

Phytoplankton Community and Abundance in High CO₂ Conditions

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Non-Technical Summary

Effects of increased concentrations of carbon dioxide on marine microorganisms were researched in Friday Harbor, Washington in April 2013 through a mesocosm experiment. Mesocosm experiments bridge the gap between the natural environment and controlled experiments by creating an observational field study that mimics the natural environment. Phytoplankton, or photosynthetic microscopic organisms, are the primary producers of energy in marine environments and therefore, play an integral part of the food web. Controlled carbon dioxide levels in the mesocosms allowed researchers to analyze the effects of high carbon dioxide conditions on the phytoplankton by examining their cell densities and genus composition. No significant difference was found between differing carbon dioxide treatments on the cell density and genus composition over time. Major changes in cell density and genus composition were seen across all treatments due to natural ecosystem variations.

Abstract

Deleterious effects of anthropogenic carbon dioxide are not yet fully understood at ecosystem or food web level. A large volume mesocosm experiment conducted in Friday Harbor, Washington aimed to research the effects of increased pCO₂ levels (650 ppm maintained, 1250 ppm maintained, and 1250 ppm drift) on phytoplankton communities to better understand the future ocean acidifications conditions. Research was focused on effects of pCO₂ treatments on phytoplankton densities, genera composition, and correlations between dominant genera and pCO₂ treatments. Phytoplankton densities were analyzed for three treatments of pCO₂ over a 22-day experiment. No significant difference was found in phytoplankton densities among

treatments or genus composition due to variability within treatments. However, it was found that a distinct phytoplankton community shift occurred in all treatments during the experiment due to natural variations in the ecosystem.

Introduction

The effects of anthropogenic carbon dioxide on marine environments and community dynamics have become a growing interest and focus of ocean acidification research. Before the industrial revolution began, densities of atmospheric CO₂ varied naturally from glacial 180 μatm to interglacial 280 μatm (Engel et al., 2008). Today's atmospheric CO₂ recently surpassed the 400ppm milestone in Mauna Loa, Hawaii (Ewald 2013). The ocean has taken up about 30% of the anthropogenic CO₂ produced and this has led to a measurable decrease in pH of 0.1 (Riebesell et al., 2008). The absorption of atmospheric CO₂ has reduced the greenhouse gas effect and global warming to date but deleterious effects are starting to be seen in our marine environments (Feely et al., 2009). A decrease of 0.1 on the pH scale doesn't seem like a large amount but considering that pH is measured on a logarithmic scale, this means that the concentration of H⁺ is already about 30% larger. As CO₂ is projected to continue to increase the changes in future pH could be enormous (Feely 2009). CO₂ enters seawater by gas exchange and it then reacts to form carbonic acid (H₂CO₃), which then reacts further to form a bicarbonate ion (HCO₃⁻) and hydrogen atom (H⁺) (Feely 2009). The increase in H⁺ causes carbonate ions (CO₃²⁻) to react with H⁺ and to form more bicarbonate (Feely 2009). In summary, addition of CO₂ to seawater leads to an increase in H⁺, carbonic acid, and bicarbonate, which results in a decrease in pH (increase in acidity), and a decrease in concentrations of carbonate ion. A decrease in carbonate ions can lead to dissolution of carbonate minerals

in order to buffer the pH (Feely 2009). Effects of carbon dioxide on marine organisms that use calcium to survive have been thoroughly researched using oysters (Kurihara et al., 2007) and coccolithophores (Riebesell et al., 2000), but there is a little data on how ocean acidification will affect food webs, microbial communities and carbon cycling.

Background

Large volume mesocosm experiments are one important way to achieve a better understanding of how the phytoplankton community changes with time during a bloom and how they respond to high CO₂ conditions. Such experiments give clues about possible future changes in the biological communities in our oceans. Photosynthetic plankton can be limited by light and nutrients such as nitrogen and phosphorus (Fenchell 1988). The seawater around San Juan Island is rich in nutrients (NO₃ = 25 μM) and CO₂ (pCO₂ = 650 μatm) and low in pH (pH = 7.8), so nutrients are not generally limiting to plankton. This is a natural ocean acidification condition due to upwelling of NO₃ and CO₂ rich water on the Washington coast. This water is low in chlorophyll. Open ocean areas that are high in nitrate and low in chlorophyll are referred to as HNLC ocean regions (Fenchell 1988). They characteristically have food-webs dominated by small phytoplankton and small grazers (Fenchell 1988). Seasonal variability in nutrients, mixing layer depth and light availability also drive population variability, which in turn affects the food web and growth rates (Fenchell 1988). Oscillations of populations of phytoplankton and zooplankton communities can follow predator-prey cycles, which has reflected a trend that more productive communities have larger oscillations (Fenchell 1988). Phytoplankton generally follow a succession during a bloom (Merico et al., 2004). From previous studies, the dominant phytoplankton group at the start of a bloom was

diatoms, followed by flagellates and dinoflagellates (Merico et al., 2004). In terms of the carbon cycle, diatoms are known best as a sink of carbon from the euphotic zone. This is because their large, dense silica walls act as ballast to enhance sinking to depth after the diatoms are grazed. This makes them important in research concerning carbon and ocean acidification (Horner 2002). Shifts in communities could mean changes for entire ecosystems and the nature of the ocean carbon sink.

The initial phytoplankton community in Friday Harbor differed from previous mesocosm experiments from Korea (Kim et al., 2006) and Norway (Engel et al., 2008) due to different naturally occurring communities and coastal conditions. The surrounding water of the San Juan Islands is a naturally occurring upwelling zone, which causes CO₂ and nutrient rich waters from in the deep ocean to upwell off our coast. This upwelling system provides an environment that resembles predicted water chemistry and pH of “ocean acidification” conditions. The Korean mesocosm experiments found a shift from microflagellates to skeletonema diatoms (Kim et al., 2006) while the Norway experiments frequently have a bloom of *E. huxleyi* (Engel et al., 2008), a genus not found near the San Juan Island. One Norway mesocosm experiment saw a community shift from diatoms to coccolithophores to dinoflagellates and cyanobacteria through chlorophyll a analysis over 24 days (Riebesell et al., 2007). These mesocosm experiments provided background information on conducting experimental mesocosm studies and previous methods of analyzing phytoplankton communities. The main goal of my research was to determine how the population and composition of phytoplankton changed during a 22-day mesocosm experiment that included different treatments of CO₂.

Sampling and Analytical Methods

Mesocosms

The design of the mesocosm experiment consisted of nine 3500-liter polyethylene bags placed in a mesh bags to reduce the light by more than 55%. After successfully reducing the light for half of the experiment, the mesh was removed on T10 to allow the biology to grow without light as a limiting factor for bloom data. The experiment was broken into two phases, phase one being T0 to T10 when the mesh bags were on the mesocosms, and T12 to T20 when the mesh bags were lowered and the mesh caps were removed. Each bag was six meters long, one meter above water level and five meters submerged, with a mesh cap to cover the top from direct sunlight and a clear acrylic dome to prevent contamination. The mesh-covered bags were secured to floating cylindrical cages and secured to a portable floating dock. The portable floating dock was attached to the Friday Harbor Laboratories' main dock, facing the inlet and oriented towards the East to provide equal sunlight to each mesocosm. Seawater that filled the bags was collected from 2 meters off the main dock, and filtered with a 500 μ m filter to keep larger zooplankton and jellyfish from entering the mesocosms. The seawater was pumped into a large 1500L reservoir on the main dock with an air compressor powered Teflon diaphragm pump to reduce damaging biology. Each mesocosm was filled in situ with water from the reservoir at 1L per minute. The large reservoir reduced the initial variability between bags by simultaneously filling them through nine tubes that ran from the reservoir to each mesocosm. To maintain the reservoir level, the bags were filled constantly for about 50 hours. Once the mesocosms were filled, 15 L of a brine solution of 3500g NaCl was added each bag to increase the salinity and make them denser than

the surrounding water. The increased density increased the stability of the bags in comparison to the surrounding water. A homemade “spider” apparatus consisted of a 1 ft PVC pipe with staggering holes drilled throughout the pipe. Four-inch long plastic tubes were glued on to the drilled holes. The PVC pipe was screwed onto a tube leading to buckets filled with the brine solution. The “spider” apparatus was used to gently disperse brine solution evenly through out the bags by pumping water into the PVC pipe and out through the smaller tubes glued to the drilled holes. The change in salinity of the bags allowed us to calculate the volume of seawater in the mesocosms. There were triplicate mesocosms with three treatments pCO₂ levels: control (650ppm), drift (1250ppm) and high (1250 ppm). Bags were numbered from 1 to 9, with bags one, four and seven as the control, two, five, and eight were the high treatments and three, six, and nine were the drift treatments. Staggering the treatments reduced environmental variance among the treatments. The control mesocosms maintained an ambient CO₂ level of 650 ppm through CO₂ additions as needed in the afternoon after sampling. The drift mesocosms CO₂ level was raised to 1250 ppm initially but no CO₂ saturated water was added for the rest of the experiment. The high treatment group maintained a CO₂ level of 1250 ppm through CO₂ additions as needed in the afternoon after sampling. CO₂ was added in the form of 0.2 μm CO₂ saturated water that was generated and maintained on the portable dock and added to the mesocosms with a peristaltic pump. The “spider” apparatus used to increase salinity was also used for even dispersal of CO₂ saturated water. Every morning, light was measured with a Li-Cor light meter, with the mesh caps still on, and mesocosms were then mixed with a circular slice of Plexiglas tethered to a rope that was placed in each mesocosm bag three times. A Sea-Bird Model 37SMP CTD, that measured the density

(kg/m³), depth (m), salinity and temperature (°C), integrated sampler, and a Niskin bottle submerged 2 meters provided the water samples and data.

Phytoplankton

Phytoplankton samples were taken daily using an integrated sampler. The integrated sampler consisted of a three-meter tube with a one-way valve on one end. The tube was lowered with the one-way valve end first, allowing seawater to flow into the tube. The tube was capped at the other end and pulled out, with the water sample inside the tubing. The water in the sampler was poured gently into a three-liter jug and distributed to researchers as needed. To sample for phytoplankton, one 50ml centrifuge test tube per mesocosm was labeled and filled with 50ml of water from the three-liter jug of water retrieved from the integrated sampler. In the lab, 1ml of acid Lugol's solution was added to each test tube and the tubes were inverted to mix. Acid Lugol's solution was made with 1 L deionized water, 50g iodine, 100g potassium iodide, and 100 mL acetic acid. Fixed samples were then transferred to homemade settling chambers and left in a four degree Celsius fridge for 48 hours. To create the settling chambers, holes were drilled in the tip of 50ml centrifuge test tubes and attached with epoxy to a drilled cap from a 2ml centrifuge tube as shown below.

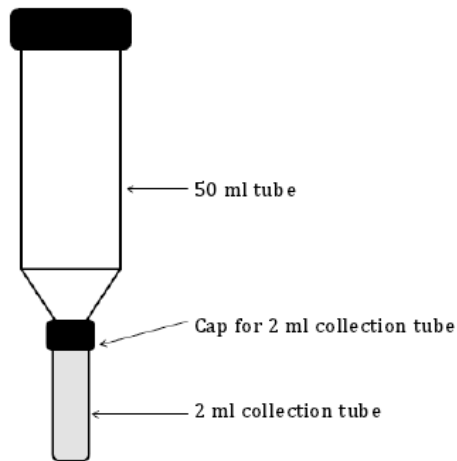


Photo Credit: Robin Kodner

The bottom part of the 2ml centrifuge tube was then screwed on to the glued cap and used to collect settled plankton. After 48 hours of settling, the bottom 2ml centrifuge tubes were twisted off and secured with a new cap. The 2ml settled tubes were inverted for mixing. 100 μm of the settled plankton were placed into the Palmer-Maloney counting chamber and covered with a cover slip. Ten randomly chosen fields of view of the counting chamber at the 20x objective were counted. Cells were identified to genus for diatoms and dinoflagellates. This process was done six times for each sample. Average counts/ml were calculated and then adjusted for settling concentration to get the cells/ml in the bags.

$$\text{Volume of Palmer-Maloney Counting Chamber} = 0.1 \text{ mL}$$

$$\text{Volume of Field of View at 20x} = 0.000315 \text{ mL}$$

$$\left(\frac{\text{Average Cells}}{10 \text{ Fields of View}} \right) \times \left(\frac{10 \text{ Fields of View}}{0.00315 \text{ mL}} \right) \times \left(\frac{2.7 \text{ mL}}{50 \text{ mL}} \right) = \text{Average Cells/mL}$$

$$\left(\frac{\text{Total Cells}}{60 \text{ Fields of View}} \right) \times \left(\frac{60 \text{ Fields of View}}{.0189 \text{ mL}} \right) \times \left(\frac{2.7 \text{ mL}}{50 \text{ mL}} \right) = \text{Cells/mL}$$

The average cells/mL was calculated by averaging the cells counted per slide for each mesocosm to get an estimate of how variable the slides and cell counts were. The second formula for cells/mL was calculated to get a reliable value of the phytoplankton abundance.

Results

Treatments

Among the three treatments (high, control, and drift), there was no statistically significant difference in cells/mL (P-value= 0.809, DF=2, Friedman chi-squared= 0.424). From T0 to T10 the treatments are very similar in cell density, but after the mesh caps were removed on T10, phytoplankton began to grow exponentially as seen in Figure 1. The control treatment had the highest cell density from T14 through T20, ending with a median of 1698 cell/mL. The high treatment remained the second highest from T14 through T20, ending with a median of 1053 cells/mL, and the drift was the lowest treatment with an end median of 948 cells/mL (Figure 1).

The individual mesocosm phytoplankton cells/mL were steady from T0 to T10, but became highly variable as the experiment progressed. All the mesocosms remained very similar in cell counts from T0 to T10, but variability increased and individual mesocosm phytoplankton abundance began to diverge after the mesh caps were removed, starting on T12, as seen in Figure 2. Mesocosm 1 (control), 4 (control), and 2 (drift) had the highest cell counts of all nine bags. Mesocosm 6 (drift) and 8 (high) had the lowest cell counts.

Genus Composition

Throughout the 22-day experiment, 19 genera of phytoplankton were encountered (Figure 3). The most common encountered diatoms throughout the course of the experiment were *Skeletonema spp.*, *Thalassiosira spp.*, *Chaetoceros spp.*, *Thalassionema spp.* and *Pseudo-Nitzschia spp.* A round, peanut-shaped photosynthetic dinoflagellate was the most common autotrophic dinoflagellate. *Skeletonema* was the dominant genus, with *Thalassiosira* the next most abundant.

Through the duration of the experiment, there was a community shift between the photosynthetic dinoflagellates and diatoms. T0 to T4 were abundant in both diatoms and dinoflagellates but over time diatoms surpassed the dinoflagellates in abundance and percent composition in the phytoplankton community (Figures 4A and 4B). Within the diatom population there was also a shift in dominant genera. In the first days of the experiment, *Thalassiosira* was more abundant than *Skeletonema* in the drift and control treatments but *Skeletonema* increased in in percent composition in all treatments over time (Figures 5A and 5B). *Pseudo-nitzschia* increased in percent composition towards the end of the experiment in the drift and high treatments (Figure 3).

Correlations

A spearman test between phytoplankton and chlorophyll a resulted in a strong positive correlation. As phytoplankton cell counts increased, the chlorophyll a measured increased. The control treatment had a correlation coefficient (ρ) of 0.95 and a p-value of less than 0.001. High treatment had a correlation coefficient of 0.925 and a p-value of less than 0.001. Drift treatment had a correlation coefficient of 0.928 and a p-value of less than 0.001 (Figure 6).

A strong negative correlation between phytoplankton at nitrate showed that as phytoplankton cell counts increased, nitrate measured decreased. The control treatment had a correlation coefficient of -0.933 and a p-value of less than 0.001. The high treatment had a correlation coefficient of -0.912 and a p-value of less than 0.001. The drift treatment had a correlation coefficient of -0.888 and a p-value of less than 0.001 (Figure 7).

The correlation tests between pCO₂ and phytoplankton varied by treatments. In the control treatment, phytoplankton cell counts increased, as pCO₂ remained the same due to our CO₂ additions (Figure 8). The control treatment had a correlation coefficient of -0.582 and a p-value of less than 0.001. In the high treatment, the phytoplankton cell counts increased, as pCO₂ remained the same due to our CO₂ additions. The high treatment had a correlation coefficient of -0.624 and a p-value of less than 0.001. The drift treatment behaved differently because as phytoplankton cells increased the CO₂ decreased. The drift had the strongest correlation coefficient, -0.828, and a p-value of less than 0.001.

To test to see if genera responded differently to the treatments, the two most abundant genera, *Thalassiosira* and *Skeletonema*, and a less abundant genus *Pseudonitzschia* were plotted against pCO₂ (Figure 9). From Figure 9, it is apparent that all genera follow the same pattern. *Skeletonema* and pCO₂ control had a correlation coefficient of -0.541 and a p-value of 0.001, high had a correlation coefficient of -0.49 and a p-value of 0.004, drift had a correlation coefficient of -0.757 and a p-value of 0.000. *Thalassiosira* and pCO₂ control had a correlation coefficient of -0.71 and a p-value of 0.000, high had a correlation coefficient of -0.582 and a p-value of 0.000, drift had a

correlation coefficient of -0.649 and a p-value of 0.000. *Pseudo-nitzchia*, the least abundant of the three genera, and pCO₂ control had a correlation coefficient of -0.55 and a p-value of 0.001, high had a correlation coefficient of -0.548 and a p-value of 0.001, drift had a correlation coefficient of -0.842 and a p-value of 0.001. There is no statistical difference between the three genera's abundance and pCO₂, leading to the conclusion that there was no treatment effect of pCO₂ on *Skeletonema*, *Thalassiosira* or *Pseudo-nitzchia*.

Discussion

Although we see changes in the phytoplankton community and abundance, there is no statistically significant difference among treatments because the variability within a treatment was equal to the variability across treatments. Phytoplankton cell densities vary greatly on what is seen under the microscope, and is easily skewed by tangled groups of phytoplankton or long chains. Increased phytoplankton cell densities in mesocosms 1 and 9 could be due to their placement on the end of the portable dock, allowing for more light.

Ambient water around the dock was approximately 650 uatm, which is what the control treatment was maintained at. This could have influenced the lower phytoplankton cell densities in the high and drift treatment because the phytoplankton are less acclimated to the higher pCO₂ treatments than the control treatment. A smaller volume of water was filtered into mesocosm 6 due to unequal flow rate during unfortunate weather while filling the mesocosms. This is likely the cause of mesocosm 6 behaving so differently in comparison to other mesocosms in its treatment.

Phytoplankton genera composition is important when talking about ocean acidification because certain genera are more efficient as a carbon sink. It is important to

look at phytoplankton on a genus level to see if there are large community structure shifts and to see if some taxa respond differently to the treatment. *Thalassiosira* and *Skeletonema* are chain-forming diatoms. It is unknown how exactly a community shift between *Skeletonema* and *Thalassiosira* would effect the microbial community but it is known that it would effect the feeding, grazing, and carbon sink. These changes in genera may be accounted for by natural variations in the ecosystems given that these patterns were seen in all treatments and all mesocosms.

Correlations between chlorophyll a and nitrate with phytoplankton are not surprising by any means, but they are still important tests to run to get a better idea of the processes going on in the mesocosms. The strong positive correlation between chlorophyll a and phytoplankton density shows that most of the chlorophyll a that fluoresced was from the phytoplankton seen under light microscopy. Any deviation from a perfect correlation could be due to the variability of the phytoplankton counts or from smaller nanophytoplankton too small to count under light microscopy. The strong negative correlation between nitrate and phytoplankton shows that as the phytoplankton grow, they take up nutrients, so nitrate goes down. Imperfections from the linear relationship can be attributed the fact that they other forms of biology are using the nitrate, such as nanophytoplankton.

Correlations between pCO₂ treatments and phytoplankton varied a lot more among treatments due to our successful CO₂ additions. Our CO₂ additions maintained the high and the control pCO₂ to the specified treatment levels so the correlation in Figure 8 shows that as pCO₂ in the high and the control treatment remains the same, the phytoplankton density goes up. As previously mentioned, the cell density increased more

in the control treatment that the high treatment. Although there was no treatment effect seen, the higher cell density in the control treatment could be accounted for by the fact that the control treatment was the ambient pCO₂ of the coastal water, which the plankton could've already been acclimated to. The drift treatment behaved differently from the other treatments because no additional CO₂ was added after the initial CO₂ was increased to 1250 uatm. As the phytoplankton cell densities went up in the drift treatment, the pCO₂ was drawn down due to photosynthesis. Imperfections in these correlations are due to the fact that the phytoplankton counted under light microscopy were not the only organisms in the mesocosms photosynthesizing and all organisms in the mesocosms were respiring.

Correlations between dominant phytoplankton genera and pCO₂ (Figure 9) showed no treatment effects on *Thalassiosira*, *Skeletonema*, and *Pseudo-nitzhia*. *Thalassiosira* and *Skeletonema* were chosen because they were the two dominant genera and *Pseudo-nitzchia* was chosen because it increased in percent composition overtime (Figure 3) and was present in all mesocosms. All three genera follow a similar pattern, only varying in abundance, meaning there was no treatment effect seen on *Thalassiosira*, *Skeletonema*, and *Pseudo-nitzchia*.

Overall, the experiment was a great success in conducting a mesocosm study, maintaining the desired pCO₂ levels, and slowing biology down with light limitations. If repeated this experiment I would settle and count more volume of seawater to get a more accurate picture of what was happening with the phytoplankton in the mesocosms. Given more time, I would also perform multivariate statistics.

Conclusions

- There was no pCO₂ treatment effect seen on the phytoplankton densities.

- Phytoplankton cell counts are highly variable within pCO₂ treatments and across pCO₂ treatments. This caused there to be no statistical difference between pCO₂ treatments.
- Natural variations in community structures can account for the community shifts.

Acknowledgements

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Figures

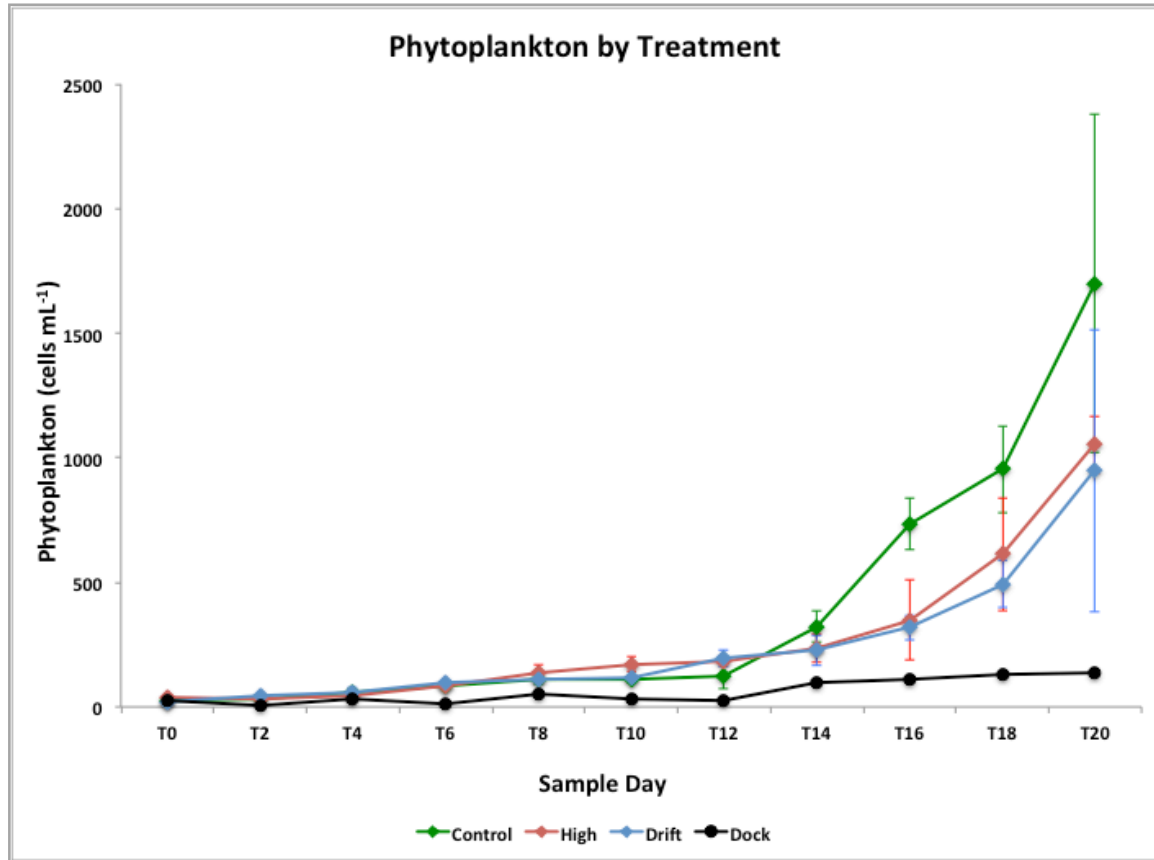


Figure 1| Phytoplankton abundance by treatment through time. Median cells/mL for the three treatments (control, high, drift) and dock from T0 to T20. Error bars are the median absolute deviation from the median. Friedman test determined no statistical difference between treatments (P-value = 0.809, Chi-squared = 0.404, DF = 2). Abundance remained very similar through T10, the light limiting mesh was removed and spurred phytoplankton growth. Control treatment (green) was the highest abundance and peaked at 1698 cells/mL, high treatment (red) peaked at 1053 cell/mL, and drift treatment (blue) had the lowest abundance with a peak of 947 cells/mL.

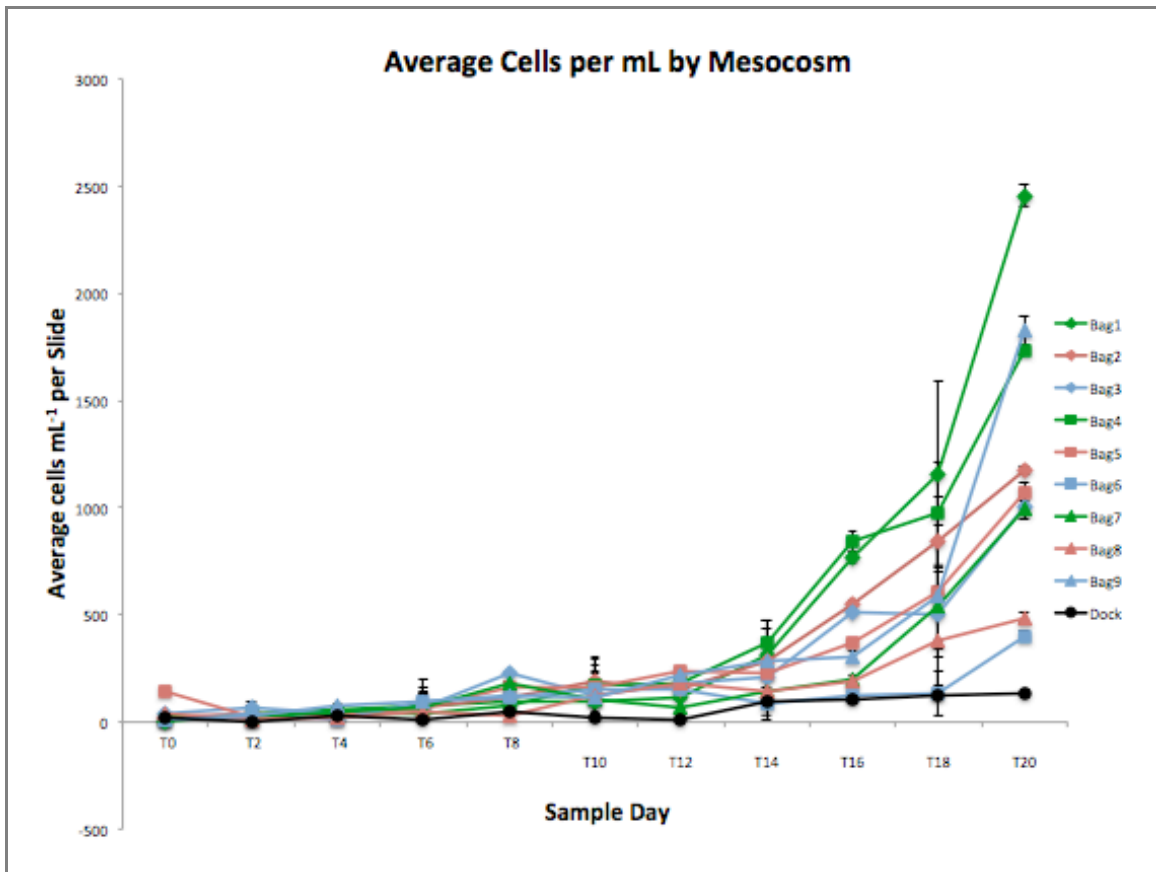


Figure 2 | Average cells/mL by mesocosm over time. Mean cell/mL of all six slides counted for each mesocosm. Demonstrates the variability of the mesocosms and the cell counts.

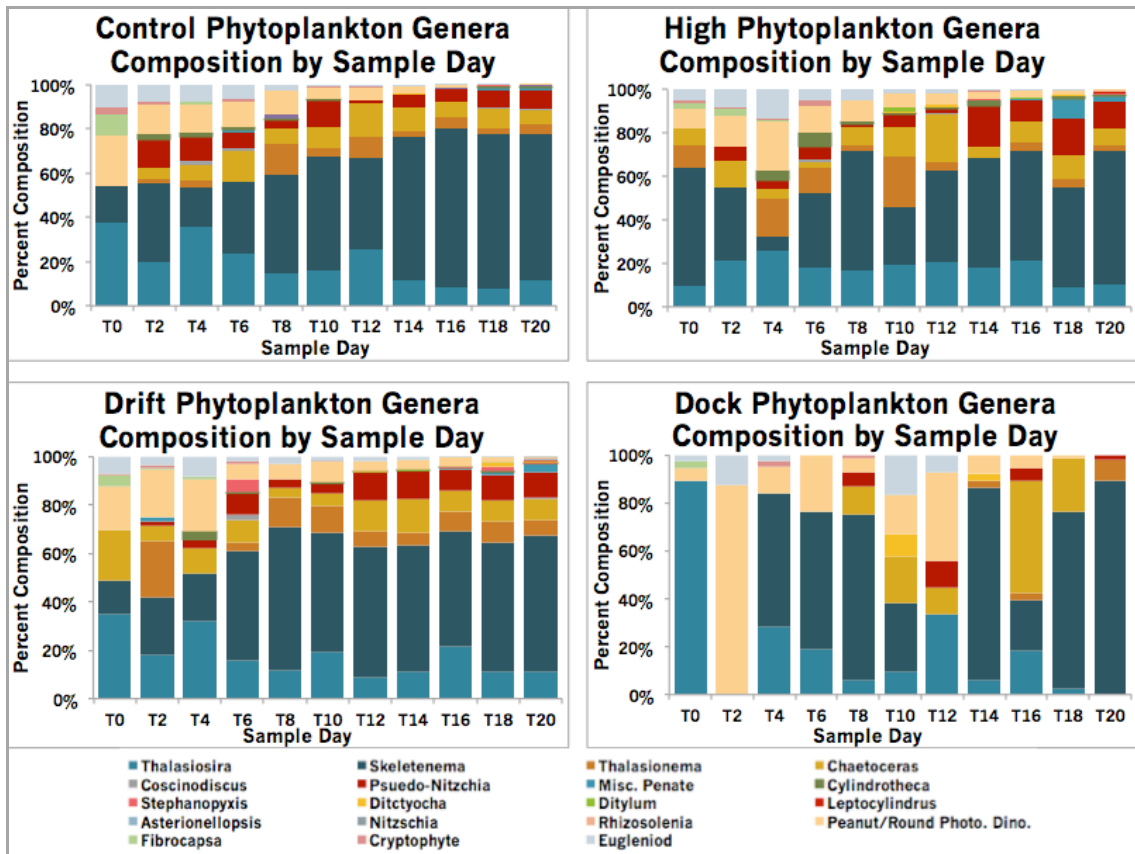
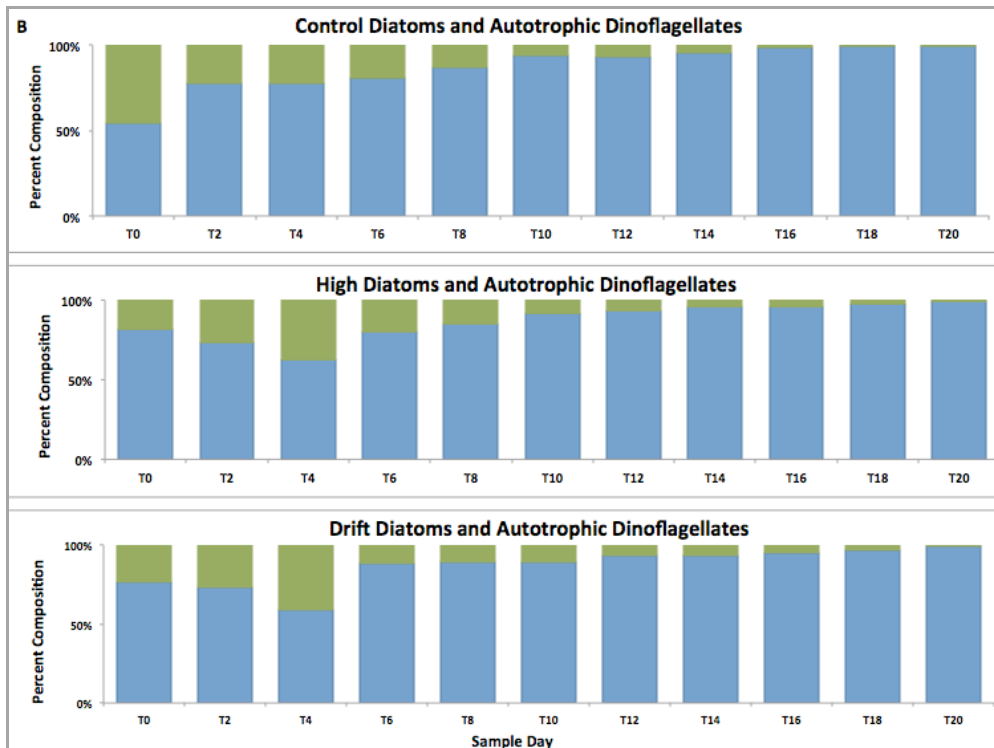
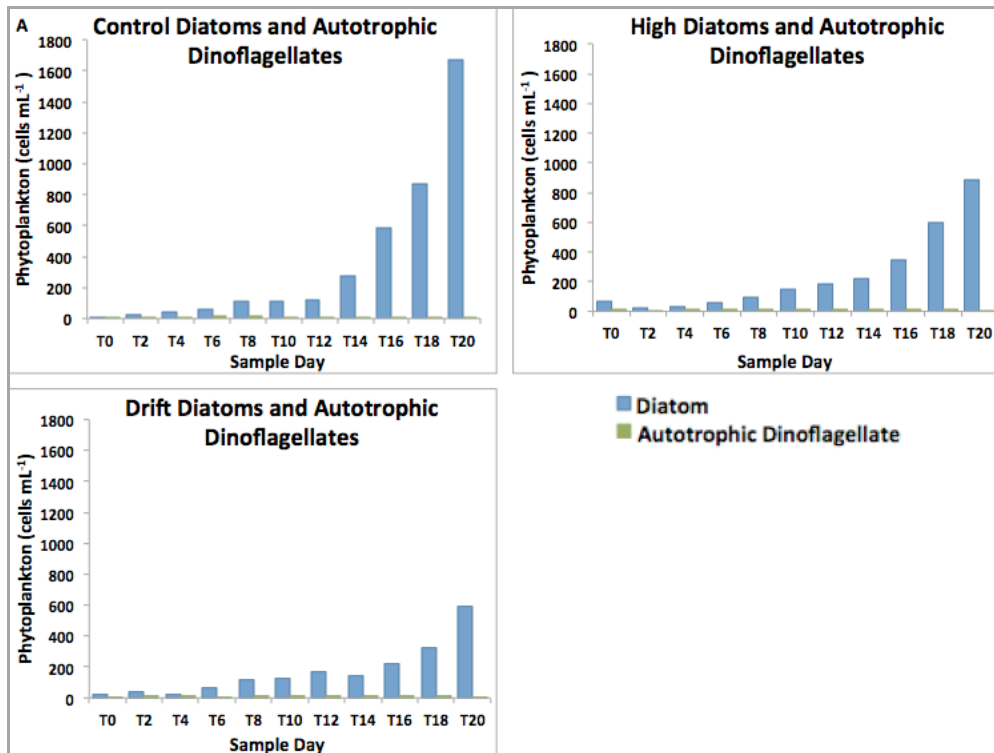


Figure 3| Phytoplankton genera composition. Cells/mL for each genera counted for each treatment over time. *Skeletonema* and *Thalassiosira* are the most common genera encountered. *Skeletonema* was the dominant genus and increased in percent composition over time.



Figures 4A and 4B| Diatoms and autotrophic dinoflagellate composition. Shift in community to a diatom dominated phytoplankton community. Dinoflagellate abundance peaked at T4 for the drift and high treatment and peaked at T0 for the control treatment. Dinoflagellates decreased over time in percent composition while the diatoms increased.

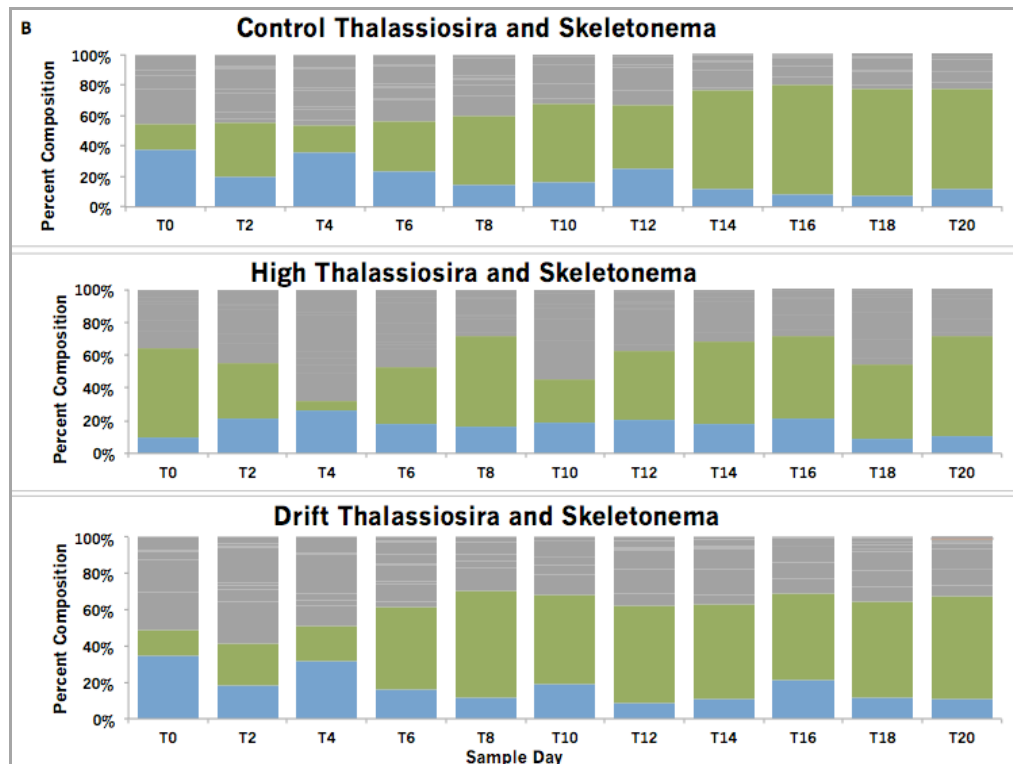
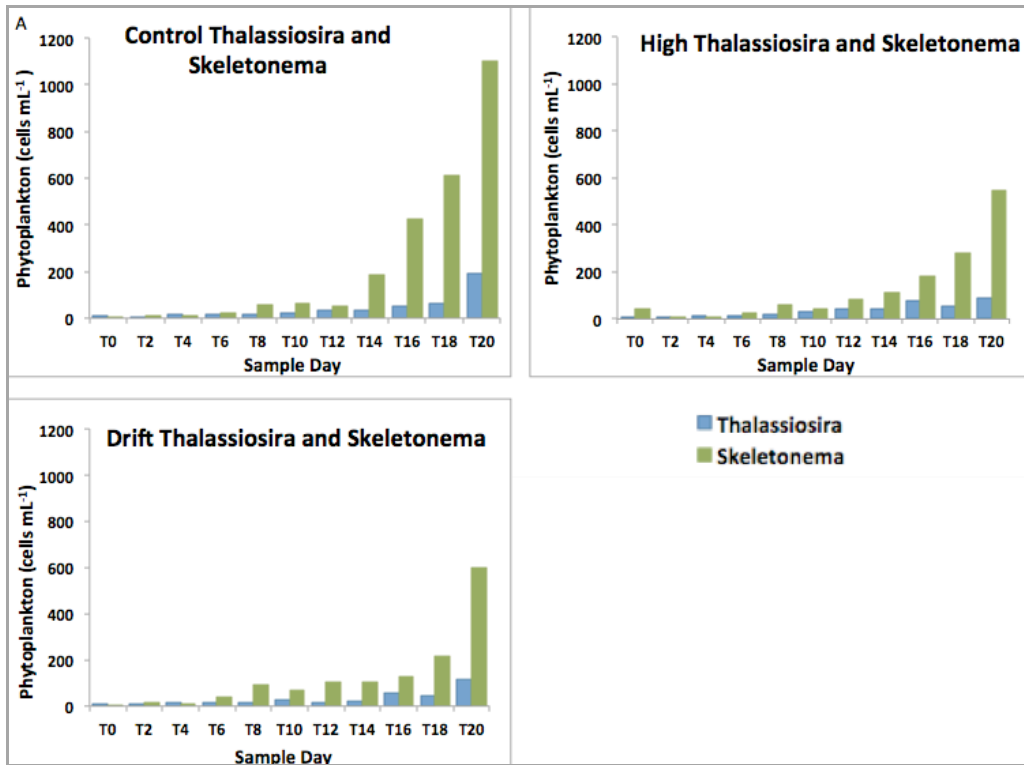


Figure 5A and 5B| Thalassiosira and Skeletonema. *Thalassiosira* and *Skeletonema* increase in abundance over time. *Skeletonema* increases in percent composition over time while

Thalassiosira decreases in percent composition over time, which demonstrates a shift in community.

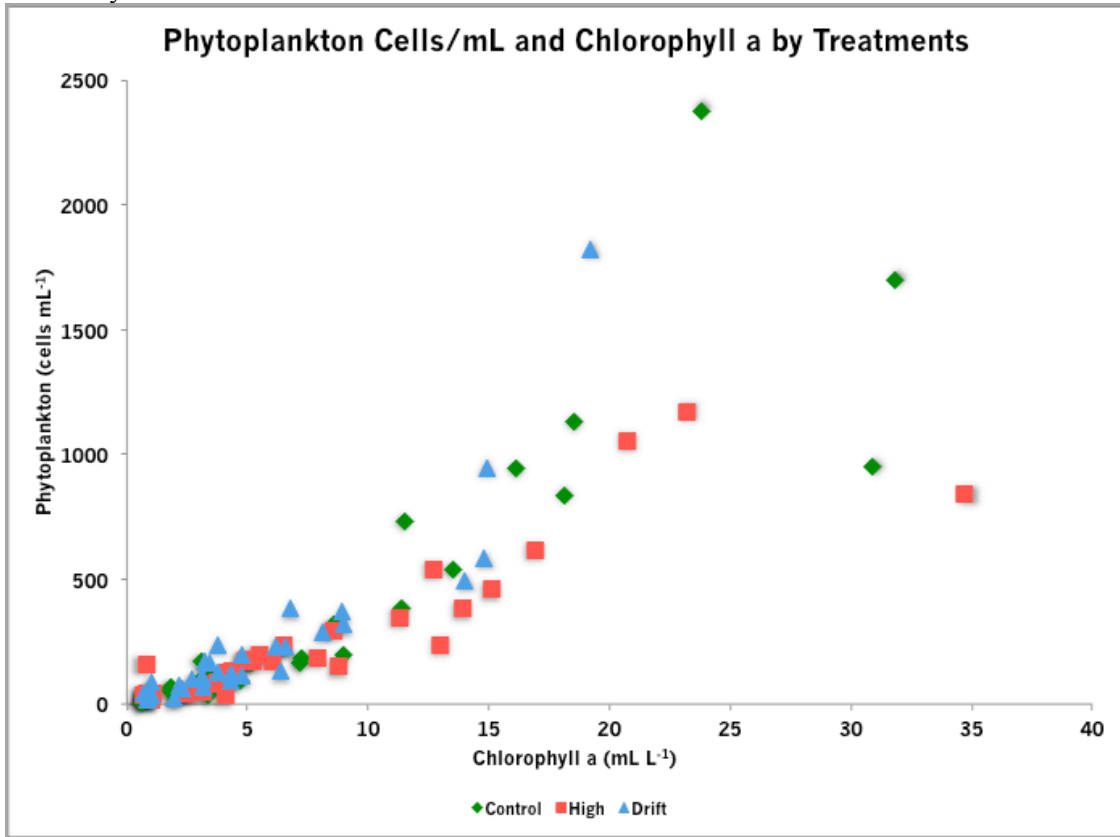


Figure 6| Phytoplankton and chlorophyll a correlation. Control ($\rho = 0.95$, $p\text{-value} < 0.001$), high ($\rho = 0.925$, $p\text{-value} < 0.001$), and drift treatment ($\rho = 0.928$, $p\text{-value} < 0.001$) had a strong positive correlation between chlorophyll and phytoplankton cells per mL.

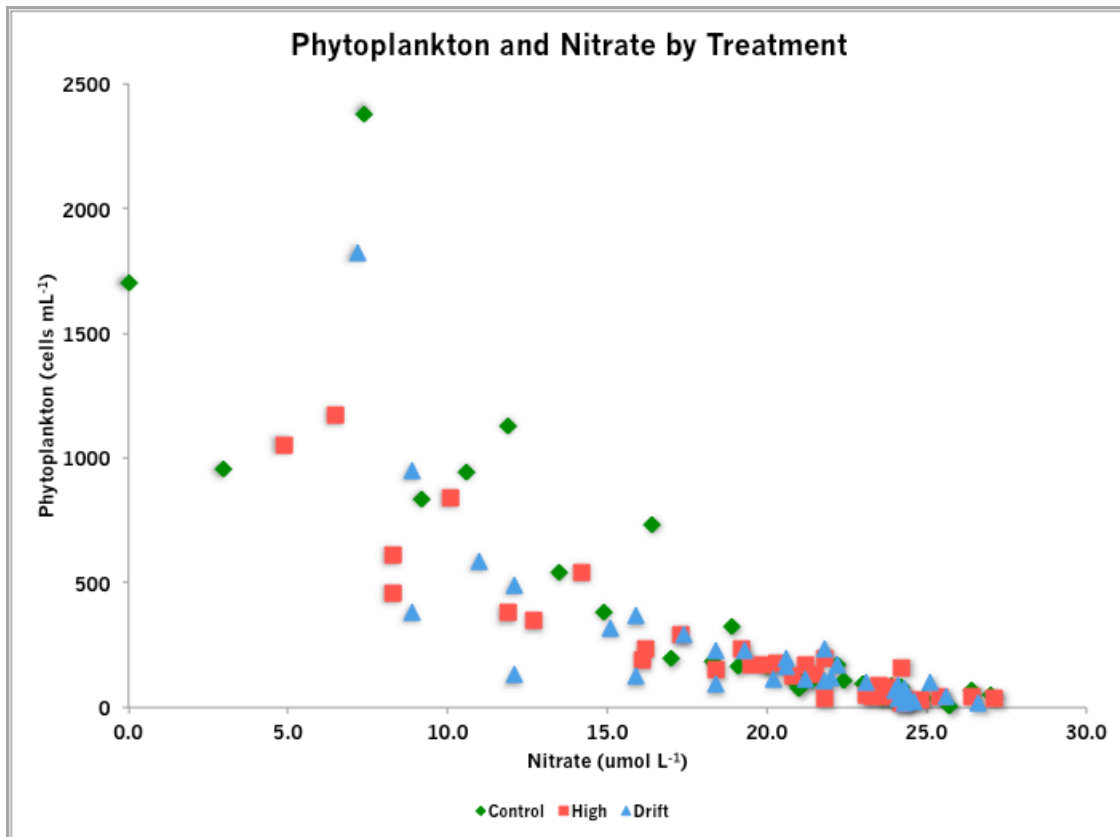


Figure 7| Phytoplankton and nitrate correlation. The control ($\rho = -0.933$, $p\text{-value} < 0.001$), high ($\rho = -0.912$, $p\text{-value} < 0.001$), and drift treatment ($\rho = -0.888$, $p\text{-value} < 0.001$) had strong negative correlation between nitrate and phytoplankton cells per mL.

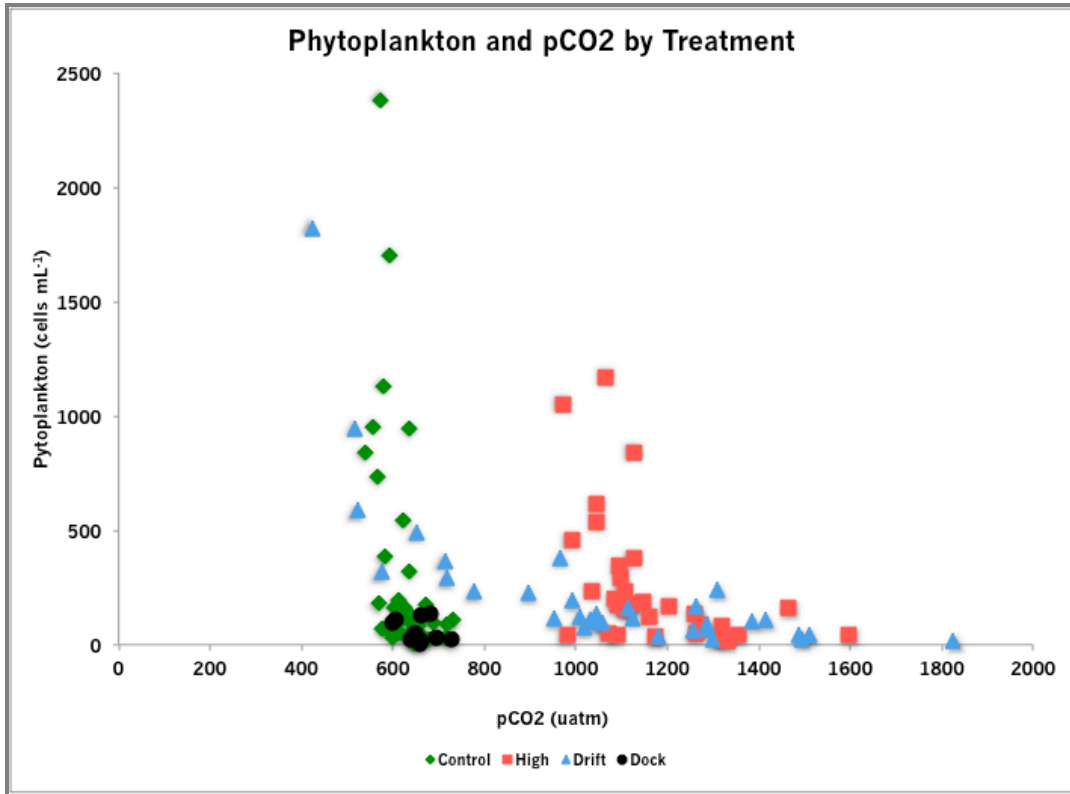


Figure 8| Phytoplankton and pCO₂ correlation. The correlation tests between pCO₂ and phytoplankton varied by treatments. In the control ($\rho=-0.582$, $p\text{-value}<0.001$) and high ($\rho=-0.624$, $p\text{-value}<0.001$) treatments, phytoplankton cell counts increased, as pCO₂ remained the same due to our CO₂ additions. The drift ($\rho=-0.828$, and a $p\text{-value}<0.001$) treatment behaved differently because as phytoplankton cells increased the CO₂ decreased..

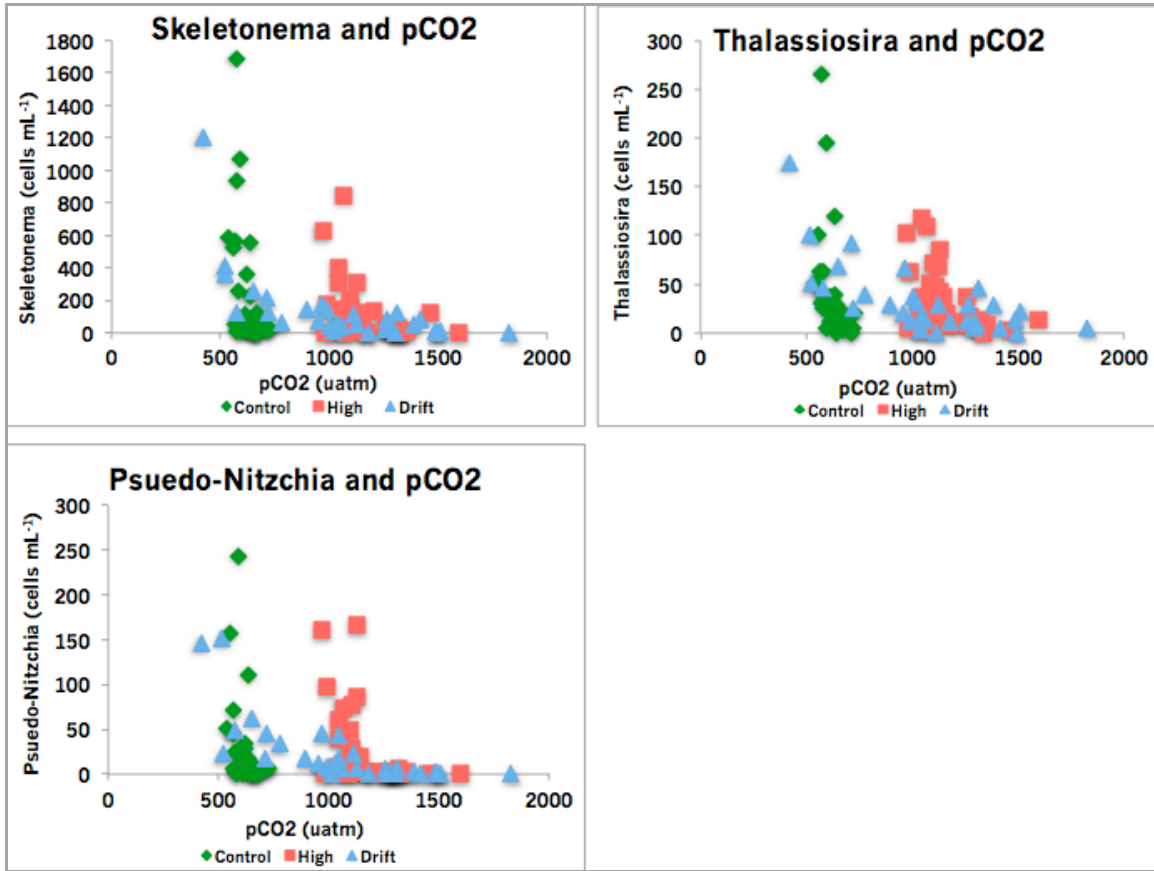


Figure 9| Diatom genera and pCO₂ correlations. *Skeletonema* and pCO₂ control (rho= -0.541, p-value=0.001), high (rho= -0.49, p-value= 0.004), drift (rho= -0.757, p-value= 0.000). *Thalassiosira* and pCO₂ control (rho= -0.71, p-value = 0.000), high (rho= -0.582, p-value= 0.000), drift (rho= -0.649, p-value= 0.000). *Pseudo-nitzschia* and pCO₂ control (rho= -0.55, p-value= 0.001), high (rho= -0.548, p-value= 0.001), drift (rho= -0.842, p-value= 0.001). *Skeletonema*, *thalassiosira* and *pseudo-nitzschia* follow that a similar pattern, showing no treatment effect on any of the three genera chosen.

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