The effects of ocean acidification on *Prochlorococcus*

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Abstract

*Prochlorococcus* is the most abundant cyanobacteria in the global ocean, and is a part of the marine microbial loop. Climate change, a stressor, presents many threats to *Prochlorococcus*, two of which are of major concern: increased temperature and increased acidity. Both pH and temperature are not constant and vary in the ocean seasonally, diurnally, and meteorologically. This variation suggests that stress related to interactions with these variables may be complex. This present study examined the effects of lowered pH and increased temperature on *Prochlorococcus* in the short term. Two strains of *Prochlorococcus*, high-light and low-light, were manipulated to experience increased temperature, decreased pH, and a combination of the effects and both strains’ responses was observed. Photosynthetic health significantly differed in the low-light clade when the pH was lowered (p = 0.045). Extracted chlorophyll showed statistical variation in the high-light clade when pH was lowered (p = 0.036), and in the low-light clade in both treatments where pH was lowered and temperature was increased (both p < 0.001). There was no statistical difference when temperature and pH were manipulated at the same time. However, more data is needed to see if these results are replicable and to see how this would affect grazing intensity and community structure.
Introduction

Both CO₂ concentration and temperature are expected to increase directly due to anthropogenic climate change. All organisms are being affected by climate change. However, the marine system also has to adapt to an acidifying ocean. Ocean acidification is the increased concentration of aqueous hydrogen ions as atmospheric CO₂ increases (Joint et al. 2010). Annually, pH has varied less than 0.1 units for the past 25 million years; one model predicts a reduction in surface pH by 0.77 units in the next 300 years (Joint et al. 2010). The acidification of the oceans is occurring at unprecedented rates and the organisms have little time to adapt to the changing abiotic conditions. Seawater that has not been acidified typically falls in a pH range of 8 to 8.3, depending on the location (Doney 2006). Lomas et al. (2012) states that a pH of 7.8 is expected by 2100 and Fu et al. (2007) estimates a 1-7 °C warming of sea surface temperatures due to climate change.

Meanwhile, the system is already experiencing short-term variation consistent with climate change. Sea surface temperature and pH vary across seasons, depths, and regions, usually due to microbial activity (Joint et al. 2010). For example, phytoplankton blooms can cause a rapid reduction in pH, and storms can lower the temperature and pH of the surface water (Joint et al. 2010). Since Prochlorococcus is able to withstand these short-term environmental changes, these suggest that marine organisms may have some adaptations to survive variable pH and temperature. It is believed that Prochlorococcus genome is under selection and contribute to fitness adaptations in a changing environment (Biller et al. 2015). One model predicts that the abundance of Prochlorococcus may increase by at least 25% with climate change, and that the range might expand towards the poles with increasing water temperature (Biller et al. 2015). However, this expansion may not be
experienced by all strains of *Prochlorococcus*. This is dependent on how the cells and the community respond to the new environment and changing conditions in the long-term, as opposed to short-term diurnal or seasonal variations.

*Prochlorococcus* is the smallest and most abundant marine phytoplankton in the ocean (Biller et al. 2015). It is approximately 0.5-0.7 μm in diameter, and it is estimated that the global population is on the order of $10^{27}$ cells (Biller et al. 2015). Its high density and biomass means that it is ecologically important in the microbial loop, generating oxygen and fixing carbon dioxide. It typically divides once a day, giving it an average growth rate of 0.5 to 0.6 day$^{-1}$ (Partensky et al. 1999). This growth rate is dependent on light, nutrient availability, and temperature (Partensky et al. 1999). *Prochlorococcus* represent 50% of the total chlorophyll in the global ocean and fixes about four gigatons of carbon each year (Biller et al. 2015). *Prochlorococcus* is predominantly found in the euphotic zone of oligotrophic ocean waters, within the 40°S to 40°N latitudinal band (Partensky et al. 1999). These cells are able to thrive in this environment because of their relationship with other microbes, as well as a high surface-to-volume ratio to generate efficient absorption of nutrients and light (Biller et al. 2015). There are two main strains of *Prochlorococcus*: high-light and low-light. Each strain has a set of at least six clades, and all function optimally under different environmental conditions (Biller et al. 2015). These differences allow for niche partitioning in the water column. Photosynthesis by *Prochlorococcus* is affected by CO$_2$ concentration in the water, irradiance, and temperature, all of which affect the relative cellular pigment content (Fu et al. 2007).

The two predominant clades of *Prochlorococcus* in the open ocean, VOL4 (also called MIT9312) and VOL7 (also called MED4), were chosen for this experiment. These are a high-
light and low-light strain, respectively, and studying their reactions can predict what may be the major response to environmental perturbations. VOL 4 is found throughout the euphotic zone and is the most abundant Prochlorococcus strain. VOL 7 is found in the upper-middle euphotic zone, usually at higher latitudes, that correspond to its lower optimum growth temperature (Biller et al. 2015).

Changes to pH and surface temperature show that the ocean is becoming more stressed. It is important to know how ecologically important organisms are going to react to these stressors. For this experiment, ocean acidification and sea surface warming were simulated in a laboratory environment to see how the photosynthetic health of two strains of Prochlorococcus fare. The goal of the experiment was to run a fully factorial model, decreasing the pH and increasing the temperature to simulate possible climate change scenarios. The temperature goal for the experiment was to increase the starting temperature of 25°C by 2°C. The pH goal was to lower the starting pH conditions from 8.0 by 0.3 units (Figure 1). The temperature increase was chosen to be from 25°C to 27°C. This decision was based on the findings from Johnson et al. (2006) that showed that this range of temperatures reduced growth rate by at least 50% for both clades. The pH was chosen to simulate a drastic condition outside of the natural pH variation in the open ocean by lowering the pH from around 8.0 to 7.7. The experimental conditions differed from the goal, due to technical difficulties discussed in the methods, with the temperature only being raised 1°C instead of 2°C, and the pH was only lowered by 0.1 units instead of 0.3 units (Figure 2). However, the high temperature still correlated with a growth rate reduction based on data from Johnson et al. (2006), and the change of 0.1 units did fit climate change models’ end date trajectory for 2100 based on data from Lomas et al. (2012).
Materials and Methods

Two test tubes of each clade were inoculated from a mother culture, leading to a total of two replicates per clade. These test tubes were used to start new bottles for each treatment, or to restart a bottle if it crashed during a trial.

Two incubators were used for this experiment. Each was set to a diurnal pattern of light: 14 hours of light to 10 hours of darkness (Figure 3). The first incubator was set to an average water temperature of 25.5 °C across both day and night. The second incubator was set to an average water temperature of about 26.5 °C. This temperature differed from the goal temperatures of 25 °C and 27 °C because the temperature could not be accurately and consistently set in the incubators; during the day, the lights heated the conditions above the settings, and during the night the temperature was lower due to the lights being turned off. While the incubator did not achieve the desired average daily temperature, it does mimic the diurnal temperature change in the surface water.

All experimental treatments were conducted in 500mL bottles with 200-400mL of culture in each, depending on the day between samples and dilution (Figure 3). On some of the days, samples were diluted when the biomass was too large or the volume of the sample was too low; other days the cultures were allowed to grow without the addition of media. The bottles all sat on individual stir plates and each had a stir bar that rotated at about the same speed to prevent the bacteria from settling. Each bottle had a 0.2 micron air filter that was attached to a split-flow valve attached to a carbon filter, which was attached to either the aquarium pump for the normal pH treatment, or the gas mixer for the lowered pH treatment. The two filters were used to achieve the purest gas possible. The carbon
filter was added halfway through the experiment, before any trials were started, because preliminary test bottles crashed unexpectedly, possibly from a contaminant in the air. A schematic of the experimental setup is shown in Figure 3.

The experiment had two modes of data collection: a daily collection and a periodic sample collection for analysis. The daily collection consisted of removing 20mL of culture from each bottle to measure fluorescence using a Turner 10-AU fluorometer. This measurement was used as a proxy for biomass in this experiment. The daily fluorescence was also used to calculate growth rate of each culture, which was used as an indicator of when to collect the periodic samples. Once the biomass was between 10 and 15 µg/L, the cultures were harvested, which was the periodic component of data collection. Harvesting included flow cytometry, pH, extracted chlorophyll, and FIRe (Fluorescence Induction and Relaxation system). If the cultures were too concentrated, they were diluted with media and left to grow for twenty-four hours before harvesting. If, after twenty-four hours, the biomass was appropriate, the culture was harvested.

The analyses were used to look at various factors of photosynthetic health of each culture. Growth rate was calculated using the following equation:

\[
growth\ rate = \frac{\ln\left(\frac{F_v}{F_m}\right)}{time}.
\]

FIRe was used as a measure of photochemical conversion efficiency, comparing the variable fluorescence and maximal fluorescence yield of a sample, denoted \( F_v/F_m \), where lower values indicate greater photosynthetic efficiency (Johnson 2004). Extracted chlorophyll measured the exact concentration of chlorophyll in the bottle when it was harvested in µg/L. Flow cytometry measured concentration of each sample in terms of the number of cells. pH was measured three times per sample and averaged to determine the
exact pH of each sample for each trial. All of the protocols for the analysis were from Dr. Johnson's laboratory (Johnson 2013b; Johnson 2013a; Johnson 2013c; Johnson 2013d).

Differences in response variables among the treatments were compared with two-way analysis of variance and Student’s t-test.

Results

The comparison data for pH experimental conditions show that while there was a significant difference between control and low pH for both VOL 4 and VOL 7, there was not a significant difference between the other treatments in both VOL 4 and VOL 7 (Figure 4).

The data from VOL 4 could be analyzed as fully factorial. Cell count of high-light clade VOL 4 did not vary significantly between normal and low pH treatments (ANOVA, $F_{24,15} = 0.454; p = 0.888$; Figure 5). Cell count of VOL 4 also did not vary significantly between normal and high temperature treatments (ANOVA, $F_{24,1} = 2.012; p = 0.194$; Figure 5). Chlorophyll concentration of high-light clade VOL 4 was significantly lower in low pH treatments (ANOVA, $F_{24,11} = 3.739; p = 0.036$; Figure 6). However, chlorophyll concentration of VOL 4 was not significantly affected by the increase in temperature (ANOVA, $F_{24,1} = 0.132; p = 0.726$; Figure 6). There was no significant interaction between temperature and pH for chlorophyll concentration (ANOVA, $F_{24,3} = 0.536; p = 0.670$; Figure 6). Photosynthetic health of VOL 4 did not vary significantly between normal and low pH treatments (ANOVA, $F_{14,10} = 85.962; p = 0.084$; Figure 7). Photosynthetic health of VOL 4 did not vary significantly between normal and high temperature treatments (ANOVA, $F_{14,1} = 8.464; p = 0.211$; Figure 7).
Despite the goal of running a fully factorial experiment for both strains of cyanobacteria, VOL 7 could not be grown at a high temperature and low pH, due to too low growth rates. Cell count of low-light clade VOL 7 did not vary significantly between normal and low pH treatments (ANOVA, $F_{18,9} = 1.320; p = 0.365$; Figure 5). Cell count of VOL 7 also did not vary significantly between normal and high temperature treatments (ANOVA, $F_{18,1} = 2.129; p = 0.188$; Figure 5). Chlorophyll concentration of VOL 7 did vary significantly between normal and low pH treatments (ANOVA, $F_{18,9} = 110.675; p < 0.001$; Figure 6). Chlorophyll concentration of VOL 7 also varied significantly between normal and high temperature treatments (ANOVA, $F_{18,1} = 80.406; p < 0.001$; Figure 6). Photosynthetic health of VOL 7 also varied significantly between normal and low pH treatments (t-test, d.f. = 1; $p = 0.045$; Figure 7). However, photosynthetic health of VOL 7 did not vary significantly between normal and high temperature treatments (t-test, d.f. = 1; $p = 0.398$; Figure 7).

**Discussion and Further Research**

The aim of this experiment was to see how two clades of *Prochlorococcus* respond to simulated ocean acidification. While the entire experiment could not be run for VOL 7 due to insufficient growth, a fully factorial experiment could be performed for VOL 4. For VOL 4, the only significant effect on photosynthetic health or growth was lower chlorophyll concentration in low pH treatment (Figure 6). All other measures were similar across treatments, possibly indicating that VOL 4 was able to cope with the stressful experimental conditions. The data that could be run for VOL 7 showed that the significant effects on photosynthetic health or growth was chlorophyll concentration for both low pH and high temperature treatments, and $F_v/F_m$ for the lower pH treatment (Figures 6 and 7).
This data suggests that VOL 7 seemed to struggle to grow significantly when only one of the environmental conditions was manipulated. This struggle to grow culminated in an inability to grow in the double-stress environment. This could suggest that VOL 7 will not be able to survive in the changing ocean. Both strains grew successfully in control conditions. Prochlorococcus does experience changes in pH seasonally, diurnally, and meteorologically (Joint et al. 2010), but the constant exposure to the stressor or whether the stressor was outside of the Prochlorococcus range of tolerability could have caused the differences observed in this study. If the stressor was outside the range of tolerability for Prochlorococcus, this could mean a cascade of effects in the global ocean when these conditions occur.

There were many difficulties growing VOL 7 at a high temperature—which was expected from the data from Johnson et al. (2006). One of the main difficulties was that the salinity of the high temperature bottles was much higher than the normal range, and when the repetition of the trial was attempted while monitoring salinity, the cultures did not take and would not grow. If this experiment were to be run again, attempting to grow VOL 7 at a high temperature with low pH would be an area of focus to complete the fully factorial experiment and analysis for VOL 7.

Even though the pH and temperature in both strains was not changed as drastically as intended (Figure 1 and Figure 2), a drop of 0.1 units in pH and an increase of 1°C still provide valuable information to assess how these changes affect Prochlorococcus. Both VOL 4 and VOL 7 showed a significant change in extracted chlorophyll concentration when pH was lowered. However, these results differed from Fu et al. (2007). This experiment was done with larger bottles of cyanobacteria and different experimental parameters and tests
were performed, so a complete comparison cannot be drawn. There was no difference in experimental tests of *Prochlorococcus*, comparing the acidic, high temperature, and both acidic and high temperature treatments to the control.

A lower value for Fv/Fm, the measure of photosynthetic health, indicates greater photosynthetic efficiency. Fv/Fm only showed statistically significant differences for VOL 7 when the pH was lowered. This means that the lowered pH experimental condition did have an effect on photosynthetic health of VOL 7. However, this effect was positive—as the environmental conditions became more stressful, photosynthetic health increased. VOL 4 in all of the treatments and VOL 7 high temperature treatment also showed this positive effect as well. However there was not enough of a difference across the treatments to be statistically significant. This increased efficiency could be explained by an increase in RUBISCO-enzymatic efficiency; as temperature was increased and pH was lowered, RUBISCO may have been able to function more efficiently, similar to when it was first evolved in a high CO₂ environment (Fu et al. 2007).

The pattern of Fv/Fm differs from the data for extracted chlorophyll of both VOL 4 and VOL 7 across the experimental treatments. For VOL 7, extracted chlorophyll was significantly decreased across the experimental treatments (Figure 6). However, VOL 4 showed that extracted chlorophyll decreased with lowered pH, but increased with increased temperature (Figure 6). The trend in VOL 4 is also seen in cell count (Figure 5). This trend is not observed in cell count of VOL 7 across the experimental treatments (Figure 5). This correlation in extracted chlorophyll and cell count in the VOL 4 strain could be because each cell had the same concentration of chlorophyll, and there was a difference in the total number of cells. For VOL 7, this disjuncture between an increase in cell count
and decrease in extracted chlorophyll concentration could be because the cells were more efficient, as seen in the data of $F_v/F_m$ in Figure 7, and did not need as much chlorophyll.

If ocean acidification does not negatively affect photosynthetic health of *Prochlorococcus*, this could be a silver lining for the health of the oceans. *Prochlorococcus* is part of the microbial loop; if it is able to sustain its rates of growth despite perturbations from ocean acidification, then there may not be a ripple effect higher in the food web from a lack of food to higher trophic levels. This might help alleviate some stress that higher trophic levels will have to face with ocean acidification, as it has been shown that higher trophic levels are more susceptible to climactic stress (Voigt et al. 2003). A study by Lomas et al. (2012) looked at ocean acidification in the open ocean and its effect on producers and grazers. This study found that while there were small changes with environmental conditions, as long as the picoplankton community did not change much, grazers were able to control the population.

However, this is not the end of the research. Because cyanobacteria are sensitive to environmental conditions, this experiment needs to be repeated, in both a controlled laboratory setting and in an uncontrolled setting like the Lomas et al. (2012) study. This replication would show if the results are an isolated event, or if there is a pattern of survival. It is also necessary to figure out why *Prochlorococcus* may not be as susceptible to the harmful effects of ocean acidification as other marine phytoplankton. Another important note is that the two strains studied, VOL 4 and VOL 7, behave differently and have different necessary environmental conditions. Their behavior to these environmental changes may not be true for all strains within the high and low-light clades. It is also important to note that changing pH and temperature will also have effects on the water
column chemistry that will be difficult to study in a laboratory setting, such as altering nutrient flux and mean light levels (Joint et al. 2010). This research, in its current state, only looked at the first factor in a small, isolated system. However, to understand the whole picture of what could possibly happen, all of these factors would have to be controlled and observed as well as including other microbes and grazers to see how interactions change.

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References


https://wiki.duke.edu/display/ZIJLAB/Flow+Cytometry++Fix+and+Freeze


https://wiki.duke.edu/pages/viewpage.action?pageId=93979717


**Appendix**

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<th>Lower pH and Higher Temp</th>
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Figure 1: A visual representation of the goal of the experimental treatments.

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<th>Higher Temp</th>
<th>Lower pH and Higher Temp</th>
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Figure 2: The actual experimental conditions of this research. The temperature was not as high as the goal, nor was the pH low enough and consistent across manipulations.
Figure 3: A schematic drawing of the experimental setup inside the incubator.
Figure 4: pH data (means ± sd; n = 6) for VOL 4 and VOL 7. These differences are significant for comparing experimental treatments to the control for VOL 4 and VOL 7.
Figure 5: Cell counts (means ± sd; n = 6) for VOL 4 and VOL 7. These differences are not statistically significant for VOL 4 or VOL 7 across the treatments.
Figure 6: Chlorophyll concentration (means ± sd; n = 6) for VOL 4 and VOL 7. These differences are statistically significant for the low pH treatment for VOL 4, and both experimental treatments for VOL 7.
Figure 7: \( F_v/F_m \) (means ± sd; \( n = 6 \)) for VOL 4 and VOL 7. These differences are statistically significant for the high temperature treatment for VOL 4, and for the low pH treatment for VOL 7.