

**Impact of elevated  $p\text{CO}_2$  on heterotrophic bacterioplankton:  
a mesocosm experiment**

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Ocean Acidification Apprenticeship 2013

Spring 2013

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*Keywords:* ocean acidification, mesocosm, bacterioplankton,  $p\text{CO}_2$

## **Impact of elevated $p\text{CO}_2$ on heterotrophic bacterioplankton: a mesocosm experiment**

**Abstract** The rapid rise in anthropogenic atmospheric carbon dioxide ( $\text{CO}_2$ ) and the subsequent acidification of the world oceans has increased the need to determine if these changes will seriously and adversely impact the microbial food web and its constituents. A mesocosm study was conducted to test the effects of elevated partial pressure  $\text{CO}_2$  ( $p\text{CO}_2$ ) on the community composition and abundance of bacterioplankton. Three levels of  $p\text{CO}_2$  were established in triplicate: 650  $\mu\text{atm}$  (Control), 1250  $\mu\text{atm}$  (High), and 1250  $\mu\text{atm}$  allowed to drift (Drift). Initial light levels were reduced to slow biological processes and were returned to ambient conditions on T10. Results of bacterial abundance were statistically significant between treatments with the High and Drift treatments showing the lowest abundance. Community composition was shown to change temporally with distinct shifts in structure from T0 to T13 to T21. There was no change in community species composition among the treatments. Study indicates elevated levels of  $p\text{CO}_2$  suppresses abundance of bacterioplankton without a corresponding change in community structure.

### **Introduction**

The rapidly accelerating production of anthropogenic  $\text{CO}_2$  primarily from the burning of fossil fuels and agricultural practices (Feely et al., 2004; Guinotte and Fabry, 2008; Sabine and Feely, 2007) has a corresponding impact on the concentrations of partial pressure  $\text{CO}_2$  ( $p\text{CO}_2$ ) in the world oceans with the potential to decrease pH and change the composition and chemical state for many generations to come (Caldeira and

Wickett, 2003; Riebesell et al., 2008). With the daily high reading of atmospheric CO<sub>2</sub> reaching 400 ppm in May 2013 (NOAA Earth System Research Laboratory, 2013) there is increasing concern limits are now above levels experienced by all extant taxa or their predecessors (Ehleringer et al., 2002). Within the oceans the uptake of *p*CO<sub>2</sub> leads to changes to the biogeochemical processes and ocean acidification (Doney et al., 2009). This has the potential to change the structure of the communities not only at a higher trophic level, but on a microscale as well (Feely et al., 2004). As primary producers are a driver for the health of the oceanic food webs, so too are the bacterioplankton closely coupled to them. The scientific community is working to describe not only the potential changes to the microbial community, but also those interactions and processes not historically well understood (Joint et al., 2011; Yokokawa and Nagata, 2010).

Bacteria account for a large proportion of biomass in the oligotrophic strata of the ocean (Cho and Azam, 1990) and research indicates bacteria process upwards of 50% of the daily primary production (Ducklow and Carlson, 1992). This keystone role of bacterioplankton within the microbial food web is increasing in recognition, but to this point the underlying mechanisms of their involvement in the carbon cycle have not yet been elucidated (Azam and Malfatti, 2007) and the bacterial research is in its infancy (Guinotte and Fabry, 2008). While many factors influence the bottom-up and top-down control of bacterial abundance and community structure (Ducklow, 1992), these complex components of the microbial food web cannot easily be separated for individual study. Nevertheless, with rising *p*CO<sub>2</sub> levels it is more important than ever to understand not only the intricacies of the interactions of phytoplankton and bacterial communities, but

also the community composition of the bacterial species present and their relative abundance.

A study design employed to this end is exploring ocean acidification by way of mesocosms. This experimental technique provides the opportunity to study a closed system, outside of the laboratory, and allows us to investigate the biogeochemical processes and impacts to bacterial abundance, community composition and their interactions. Previous mesocosm experiments – such as the Bergen, Norway experiments known as the Pelagic Ecosystem CO<sub>2</sub> Enrichment (PeECE I-III) studies (Riebesell et al., 2008) – have examined a number of questions regarding bacterioplankton and their role in the microbial community. These international collaborations resulted in a suite of literature, from which a limited number of articles describing aspects of bacterial processes within the microbial loop have been published. These included a study on the coupling of bacteria with phytoplankton (de Kluijver et al., 2010) which showed no statistical difference in the response to higher levels of *p*CO<sub>2</sub>. Another examined nutrient availability (Tanaka et al., 2008), again with no statistical differences among treatments. Coupling of heterotrophic bacteria to phytoplankton bloom (Allgaier et al., 2008) and abundance and bacterioplankton dynamics within the mesocosms (Grossart et al., 2006) have also been studied. While no differences in bacterial abundance were described among treatments, the elevated *p*CO<sub>2</sub> did result in increased biomass within the system. Furthermore, the mesocosm with the highest treatment, 700 µatm, produced the largest increase (Grossart et al., 2006).

On a larger scale, an experiment comprised of 50m<sup>3</sup> mesocosms moored near Svalbard found a shift in community composition in phytoplankton from larger to smaller

species (Brussard et al., 2013), and reduction of 15 rare taxa of bacterioplankton – a very small decrease in diversity (Roy et al., 2013). Their results indicate higher  $p\text{CO}_2$  levels changed the community composition of microbes, pushing primary production of smaller phytoplankton higher. Although not directly attributable to elevated  $p\text{CO}_2$  levels, the shift from larger to smaller phytoplankton clearly has the potential to increase the pressure on the microbial food web and its efficacy.

Finally, a Denmark mesocosm study (Havskum et al., 2003) examined silicate and labile dissolved organic carbon (DOC) interactions and how they impact phytoplankton and bacterial interactions. The results indicated bacterial DOC consumption depends not only on the degradability of DOC but also on the structure of the microbial food web. This research highlights the need to gather many different types of data before attempting to describe the community interactions at differing  $p\text{CO}_2$  levels.

The objective of the experiment at Friday Harbor Laboratories was to describe the abundance and diversity of bacteria within mesocosms of varying elevated  $p\text{CO}_2$  levels and conditions. The experimental design was unique. While the mesocosms were based on other experiments, there were significant differences. First, the levels of  $p\text{CO}_2$  (650 - 700  $\mu\text{atm}$ , 1250  $\mu\text{atm}$  and 1250  $\mu\text{atm}$  allowed to drift) were higher due to naturally acidified waters caused by upwelling. Also, there were three replicates of each treatment, and no nutrients were added to induce a bloom. Finally, mixing was done without bubbling, and the seawater used to fill the mesocosms was coarsely filtered to remove all large zooplankton. These innovations established distinctive experimental conditions allowing for ocean acidification and its impact on microbial interactions to be examined in new ways. Combined with data collected from other researchers, a clearer picture of

the abundance of bacteria and its community composition and distribution under elevated  $p\text{CO}_2$  can now be described.

## **Materials and methods**

### *Daily Sampling*

The mesocosm study was carried out between 9 April and 30 April 2013 at the University of Washington Friday Harbor Laboratories in Friday Harbor, Washington, United States of America (48.53° N, 123.01° W). Nine mesocosms were constructed of 6 meter X 1 meter commercially sealed polypropylene. They were filled with approximately 3,500 liters of coarsely filtered (500 $\mu\text{m}$ ) seawater to remove cnidarians, ctenophores and mesozooplankton over 57 hours from nine individual tubes attached to a reservoir that was filled using a plastic and Teflon peristaltic pump (Wilden P-1 double diaphragm). The mesocosms were filled 1 meter from the surface and held at this level within steel cages suspended with buoys. To reduce light (by ~55%) to the mesocosms, the sides and tops were covered with mesh bags and caps for the first 11 days (T0 – T10) of the experiment. Salinity readings before and after the addition of brine solution (3500 g NaCl/15 L water) determined the final volume of mesocosms. To achieve experimental control, high and drift conditions within the enclosures  $\text{CO}_2$  was taken from a holding tank of filtered seawater augmented with  $\text{CO}_2$  and was added to each mesocosm using a Cole-Parmer MasterFlex L/S Easy Load II pump attached to polyethylene tubing. The mesocosms were then covered with acrylic domes to keep airborne contaminants from the interior. On day T10 the mesh bags were lowered and the mesh caps were removed to increase light level and biological activity within the mesocosms.

Sampling occurred daily for 22 days. Light readings were taken using a LICOR LI-700 prior to mixing with mesh caps in place (T0-T10). Mixing was conducted using a perforated acrylic disk which was raised and lowered to gently mix contents for three minutes. Salinity and temperature were measured daily using a Sea Bird Electronics 37-SMP MicroCAT recorder. A 3 meter plastic tube (4.4 cm diameter) depth-integrated sampler was lowered into the center of each mesocosm and decanted into an acid-washed carboy for more precise sampling. For bacterioplankton, 1 liter was measured daily from the reservoir into amber bottles for each mesocosm. Dissolved organic carbon (DOC) and particulate organic carbon (POC) were sampled daily from the reservoir into 125 ml amber bottles through day 12 and sampled every other day to the end of the experiment. Oxygen, dissolved inorganic carbon (DIC), alkalinity and nutrients ( $\text{NH}_4$ ,  $\text{NO}_3$ ,  $\text{NO}_2$ , and  $\text{PO}_4$ ) were sampled daily taken from a 2 or 5 liter Niskin (General Oceanics, Inc.). Chlorophyll was sampled daily (Porcino, 2013). Biogenic silica was sampled every other day per previously described protocols (Paasche, 1980; Strickland and Parsons, 1968).

DOC and POC samples were prepared by filtering 20 to 25 ml seawater through combusted GF/F filters into pre-chilled vials. An additional volume to equal 60 ml (prior to day 12) and 120 ml (days 12 to 22) was syringed through the filter, folded with particulates inside and stored on an analyslide. DOC and POC samples were stored at  $-20^\circ\text{C}$  until delivered to the Marine Chemistry Laboratory for analysis. DOC samples were analyzed on a Shimadzu TOC-Vcsh carbon analyzer, using the high temperature catalytic oxidation method (HTCO) and measured on a non-dispersive infrared (NDIR) detector. Samples for organic carbon analysis were acidified (w/6N HCl), sparged and

injected into the system (Sugimura and Suzuki, 1988). POC samples were fumed overnight with saturated HCl, dried, and measured with an elemental analyzer.

### *Bacterial Abundance*

Bacterial abundance was sampled daily within six hours of collection. 400 µl unfiltered mesocosm water was combined with 20 µl 10% glutaraldehyde in a 2 ml cryotube to fix the sample. Cryotubes were chilled for 30 minutes at 8°C, then stored at -80°C. After delivery to the University of Washington Environmental Genomics Laboratory fixed samples were thawed and prepared for flow cytometry. For each full-strength microplate 200 µl fixed sample was pipetted into each well. For each diluted microplate well 20 µl sample plus 180 µL filtered seawater was pipetted into each well. A reagent of Tris and SYBR-Green (1:39 ratio) was prepared and 4 µl was added to each well. Microplates were placed in dark to process from several hours to overnight. Microplates were analyzed on a Guava EasyCite Pro flow cytometer running Cyto Soft 5.2 software.

### *Bacterial Community Composition*

Bacterial community composition sampling was conducted daily. A 1 liter mesocosm sample was vacuum filtered through 0.2µm 47mm Supor200 membrane filter to collect DNA. Filters were rolled and stored in 2 ml cryotubes at -80°C. Based on flow cytometry analysis, days T0, T13 and T21 were selected to determine community composition. Filters for selected sample days were thawed and each cryotube treated with 1 ml sucrose lysis buffer (20mM EDTA, 400 mM NaCl, 0.75 M sucrose, 50 mM Tris-HCl, pH 8.4), 200 µl sodium dodecyl sulfate (SDS) and 20 µl proteinase K. The cell



lysate was incubated at 37°C for 30 minutes, then at 57°C for 30 minutes. Community genomic DNA was extracted from 200 µl of cell lysate using a DNeasy kit (QIAGEN) per previously published protocol (Morris et al., 2012). In brief, isolate and community screening was conducted by polymerase chain reaction (PCR) using 2 µl of DNA template with 23 µl of Master Mix (dH<sub>2</sub>O, 10X buffer, 2 mM dNTP, 50 mM MgCl<sub>2</sub>, 50% Acetamide, 519R primer, and 8F primer) with Taq polymerase. After 16S rRNA genes were amplified for terminal restriction fragment length polymorphism (T-RFLP) analysis they were restricted with HaeIII enzyme and cleaned. The T-RFLP fragments were sent to Fred Hutchinson Cancer Research Center for resolving. Peak Scanner version 2.0 (Applied Biosystems) was used to size the fragments.

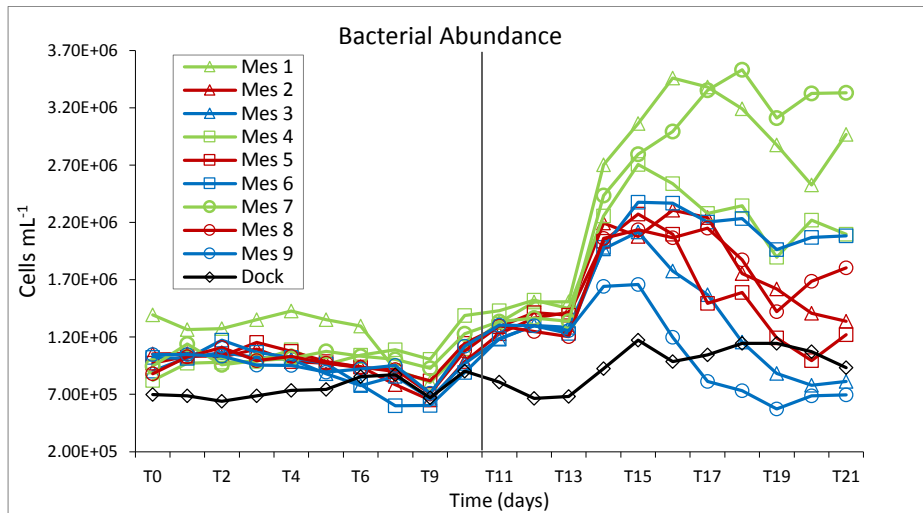
#### *Statistical Analysis*

Statistical analysis for bacterial abundance and its association with experimental variables was conducted using IBM SPSS Statistics software. Non-metric multidimensional scaling (NMS) analysis of the community composition data was done using PC-ORD version 4.0 (MjM Software). Data was relativized by adding the peaks and dividing by the total. Ordination NMS was performed using a Sorensen distance matrix and the slow and thorough option. NMS was run on data grouped by treatment day (T0, T13 and T21) and treatment type (control, high and drift).

#### **Results**

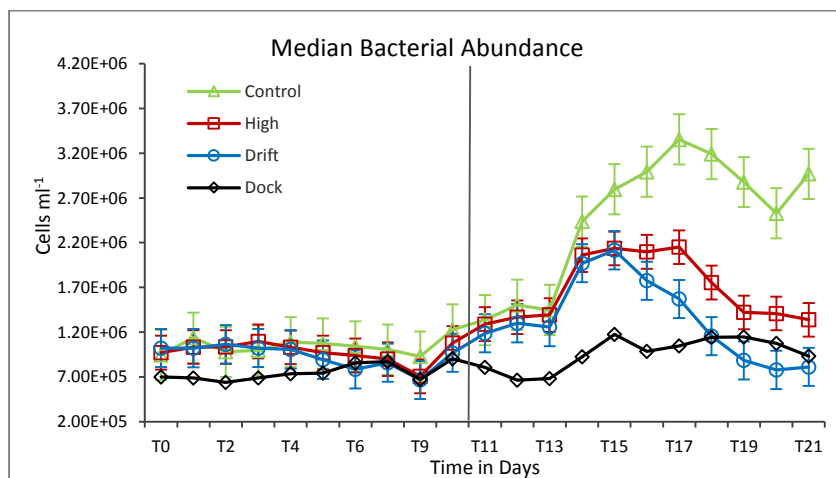
With regard to bacterial abundance, the study progressed in two phases. During the first phase, when the light within the mesocosms was reduced by the mesh bags and caps, bacterial abundance was similar among the treatments and not statistically different. Once the light was returned to ambient conditions, the biological activity increased and the difference in bacterial abundance among the treatments changed rapidly. Results for T7 were not used due to a pipetting error adding too much glutaraldehyde fixative to

cryotubes. Results from the individual mesocosms show differences in bacterial abundance between mesocosms with the Control exhibiting the greatest abundance, followed by the High and the Drift treatments. Of note is Mesocosm 6. It had the lowest volume and did not follow trends in this or other sampled data sets (Figure 1).



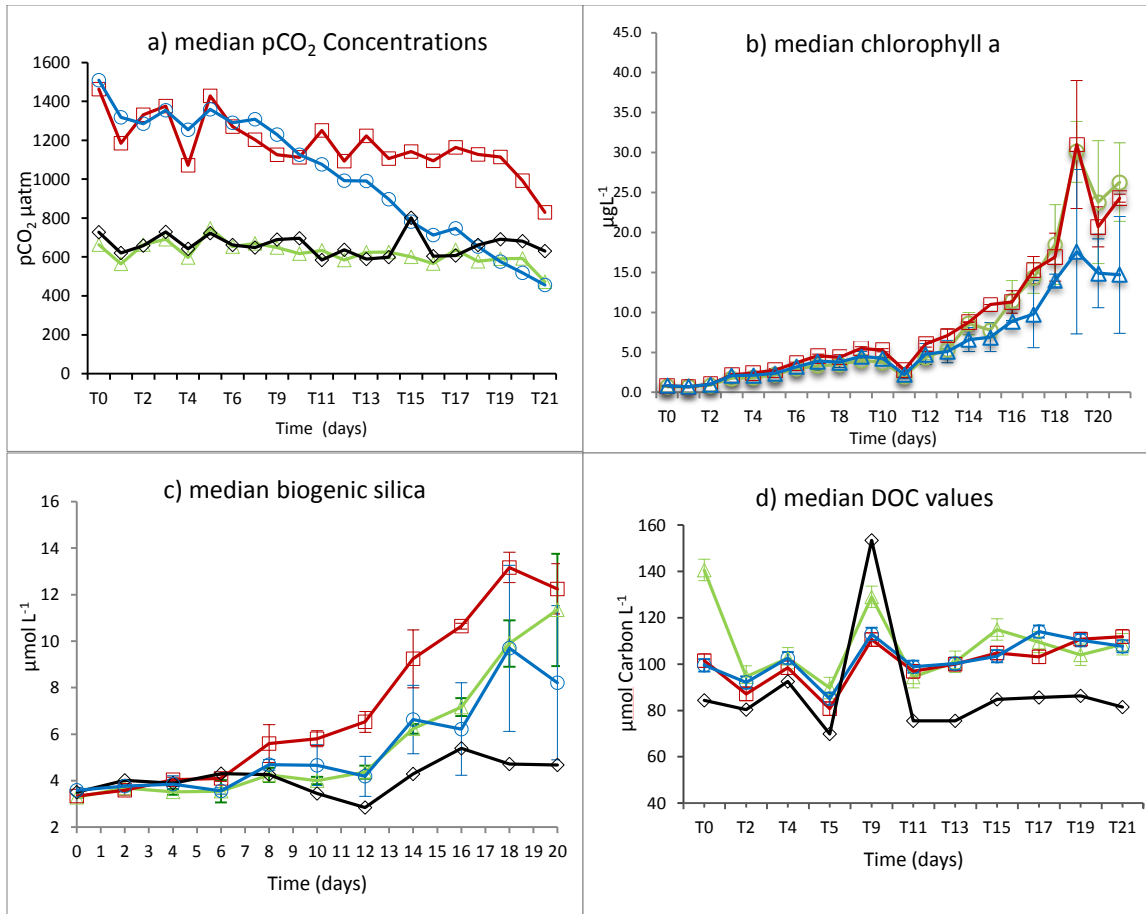
**Figure 1.** Bacterial abundance per mesocosm per treatment. Colors indicate treatment type. Green = Control, Red = High, Blue = Drift, Black = Dock. Time on the X-axis is in days sampled. Bisecting line indicates date mesh caps removed from mesocosms. Control treatments had higher abundance than either the High or the Drift treatments. Mesocosm 6 did not follow trends of other mesocosms within the Drift treatment due to lower volume and higher  $p\text{CO}_2$  measurements throughout course of study.

The mesocosm raw data was combined to analyze the overall trend among the treatments. A statistical difference was measured among all three treatments, with the largest delta between the Control treatment and the High and Drift treatments (Figure 2). The 650 – 700  $\mu\text{atm}$  treatment (Control) produced the greatest abundance. The High treatment (1250  $\mu\text{atm}$ ) was significantly below the Control. Of note is the Drift treatment that began at 1250  $\mu\text{atm}$  and ended below 500  $\mu\text{atm}$  (Mesocosm 6 ended at 934  $\mu\text{atm}$ ). The bacterial abundance for the Drift treatment fell below even the Dock values. The dock samples were taken outside of the mesocosms and was not filtered to remove higher trophic level organisms.



**Figure 2.** Median bacterial abundance in cells ml<sup>-1</sup> by treatment. Green triangle = Control, Red square = High, Blue circle = Drift, Black diamond = Dock. Median absolute deviation (MAD) given for Control, High and Drift treatments. Bisecting line indicates date mesh caps removed from mesocosms. Control treatment shows greater abundance than High or Drift treatments. Of note, Drift treatment abundance ended with cells ml<sup>-1</sup> below that of Dock. Statistical analysis (Friedman test)  $F_{(2, 66)} = 58.084$ ,  $p < 0.001$ .

Measurements taken for  $p\text{CO}_2$ , chlorophyll a, biogenic silica and DOC provide context for the abundance results (Figure 3a-d). Levels of  $p\text{CO}_2$  measured remained within acceptable pre-established levels (Figure 3a). Initial (light limited) phase is evident in the gradual then rapid increase in chlorophyll a and biogenic silica (Figures 3b and 3c respectively). The DOC remains relatively stable throughout the course of the study fluctuating very little, but with the notable peak on T9 (Figure 3d).



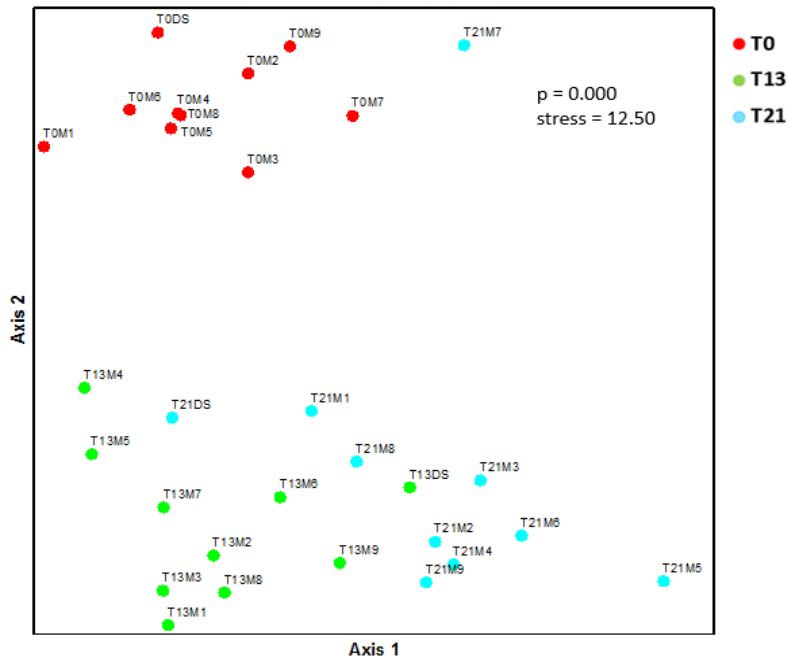
**Figure 3.** Median values by treatment type for a)  $p\text{CO}_2$  in  $\mu\text{atm}$ , b) Chlorophyll a in  $\mu\text{g L}^{-1}$ , c) biogenic silica in  $\mu\text{mol L}^{-1}$  and d) DOC in  $\mu\text{mol L}^{-1}$ . Green triangle = Control, Red square = High, Blue circle = Drift, Black diamond = Dock. Graphs indicate a)  $p\text{CO}_2$  remained within acceptable range for duration of experiment, b) Chlorophyll increased gradually before T10 and more rapidly during phytoplankton bloom (T11-T20), c) Biogenic silica was suppressed early in the experiment and increased after T10, and d) DOC fluctuated very little over course of experiment with exception of peak at T9.

**Table 1.** Spearman's rank correlation coefficient and reported  $p$ -values for factors thought to influence bacterial abundance. NS = non-significant.

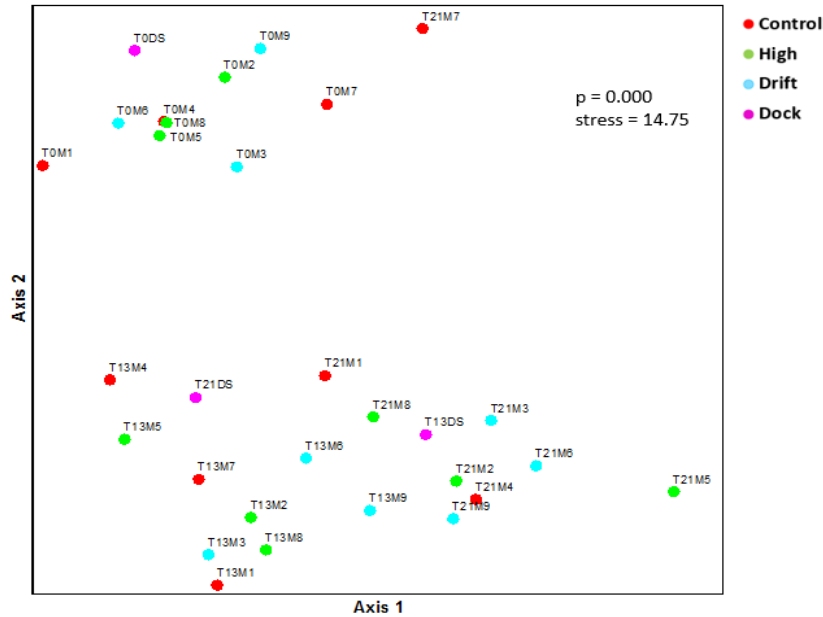
Mesocosms	$p\text{CO}_2$	Chlorophyll	Biogenic Silica	DOC
Variables				
Control	$\rho = -0.569$ $p < 0.001$	$\rho = 0.773$ $p < 0.001$	$\rho = 0.784$ $p < 0.001$	NS
High	$\rho = -0.402$ $p = 0.001$	$\rho = 0.605$ $p < 0.001$	$\rho = 0.584$ $p < 0.001$	NS
Drift	NS	NS	NS	NS

Multivariate analysis of community composition determined that between days T0 - T13 and T13 - T21 a shift in species occurred (Figure 4). This temporal shift was

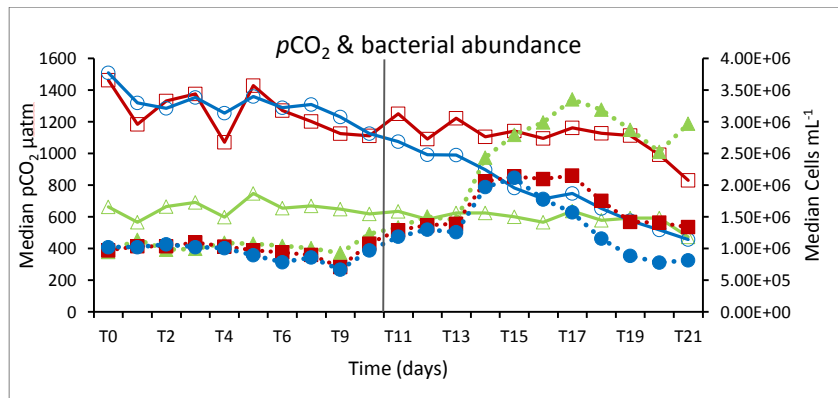
seen in the phototrophic and heterotrophic plankton communities as well (Gravinese, 2013; Stephens, 2013). Further analysis grouped the data by treatment type. The ordination plot showed elevated  $p\text{CO}_2$  levels did not contribute to any change in community structure among the treatments (Figure 5). While bacterial abundance was suppressed under High and Drift treatments, the species present did not change under elevated  $p\text{CO}_2$  conditions.



**Figure 4.** Non-metric multidimensional scaling (NMS) ordination plot of 16s rRNA T-RFLP results grouped by sample day (T0, T13, T21). Red = T0, Green = T13, Blue = T21 sampling days. Community composition showed similar species shift over course of experiment.



**Figure 5.** NMS ordination plot of 16s rRNA T-RFLP results grouped by treatment. Community composition did not change within the treatment groups. All mesocosms represented by day and treatment. Red = Control, Green = High, Blue = Drift, Pink = Dock.



**Figure 6.** Measured  $p\text{CO}_2$  ( $\mu\text{atm}$ ) on the primary Y-axis with solid lines graphed against bacterial abundance ( $\text{cells mL}^{-1}$ ) on the secondary Y-axis with dashed lines. Green triangle = Control, Red square = High, Blue circle = Drift treatment. Bisecting line indicates date mesh caps removed from mesocosms. Graph indicates high median levels of  $p\text{CO}_2$  resulted in suppressed  $\text{cells mL}^{-1}$  in the High and Drift mesocosms.

## Discussion

Light, nutrition, predation, symbiosis, competition and  $p\text{CO}_2$  levels all contributed to the outcome of this mesocosm study at Friday Harbor Laboratories. While some of the factors exhibited only limited correlation,  $p\text{CO}_2$  is the most parsimonious explanation and

could not be ruled out as a direct cause of the lower bacterial abundance in the High and Drift treatments.

The first element to consider is the reduced light levels within the mesocosms. The current study produced results with two distinct phases. In the initial phase of reduced light conditions bacterial abundance was similar between treatments and mesocosms. Biotic and abiotic changes among all experimental variables did not change dramatically. When light levels increased, the biological activity within the mesocosms did as well and the subsequent bloom returned much different data. This is not to say that light was the primary driver of the results; there are many resources and processes that influence bacterioplankton of which light is only one component. Bacterial growth and diversity also differ depending upon the resources available in the niches they inhabit. Factors which impact the abundance and community composition of bacteria are often discussed in the context of how difficult they are to directly measure and how they are parts of the entire suite of microbial food web interactions. Nutrient type and availability, viral lysis, competition, physical and chemical processes, predation and symbiosis – all aspects of microbial ecology (Fuhrman and Hagström, 2008) which come into play within the context of this experiment – are not always able to be directly measured. Looking at these individually they contribute to the final outcome of the study, but cannot be given full credit for the results.

A key factor considered as part of the experiment was the availability nutrients. Sources of nitrogen were well above levels used to stimulate blooms in other mesocosm experiments –  $25 \mu\text{mol L}^{-1}$  as opposed to  $14 \mu\text{mol L}^{-1}$  (Allgaier et al., 2008; Tanaka et al., 2008) and  $9 \mu\text{mol L}^{-1}$  (Grossart et al., 2006) as examples. DOC levels were found to be

stable throughout the experiment, with the exception of the high peak on T9. This indicates the bacterial uptake of carbon was not impacted by the availability of nutrients and was not the cause of the difference in abundance. The stability of the DOC measured could be indicative of other processes involved in its uptake and release. Differences in DOC measurements between upwelling and oligotrophic regions in the Atlantic Ocean shows that this alone cannot be used to determine productivity (Teira et al., 2001) and supports using more than DOC processes to study it. While these were only some of the nutrients and cofactors measured, there are many more organic and inorganic growth factors which were not. Of interest is a growing body of evidence for the interactions of  $p\text{CO}_2$  and vitamin B<sub>12</sub> in the limiting of phytoplankton and bacterial abundance (Giovannoni, 2012; King et al., 2011). Without adequate supplies of B<sub>12</sub> as a biochemical cofactor, bacteria would not be able to grow which would have negative impact on the carbon cycle (Sañudo-Wilhelmy et al., 2012). Because the experiment did not measure B<sub>12</sub>, the relationship between vitamins and  $p\text{CO}_2$  cannot be ruled out as having significant impact on abundance in our ocean acidification study.

Within the microbial food web, an additional factor which must be considered is predation. Viral lysis is one source of top-down control of bacterioplankton; indirect evidence for this can be seen in the levels of DOC measured (Figure 3d). In this study the peak in DOC on T9 can be interpreted as having occurred due to viral lysis of bacteria reducing the uptake of carbon and releasing DOC into the system (Middleboe and Lyck, 2002). As viruses are described as specifically targeting the most abundant bacteria present in the system, the 'kill-the-winner' hypothesis (Fuhrman, 2009; Middleboe et al., 2001), this could explain a decrease in bacterial abundance without a complete removal



of bacteria from the system. This is also supported in the temporal changes in community composition between days T0 and T13 (Figure 4). However, as the DOC and abundance levels were impacted for only a single day, this factor cannot be correlated to the change in abundance over the entire experiment.

Competition for resources can also drive abundance numbers. A source of competition occurs between bacteria and diatoms in the increase in silicate uptake under stressful conditions (Havskum et al., 2003). The disruption of the microbes during the filling of the mesocosms and the subsequent restriction of light could have been a source of stress which contributed to the increase in silica per cell in the diatoms (Shutt, 2013; Taylor 1985). The initial phase of the experiment measured low levels of biogenic silica within the mesocosms (Figure 3c), which increased over time after the mesh was removed, indicating a reduction in stress to the organisms reducing their use of this mineral. A positive correlation coefficient (Table 1) indicates a relationship could exist between bacterial abundance and biogenic silica – an indirect measurement of competition within the mesocosms.

Coupling of bacteria with primary production is an example of a symbiotic process. Bacteria use chemotaxis to find and connect to phytoplankton and/or exudates within their microenvironment (Stocker and Seymour, 2012). During a phytoplankton bloom, chlorophyll a levels increase. The abundance of bacteria (Figure 2) follows the same rise in chlorophyll (Figure 3b) as would be expected in bacterial growth coupling with that of phytoplankton during a bloom. The Control treatment and the High treatment had the same levels of chlorophyll at their peak, while the abundance of bacteria in the High treatment was significantly lower than that of the Control. While the correlation

coefficient indicates a positive association, the availability of dissolved organic matter (DOM) and a phytoplankton bloom did not result in equal cells ml<sup>-1</sup> in both treatments.

Finally, physical and chemical processes such as temperature, UV radiation (UVR) and *p*CO<sub>2</sub> levels are factors that influence the abundance and composition of bacterioplankton. While there is evidence climate change will increase temperature in world oceans (Kirchman et al., 2009) and that UVR exacerbates the effects of *p*CO<sub>2</sub> (Coelho et al., 2013) these were not part of this mesocosm study. What we were able to determine were the daily values of *p*CO<sub>2</sub>. When we overlay the median bacterial abundance in cells ml<sup>-1</sup> with the daily  $\mu$ atm values of *p*CO<sub>2</sub> it is clear the High and Drift treatments – with the initial *p*CO<sub>2</sub> levels well above the Control – were the lowest in abundance (Figure 6). Of the factors influencing the growth and abundance of bacteria measured within this study, *p*CO<sub>2</sub> appears to be the only one which can account for both the High and Drift treatments' bacterial abundance. While the Friday Harbor study results showed definitive reduction in abundance at elevated *p*CO<sub>2</sub> levels, preceding studies have not yielded similar results. Although a strong correlation exists between *p*CO<sub>2</sub> levels and abundance, results indicate that a similar correlation in community composition does not exist. Further research is needed to determine if these are replicable trends.

## **Conclusion**

Bacterioplankton abundance in this mesocosm study was statistically significant among three treatments, with the High and Drift treatments returning results indicating elevated *p*CO<sub>2</sub> (often in excess of 1250  $\mu$ atm) suppressed bacterial abundance. Statistical correlation coefficients showing positive and negative associations of cofactors affecting growth (nitrogen, biogenic silica, chlorophyll a, and DOC), but these cannot be used

alone to determine their impact on bacteria present within the mesocosms. While bacterial abundance was most likely a result of differing  $p\text{CO}_2$  levels, the same cannot be said for the community composition. The community structure changed over time in all mesocosms and in the waters outside of them. There was no correlation of elevated  $p\text{CO}_2$  to species composition among the three treatments. As this is the first mesocosm study to show marked differences among levels of  $p\text{CO}_2$  future research should focus on factors which can exacerbate the effects of  $p\text{CO}_2$  on the microbial community (temperature, UVR and vitamin B<sub>12</sub> as examples) and over a longer time scale to see if the suppression of abundance changes over time. Our results notwithstanding, it cannot be ruled out that nutrient additions to stimulate a bloom could have obscured the impact of  $p\text{CO}_2$  treatments in other studies (Riebesell et al., 2008). This coupled with the species of bacterioplankton well-adapted to 650-700  $\mu\text{atm}$  in our system – often the high treatment in other studies – could have skewed our results. Future research should focus on taking the above conditions into consideration, while still maintaining elevated  $p\text{CO}_2$  levels.

Bacteria play a pivotal role in the microbial food web increasing the efficacy of primary production making nutrients available via decomposition, remineralization, and as food to other organisms within the system (Azam and Malfatti, 2007). The world's oceans are predicted to become more and more acidic as  $p\text{CO}_2$  saturation increases and pH decreases. Understanding bacteria's contributions to the microbial food web and the mechanisms of their interactions as a baseline allows science to better model how bacteria will react to these ever more rapid environmental changes. Our research indicates bacterial abundance will be suppressed under these new conditions. This has the

potential to trigger a negative cascade within the oceanic food webs vital to the future health and stability of our global marine environment.

### **Acknowledgements**

Robert Morris and the Environmental Genomics Lab for providing the equipment, materials and methods to complete my research. James Murray, Evelyn Lessard, Mike Foy, Kelsey Gaessner, Barbara Paul and Amanda Fay for their technical expertise. University of Washington - Friday Harbor Labs, the University of Washington Provost and the Henry and Holly Wendt Endowment in support of Friday Harbor Labs research apprenticeships. For their generous support of researchers and this study, thank you to the Mary Gates Endowment, Educational Foundation of America and National Science Foundation (NSF Grant # DBI 0829486). Thanks also to Aaron Morello and Kathy Kroglund in the Marine Chemistry Lab for going above and beyond in analyzing the DOC/POC samples. Finally, most importantly, I wish to thank my fellow Ocean 492 apprentices for their support and encouragement.

The author declares no conflict of interest

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