Ocean Acidification and its effect on Phytoplankton growth

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Abstract

The advent of the Industrial Revolution in the late 18th–early 19th century has slowly increased the concentration of CO$_2$ in the atmosphere. However, with the fossil fuel industry, CO$_2$ emissions increased exponentially and the concentration has reached its highest in 2016. The implications of increased CO$_2$ results in ocean acidification which causes a decrease in the pH. Phytoplankton community assemblages have become affected by this unexpected, accelerated increase and have begun to change as global warming occurs. The phytoplankton community upon which this chemostatic experiment will be using comes from the North Atlantic Ocean which feeds into the body of water surrounding Woods Hole, MA. The experiment will be inducing elevated temperatures of 30°C and ambient temperatures 15°C, alongside elevated CO$_2$ concentrations of 2000 µatm and ambient CO$_2$ concentrations of 200 µatm. The analyses that will be investigating the experiment are Chl a, CO$_2$ production, nutrient colorimetric analysis and microscopy. Resulting evidence shows that Chl a concentration decreased with elevated CO$_2$ concentrations and increased CO$_2$ concentrations. Temperature had no effect on the either Chl a, CO$_2$ production, nutrient colorimetric analysis and microscopy analyses. Nutrients were depleted in all treatments and there were significant differences between elevated and ambient CO$_2$ conditions. Future research would further extend the time period of this research to determine how phytoplankton community assemblage changes with time.

Key Words: ocean acidification, global warming, phytoplankton, growth, community assemblage, carbon dioxide

Introduction

Global warming is a completely natural phenomenon that is caused by the greenhouse effect. The greenhouse effect is the process by which gases, like CO$_2$ and CH$_4$, trap the heat coming into the Earth’s atmosphere from ultraviolet rays warming the Earth$^1$. Global warming is an Increase in temperature on Earth since the establishment of the Industrial Revolution in the early 20th century. It has increased exponentially since the 1970s due to fossil fuel emissions. As of September 2016, the current CO$_2$ concentration is 400ppm and this is the highest that CO$_2$ concentration has been. It’s never reached this high and it is continuing to increase as time progresses (Kahn and Central 2016). With fossil fuels emissions increasing, the worldwide average surface temperature has increased by 0.8 degrees Celsius$^2$. With the advent of Global warming, surface waters have been more susceptible to acidification with increasing carbon dioxide concentrations in the atmosphere. Anthropogenic CO$_2$ from the burning of fossil fuels
has increased the uptake of the greenhouse gas, causing the pH to drop from 8.2 to 8.1 (Chu 2015)

Ocean acidification has many detrimental effects to the marine life in oceans and so the consequences will only continue to occur unless CO₂ concentration decreases. The increased acidification causes decreased pH of the surface water of oceans, coral bleaching, reduced calcification or dissolution of calcifying of shell organisms and decreased productivity. It may also cause changes in the species abundance and diversity of phytoplankton (Fabry et al. 2008). Migration is also another factor of change in which scientists have seen the movement of phytoplankton to the poles (Chu 2015). The uptake of Fe (iron) in phytoplankton, such as diatoms and Coccolithophores, decreases with increasing acidification (Shi et al. 2010)

As to why this is important, phytoplankton are at the base of most food chains in aquatic systems. I want to know how phytoplankton are adapting, if they are, to changing global conditions. Global ocean acidification may change how food chains function and the cycling of nutrients in a food web may be disrupted if the productivity of phytoplankton changes. Some species of phytoplankton also cannot survive in acidic and warm environments. So, the diversity of phytoplankton may decrease and one dominant species may take over and adapt to changing conditions? The implications of decreased phytoplankton activity can cause detrimental effects on the ecosystem and the food chain. The competition for resources may increase and more dependent on the phytoplankton population.

In this experiment, I will be examining the effect of four different treatments on the phytoplankton in the water at Woods Hole, Massachusetts. These different treatments consist of two variables, temperature and carbon dioxide concentration. I will be looking at the synergistic effect of these two variables in the four treatments as: (Temperature control, CO₂ control),
(Temperature elevated, CO₂ control), (Temperature control, CO₂ elevated) and (Temperature elevated, CO₂ elevated). (Figure 1).

I will be investigating how species abundance and composition of phytoplankton, such as diatoms, and the biological effects, such as change in growth, of increased temperature and CO₂ in the samples change with time. Along with species abundance and other effects, I measured CO₂ uptake by the phytoplankton in the water throughout the day to generate a value for respiration which can be used to calculate net daytime production.

The main questions I hope will be answered is how will the synergistic effect of a multi-stressor, elevated temperatures and increased CO₂ concentrations, have on the productivity and population of phytoplankton. I am especially looking for a decrease in the phytoplankton productivity, decrease in species diversity, and a definite change in species abundance.

Methods

I conducted a pulse Chemostat experiment in order to induce the varied CO₂ concentrations and temperature. A Chemostat is a closed system in which medium that is taken out is added at the same time so as to keep the system at a constant volume. To imitate a Chemostat, 2L Erlenmeyer filter flasks were used. Each treatment was run with duplicates. To begin, I collected unfiltered seawater from Woods Hole, MA in 2L bottles. The seawater was augmented with nutrients in a concentrated solution in which, 26 mM of Na₂SiO₃·9H₂O, 18 mM KNO₃ and 1.2 mM K₂HPO₄. The nutrients were being added at 1 mL/litre of seawater. Fresh media was added at a flow rate of 200 mL/d, in which the dilution becomes 200 (mL/d) / 1000 mL or 0.2 l/d. This is a low
dilution rate in which the system took five days to turnover. An aerator will be adding carbon
dioxide to induce high (2000 µatm) or constant concentration (200µatm), and a magnetic stirrer
will be used keep the flow of water interacting with air aerobic. A water bath will be used to
cause changes in water temperature, at both ambient temperatures, (15C) and at high
temperatures (30C). In order to determine the growth of the phytoplankton in the system, I
sampled the chlorophyll a productivity following the method used in lab. To extract the
chlorophyll, seawater from each treatment and its duplicate were filtered through 25mm GF/F
filters, which were then frozen in aluminium foil for later analysis. The chlorophyll was further
extracted by putting each filter in a 50 mL Falcon tube and then adding 35 mL of 90% acetone.
A Lambda Bio 20 UV/VIS Spectrometer measured the absorbance of chlorophyll a at two
different wavelengths, 665 nm and 750 nm.

The nutrients, NO$_3^-$, NH$_4^+$ and PO$_4^{3-}$, were analysed using colormetric analysis. In 20 mL
scintillation vials, 15 mL of filtered seawater was added for each treatment and its duplicate for
each nutrient. A LACHAT (QuickChem Flow Injection Analyzer) was used to measure nitrate
concentration. To measure ammonium, the phenol-hypochlorite method was modified and then
analysed using a Cary UV Visible Spectrometer, while phosphate was measured by modifying
the method by Murphy and Riley (1962) using a UV-VIS Spectrometer.

To assess uptake of CO$_2$ and thereby net daytime production of the phytoplankton, a Li-
Cor 6200 console was used to bubble air into the flasks, changing the environment in which the
phytoplankton are living to force changes in the state of dissolved CO$_2$ in the water.

In order to assess the changes in the phytoplankton composition between each treatment, a
snapshot of the population was observed in which a sample from the most recent collection was
preserved in 25% glutaraldehyde, of which 1.0 mL was added to 9.0 mL of sample, from each
treatment and its duplicate, in a 20 mL scintillation vial. DAPI (4',6-diamino-2-phenylindole dihydrochloride) staining, using a working solution of DAPI (200µg/mL), was added to 1.0 mL of the preserved sample. It was 50µL of DAPI working solution for each milliliter of preserved seawater. A times series, a period of six days, will be created to track changes in each test so as to determine how phytoplankton react to increased acidification and warming of the water.

Results

Nutrient analyses were conducted to determine the concentrations of vital nutrients for phytoplankton growth and productivity. The concentration of nitrate in each treatment showed negative concentrations, meaning that the nutrient became depleted over the course of the experiment (Figure 1). Along with nitrate, both ammonium (Figure 2) and phosphate (Figure 3) concentration were depleted to their minimum detection levels. Figure 4, which details the concentration of chlorophyll A over a time period of 6 days, shows that there are no significant differences between the ambient and elevated temperature treatments. However, there is a significant decrease in chlorophyll a concentration between elevated CO₂ and ambient CO₂ conditions.

Net daytime production showed that there was no difference between ambient CO₂ and elevated CO₂ conditions. Elevated temperature shows that there are no significant differences either. However, when combining the multi-stressor, elevated temperature and elevated CO₂, there is a huge drawdown of CO₂ uptake by the phytoplankton, leading to low production (Figure 5). Microscopy shows that there is no difference between ambient conditions and elevated temperature conditions (Figures 6; Figures 7). However, there are major differences in Figures 8 and Figures 9, which shows that the elevated CO₂ conditions have larger, more prominent
diatoms and phytoplankton. However, though the elevated temperature and CO$_2$ treatment has large pill-like diatoms, as seen in Figures 9, the diatoms are surrounded by red-stained chlorophyll which really dominates the treatment.

Discussion

Ocean acidification increases the amount of dissolved CO$_2$ in the water. When combined with water, it becomes a very weak acid, HCO$_3^-$ . With this decrease in pH, or rather an increase in acidity, there is the dissolution of carbonate and bicarbonate ions, causing the reduction of calcification of shell organisms. Another consequence includes the decreased productivity of organisms such as phytoplankton (CITE) and coral bleaching, as the zooxanthellae of the coral leaves its host because of the increased acidity. To better understand how ocean acidification and increased temperatures are affecting the growth and population of phytoplankton in the ocean, a modelled, chemostatic system was implemented to induce the various conditions which would simulate a global warming crisis.

With an increase in CO$_2$ concentration the pH of the seawater may have dropped. Though not measured, the pH may have decreased from 8.1 units to 7.0 units with the increase in CO$_2$ concentrations. However, because it wasn’t measured, it is unsure as to how many units the pH dropped. However, the acidic conditions caused by increased CO$_2$ appears to have decreased chlorophyll a concentration. Chlorophyll a is used in photosynthesis and the amount of chlorophyll in each treatment and their samples, indicate how much the system has produced over the time period. There is no significant difference when the temperature in the system becomes elevated, but there is a significant difference when carbon dioxide concentration is
increased. This could be that phytoplankton are able to maintain their structure in warmer temperatures, but with the increase in CO$_2$, the acidic conditions disrupt the biological-carbon pump that drives photosynthesis (Collins et al. 2014).

Phytoplankton need nutrients in order to grow, produce and cycle through the food web. When augmenting the unfiltered seawater with nutrients, the vital nutrients, in addition to silica, which phytoplankton, such as diatoms and Coccolithophores need, are added to provide a resource. As colormetric analysis was used to determine the use of nutrients by the phytoplankton, it is seen that, although nutrients are added in a very concentrated solution, the nutrients are consumed to their minimum detection level. The nutrients tested for, NO$_3^-$, NH$_4^+$ and PO$_4^{3-}$, all became depleted, however, there is evidence that silica may have been used when observing the changes in the community composition between treatments using microscopy. There are no significant differences between the ambient condition and when the temperature is perturbed, rather there is less filamentous cyanobacteria. The filamentous cyanobacteria may not be able to survive higher temperatures, hence, the reason for a decreased presence. However, the pennate diatoms are still present in all treatments, suggesting that they are able to survive most of the simulated conditions. Pennate diatoms, also known as *Bacillariophyceae*, are diatoms with bilateral symmetry, forming along a line, rather than a point such as radial diatoms. (CITE)

Along with pennate diatoms that persisted in all treatment conditions, larger diatoms survived in the elevated CO$_2$ conditions and this may be as a result of the addition of silica into the closed system every day. The reason for the persistence of diatoms in elevated CO$_2$ conditions is that they use silica, which is added, rather than carbonate or bicarbonate, compounds which dissolve in high acidic conditions. However, not present in any treatment is Coccolithophores. Coccolithophores are calcifiers and so with increased CO$_2$ conditions and
warmer temperatures, the ability of the phytoplankton to exist becomes harder and they disappear and are degraded in the model system. The presence of larger diatoms, such as the chain forming diatom in Figure 8, and the large pill-like diatom surrounded by red-stained chlorophyll in the multi-stressor treatment (Figure 9) suggests that there may be the presence of larger plankton, such as flagellates and ciliates, which are grazers, that prey on smaller phytoplankton. Hence, reducing the number and presence of smaller phytoplankton in the elevated CO₂ treatments.

In regards to future research, it is possible that conditions can be simulated and maintained and will have major implications on aquatic systems. If global warming were to continue to accelerate, then it is possible that the induced conditions and the results evident in this pulse Chemostat model system could greatly affect the most diverse and most abundant food resource in aquatic systems. Food webs may change and phytoplankton may begin to disappear from food chains. Implementing researching on combatting this outcome would greatly benefit coastal ecosystems productivity as they are more dynamic systems than open ocean systems.
Figures

Figure 1. Nitrate concentration over time. Nitrate was undetectable throughout every treatment, with each value measured being negative.

Figure 2. Phosphate concentration over time. Phosphate concentration was detected at the minimum level of detection by the spectrometer, with all treatments showing a depleted phosphate system.

Figure 3. Ammonium concentration over time. The concentration was detected at the minimum level of detection by the spectrometer, with all treatments showing a depleted system.

Figure 4. Chlorophyll A concentration over a period of 6 days. Solid lines represent ambient CO₂ conditions and the dotted lines represent elevated CO₂ conditions. Ambient carbon dioxide conditions saw no significant difference and had higher production, while elevated carbon dioxide had lower production and saw significant difference between ambient and elevated CO₂ treatments.

Figure 5. Net Daily Production of each treatment. The uptake of CO₂ for production was measured and shows no correlation with ambient CO₂ or elevated temperature conditions. However, when combining the stressors, production greatly decreases as compared to the other treatments.

Figure 6. Ambient treatment pictured under microscope to visualise community assemblage. Qualitative analysis was done to observe differences among treatments and in between duplicates. There are more filamentous cyanobacteria or green algae, along with pennate diatoms observed in this treatment.

Figure 7. Elevated temperature treatment pictured under microscope to visualise community assemblage. Qualitative analysis was done to observe differences among treatments and in between duplicates. There are less filamentous bacteria observed, but there is no significant difference between this treatment and the ambient treatment.

Figure 8. Elevated CO₂ treatment pictured under microscope to visualise community assemblage. Qualitative analysis was done to observe differences among treatments and in between duplicates. This treatment differs more in that mostly observed was chain-forming diatoms.

Figure 9. Elevated temperature and elevated CO₂ treatment pictured under microscope
visualise community assemblage. Qualitative analysis was done to observe differences among treatments and in between duplicates. This treatment differs more in that mostly observed was pill-like diatoms surrounded by red-stained chlorophyll. Picture taken at 10x was magnified to 60x.

Literature Cited

3. https://westerndiatoms.colorado.edu/about/what_are_diatoms


Appendix

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