filtration, experimental animals still need to be fed, so water chemistry from these systems is also prone to errors in measurement. Quantifying the error associated with filtration and feeding could help in preventing error in carbonate chemistry measurements of these systems. While these experiments indicate some general trends, further investigation is needed to make absolute conclusions regarding the amount of error filtration, UV sterilization and feeding add into carbonate chemistry measurements.

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