Diatom silicification changes in high pCO_2 seawater: a mesocosm experiment

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Ocean Acidification Research Apprenticeship 2013
Spring 2013

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Keywords: ocean acidification, mesocosm, pCO_2, diatoms

Abstract:

Diatoms contribute significantly to the transfer of dissolved organic carbon from the surface ocean to the deep in a system called the biological pump. Understanding how diatoms silicify (incorporate silica into their cell wall) in response to high partial pressure CO_2 (pCO_2) seawater is important to understand how ocean acidification will affect this biological pump. A mesocosm experiment was performed at University of Washington's Friday Harbor Laboratories to investigate these effects. Three pCO_2 treatments were used in triplicate to determine whether or not diatom silicification is directly affected by ocean acidification. The control and high treatment, at 650ppm and 1250ppm, were held at constant pCO_2 levels with additions of highly saturated CO_2 seawater. The drift treatment started at 1250ppm and was allowed to drift as biological processes used the CO_2. Biogenic silica was measured every other day and integrated with diatom cell counts to calculate silicification. Results showed no differences between pCO_2 treatments for total silica in a population and silicification per diatom cell. A variety of potential stressors were explored to understand the changes in diatom silicification throughout the experiment. Factors other than CO_2, such as light limitation, are more likely to explain data trends. Further research is necessary to see how CO_2 will affect diatoms' ability to sequester carbon especially when coupled with temperature increases.

Introduction:

Increasing carbon dioxide in the atmosphere due to anthropogenic activities leads to increased uptake of CO_2 by the ocean reservoir (Siegenthaler & Sarmiento 1993). More than one third of anthropogenic CO_2 has been taken up by the ocean reservoir in the

past century (Sabine et al., 2004). This oceanic uptake leads to increased bicarbonate ion concentration and decreased pH (Riebesell et al., 2007). Mesocosm experiments work to study food web structures and chemical changes in high pCO_2 conditions. Within the confines of a mesocosm experiment many variables can be measured and controlled. While the negative effect of acidifying oceans on calcifying phytoplankton has been widely studied (Riebesell et al., 2000) it is also important to understand how other major primary producers, such as diatoms, will be affected. Diatoms are responsible for 40% of primary production in ocean systems (Nelson et al., 1995). Diatoms have cell walls, called a frustule, composed of silica allowing them to sink more efficiently than most phytoplankton, contributing significantly to the biological pump or transport of organic carbon from the euphotic zone to the ocean interior (Buesseler 1998; Ducklow et al., 2001). Diatoms are at the base of the marine food web and CO_2-induced changes could prove unfavorable for higher trophic levels (Riebesell 2004). A change in diatom fitness could negatively affect primary production as well as carbon export.

The goal of this experiment was to better understand if there would be changes in diatom silicification under ocean acidification conditions. Having a grasp on potential changes in diatom silicification under high pCO_2 conditions could be significant in understanding future carbon cycling changes due to the addition of anthropogenic CO_2 to the atmosphere. As the ocean acts as the second largest sink for anthropogenic carbon (Riebesell et al., 2007), a silicification decrease in diatoms could affect how carbon is taken up by the oceanic reservoir. Calculating biogenic silica per cell using biogenic silica measurements and total diatom counts (Stephens 2013), any changes in silicification between treatments can be measured quantitatively.

Previous studies show increased silicification under zinc and iron stress (De La Rocha et al., 2000) as well as light stress in diatom species *Coscinodiscus granii* (Taylor 1985). Stress from zooplankton grazing pressure was shown to increase cell wall silicification in a marine diatom (Pondaven 2007). Environmental stress caused by the increased pCO_2 could lead to similar results. Mesocosm studies in Korea found evidence for enhanced diatom growth rates for one species under high pCO_2 conditions (Kim et al., 2006). As silicification increases, diatom sinking rates also increase (Raven & Waite 2004). If silicification increases under ocean acidification conditions, diatoms could positively impact carbon sequestration to the deep ocean.

This experiment differs from other mesocosm experiments in that we successfully maintained the control and high treatment pCO_2 levels, the water was very high in NO_3 and mesh was used to reduce light by ~55% in order to stall a phytoplankton bloom. No nutrients were added to any mesocosm for the experimental duration. The water was filtered with a biologically safe pump that excluded large grazers such as copepods and other mesozooplankton. Also, seawater off the Friday Harbor Laboratories dock tends to be around 650-750ppm CO_2 due to coastal upwelling. No data has been found for silicification in diatoms in an ocean acidification mesocosm experiment, which is why the data presented in this report is very important in understanding the future of diatoms' contribution to carbon sequestration. The following paper attempts to ask two questions (1) does biogenic silica differ between pCO_2 treatments (2) what environmental stressors account for changes in diatom silicification in our mesocosms?

Methods and Materials:

Experimental Design

Nine 6-meter long polyethylene mesocosm bags were used, each holding approximately 3,500 liters of filtered seawater. Bags were supported using a 3-meter long metal support cage and were covered with clear acrylic domes to prevent contamination. Mesh bags and tops were placed around each polyethylene bag in attempt to reduce light by ~55%. This mesh was used to slow down phytoplankton growth to prevent an immediate bloom. The mesh tops were removed on T10, and the mesh bags were lowered 1 meter to allow greater light penetration.

Each bag was filled simultaneously at 1L per minute from a common reservoir (1500L filled with a plastic/Teflon diaphragm pump) over a period of ~55 hours. After the filling process, CTD data was used to determine the salinity in each mesocosm. Supersaturated NaCl brine (3500g NaCl/15L H_2O) was used to dispense ultra saline water into each mesocosm. This was necessary in order to keep the mesocosms denser than the surrounding seawater. The addition of the brine and subsequent salinity increase was used to determine the final volume of the mesocosm bags after filling. Three different pCO_2 treatments were used for the nine mesoscosms. Mesocosms 1, 4, and 7 were control treatment bags and maintained at approximately 650ppm CO_2. Mesocosms 2, 5, and 8 were high treatment bags and maintained at approximately 1250ppm CO_2 using additions of CO_2-saturated seawater. Mesocosms 3, 6, and 9 were drift treatment bags that were initially given extra CO_2 saturated water to increase their pCO_2 levels to around 1250ppm but were not kept at that level using additional supersaturated CO_2 seawater. Alkalinity and DIC were measured in each mesocosm to calculate pCO_2 and pH. For the control and high treatments, these measurements were used to determine how much CO_2 saturated water would be added to these treatments. CO_2 additions were made

using a peristaltic pump and a “spider” device evenly distributed the water throughout each mesocosm.

Prior to sampling the mesocosms using a depth-integrated sampler and a Niskin bottle, light measurements were taken in each bag as well as on the dock for comparison using a LI-COR LI-700 light meter. These measurements were done with the mesh tops in place prior to T10. Next, the bags were mixed using a perforated plexi-glass disc. CTD data was taken after mixing using a Sea-Bird Model 37SMP to measure salinity, temperature, density and depth. Integrated sampling was the first sample technique used each day. This involved lowering a five-meter pump tube into the center of each mesocosm bag, capping it, and filling a plastic carboy for multiple samples. This technique aimed to obtain a water column sample in each mesocosm. A 2L or 5L Niskin cast was done after integrated sampling, which concluded the sampling process.

Biogenic Silica

Each mesocosm was sampled every other day along with a dock sample. Samples were taken with the Niskin bottle, deployed to a depth of 2 meters, and samples were kept in 125mL brown nalgene bottles and filled to the brim to obtain a volume of 150mL. Filters (25mm 1.0um polycarbonate membrane) were loaded onto a 6-place filter manifold. The filters were transferred to 50mL Falcon tubes. 15mL of 0.5% Sodium Carbonate solution was added to the tubes using a syringe. Samples were incubated for 2 hours in an 85° C water bath. After cooling, the samples were filtered again using a Millipore (0.45um) filter.

Samples were then prepared with methyl orange and HCl according to Paasche (1980). Samples were prepped for spectrometer measurements using the *Determination*

of reactive silicate from A Practical Handbook of Seawater Analysis with a few modifications. Standards were created using 10mL of synthetic seawater with various amounts of hexaflourosilicic acid. One standard had no acid, the others varied from 50 to 250µmL. Each test tube (10mL polyurethane) prepared for spectrometry included 1 mL ammonium molybdate, 2.5mL sample or standard, and 1.5mL reducing solution. The reducing solution was made up of 10mL metol sulfite, 6mL sulfuric acid, 6mL oxalic acid, and 8mL DI H_2O. After waiting for two hours, these samples and standards were run in a spectrometer read at 810nm for absorbance. These amounts were scaled down from the methods used in Strickland and Parsons (1972) due to the smaller sample size. The standards were run twice and these values determined the conversions needed for absorbance to µmol BSi/L.

Biogenic Silica per diatom cell (silicification) calculation

Values for biogenic silica were converted to biogenic silica $cell^{-1}$ using Stephens (2013) phytoplankton cells mL^{-1} and raw diatom cell counts. The equation used was: Biogenic silica diatom $cell^{-1}$ = Biogenic silica L^{-1} ÷ (Phytoplankton cells L^{-1} × (# Diatoms ÷ # Phytoplankton cells)). This was done for each mesocosm for each day measured (T0-T20, every other day).

Statistics

Statistical tests were run on IBM SPSS Statistics 19. Shapiro-Wilk tests were used to determine normality. Friedman tests were run to determine statistical differences between treatments. The significance level was set at p=.05. Spearman ranked sum tests were used to determine correlations and associations between variables.

Results:

Biogenic silica was shown to increase in all treatments, peaking on T18 for the High and Drift treatments and going up towards a peak on T20 for the Control treatment (Fig 1). The control treatment ranged from values of 3.28 to 11.34 µmol BSi L^{-1} (Fig 1). The high treatment mesocosms ranged from values of 3.33 to 13.17 µmol BSi L^{-1} (Fig 1). The drift treatment mesocosms ranged from values of 3.58 to 9.69 µmol BSi L^{-1} (Fig 1). Shapiro-Wilk tests for normality determined non-normal distribution for all treatment groups for both biogenic silica and biogenic silica $cell^{-1}$ with all p-values being <0.001. There was no statistical difference between pCO_2 treatments for biogenic silica ($F_{(2,32)}$= 5.250, p=0.072).

Figure 2 displays the raw data for each mesocosm in order to show the variation between treatment groups specifically in mesocosms 4 and 6. Mesocosm 4 was much higher than other mesocosm, peaking at a value of 17.32 µmol L^{-1} on T18 (Fig 2). Mesocosm 6 is shown to peak at a value of 5.15 µmol L^{-1} on T14 (Fig 2). Mesocosm 4 had much higher BSi L^{-1} than other control treatment mesocosms (1,7). Mesocosm 6 had much less BSi L^{-1} than other drift treatment mesocosms (3,9).

Figure 3 shows the results of calculated pmol BSi $cell^{-1}$ from T2 to T20. All treatments showed a decrease in silicification after T4 (Fig 3). The treatment groups were not statistically different for pmol BSi $cell^{-1}$ ($F_{(2,30)}$= 4.200, p=0.122). Control treatment bags had a peak on T2 with a value of 161.13 pmol BSi $cell^{-1}$. High treatment bags had a peak on T4 with a value of 152.49 pmol BSi $cell^{-1}$. Drift treatment bags had a peak on T0 with 106.48 pmol BSi $cell^{-1}$. Each treatment group showed a minimum at T20 with values of 9.21, 11.68 and 8.60 pmol BSi $cell^{-1}$ for control, high, and drift treatments respectively (Fig 3).

Figure 4 shows biogenic silica (µmol L^{-1}) and nitrate (µmol L^{-1}) plotted through time on the same graph. Statistical correlations can be seen in Table 1a showing strong negative correlations for all treatment groups.

Figure 5 shows biogenic silica (µmol L^{-1}) and silicate (µmol L^{-1}) plotted through time on the same graph. Statistical correlations for nutrient data and biogenic silica can be seen in Table 1b showing strong negative correlations for all treatment groups.

Figure 6 shows irradiance inside mesocosms at T0 with mesh caps still in place and at T11, which was the day after the mesh caps were removed. Greater light penetration for the top ~2m can be seen after removal of mesh caps.

Table 2 displays Spearman correlation coefficients for a variety of variables against biogenic silica and biogenic silica per diatom cell (silicification). Grazing rates and silicification had no correlation in the control and high treatment (Table 2a). Biogenic silica and phytoplankton cells mL^{-1} had strong positive correlations for all treatment groups (Table 2b). Biogenic silica and chlorophyll had strong positive correlations for all treatments (Table 2c). Silicification and pCO_2 had slight correlations with all ρ values above 0.5 (Table 2d). Biogenic silica and bacterial abundance had a strong correlation for the control treatment, a slight correlation for the high treatment, and no correlation for the drift treatment (Table 2e).

Discussion:

Biogenic Silica

Results show that biogenic silica did not differ between pCO_2 treatments in our mesocosm experiment. The diatoms were not limited by nitrate (Figure 4). Silicate, necessary for diatom growth (Lewin 1955) was not limiting in our mesocosms either

(Figure 5). In fact, diatoms should dominate a bloom if silicate concentration is above 2μmol L^{-1} (Egge & Aksnes 1992) and silicate was above 40 μmol L^{-1} to start in the mesocosms and did not go below 15 μmol L^{-1} (Figure 5).

Figure 5 also shows silicate being drawn down at a higher rate than biogenic silica increases. This could be due to bacteria using silicate as an energy source (Havskum et al., 2003), perhaps describing the strong correlation present between biogenic silica and bacterial abundance in the control treatment (Table 2E). There is also the possibility of benthic diatoms present in the mesocosms and these would not have been present in biogenic silica samples.

In each treatment, biogenic silica values for all treatments accelerated after removing the light-limiting mesh toppers after T10 as seen from Figure 1. This is consistent with chlorophyll data and phytoplankton cells mL^{-1} (Table 2B, C). We see higher correlation coefficients for all treatments with chlorophyll rather than cell mL^{-1}. This is most likely due to there being smaller autotrophs present in the mesocosms that were unable to be counted with light microscopy, and thus would not be included in phytoplankton cells mL^{-1}.

Silicification

Our results show that increased pCO_2 does not necessarily pose a threat to diatom silicification and that other factors could be responsible for the trends we see in our data. Even though ρ values for pCO_2 and silicification were over .5 for each treatment, the results of the Friedman test which showed no statistical difference between treatments nullifies this slight correlation.

Silicification has been shown to increase in diatoms as a result of low growth rates (Claquin et al., 2002). This correlates with the results in Figures 1 and 3, which show high silicification per cell during the first half of the experiment (T2-T10) coupled with low biogenic silica values during that same time period.

Previous experiments have shown an increase in diatom cell wall silicification in response to a variety of stressors whether it be nutrient (macro and micro) or grazing pressure and light limitation (Taylor 1985; De La Rocha et al., 2000; Pondaven 2007). The diatoms in our study were not macronutrient limited, but we had no way to measure micronutrient concentrations. Grazing rates were only measured (Govenar 2013) in two mesocosms (4, 5) so it is difficult to rule out grazing pressure as a stressor that could've affected diatom silicification. Light limitation is the most likely stressor to account for the silicification increase at the beginning of the experiment. This along with other natural variations or unmeasured variables could be the reason for data trends.

Conclusions

Taking the mesh tops off a few days sooner would've sped up the phytoplankton bloom and allowed for some 'post-bloom' data interpretation. Sampling every day instead of every other day might have changed our results due to increased sample size. Grazing rates measured in more than two mesocosms could've demonstrated that grazing pressure increased silicification at the beginning of the experiment. Maintaining the pCO_2 levels in the control and high treatments could've altered results since this doesn't occur in nature. Sampling for micronutrients such as iron or zinc could have shown that these nutrients were limiting to diatoms possibly providing more conclusive results.

Future research is necessary in order to understand the effects of ocean acidification on diatoms and diatom silicification. Diatoms are not entirely sensitive to CO_2 and will most likely be affected by the rising temperature, subsequent stratification, and nutrient and light changes that come with climate change (Rost et al., 2008). Temperatures in seawater have risen 1°C over the past one hundred years and are continuing to increase at 1°C per century (Hoegh-Guldberg 1999). It would be interesting to see how results would be different if we had a 'greenhouse' treatment as they have in the Korean mesocosms (Kim 2010) since temperature has a negative effect on diatom growth rate (Motagnes & Franklin 2001). Future implications of seawater warming and subsequent stratification of the water column will be necessary to study any negative effects on diatom productivity and growth.

It is inconclusive as to whether or not pCO_2 had a direct effect on diatom growth or silicification in this experiment. No single factor can be attributed to showing the trends present in this data. How the biological pump will function in the acidified and warming waters of the future remains unknown. Further research is vital to fully understand how rising CO_2 levels in the atmosphere will affect diatoms and carbon sequestration via the biological pump.

Acknowledgements:

I would like to give thanks to the University of Washington and Friday Harbor Laboratories for providing space to run our experiment. Dr. James Murray, Dr. Robin Kodner, and Kelsey Gaessner for providing assistance and mentorship throughout this experiment. Mike Foy, Barbara Paul, and Amanda Fay for their support. All my fellow apprentices for their support. The University of Washington Provost and the Henry and

Holly Wendt Endowment to support apprenticeships at Friday Harbor Labs. The Mary Gates Endowment for additional funding as well as the Educational Foundation of America and National Science Foundation (NSF Grant # DBI 0829486).

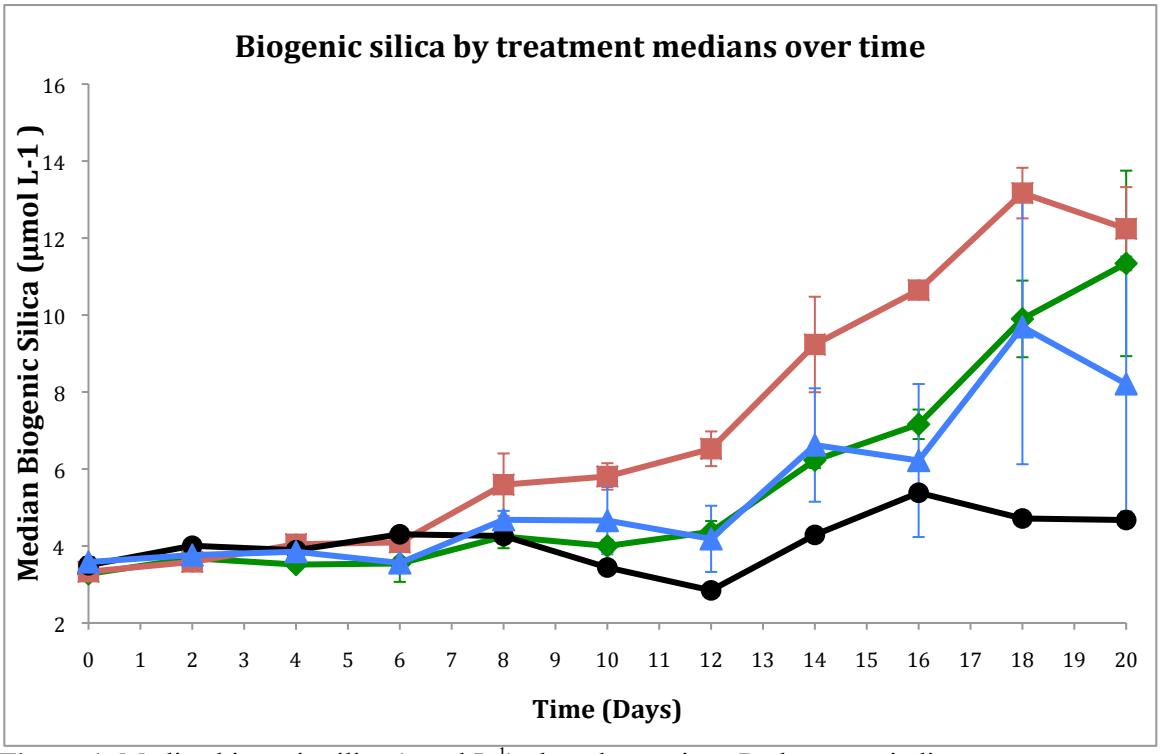

Figure 1: Median biogenic silica (µmol L^{-1}) plotted over time. Red squares indicate median values for high treatment, green diamonds indicate median values for control treatment, and blue triangles indicate median values for drift treatment. Black circles refer to dock samples. Error bars represent median absolute deviations for each treatment for each sample day.

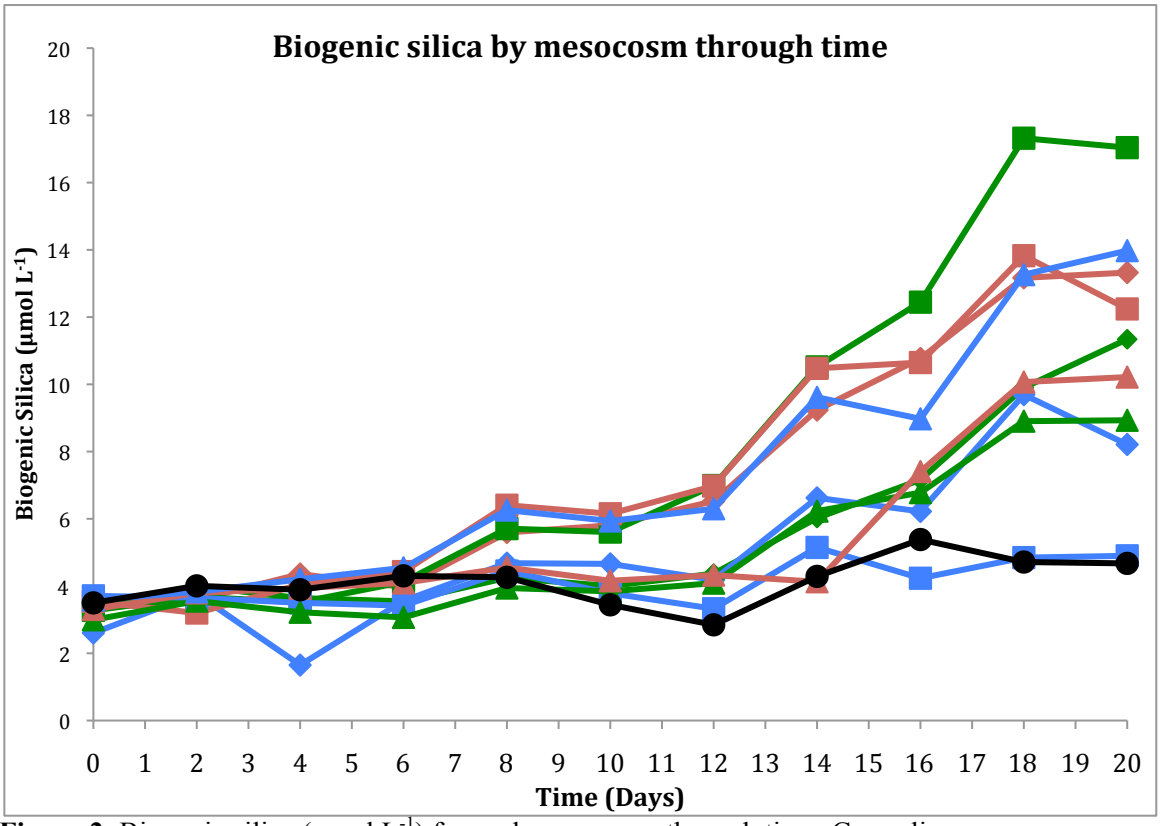

Figure 2: Biogenic silica (µmol L^{-1}) for each mesocosm through time. Green lines represent control treatment bags. Red lines show high treatment bags. Blue lines show drift treatment bags. Diamonds represent bags 1-3, squares represent bags 4-6 and triangles represent bags 7-9. The black dotted line represents the dock.

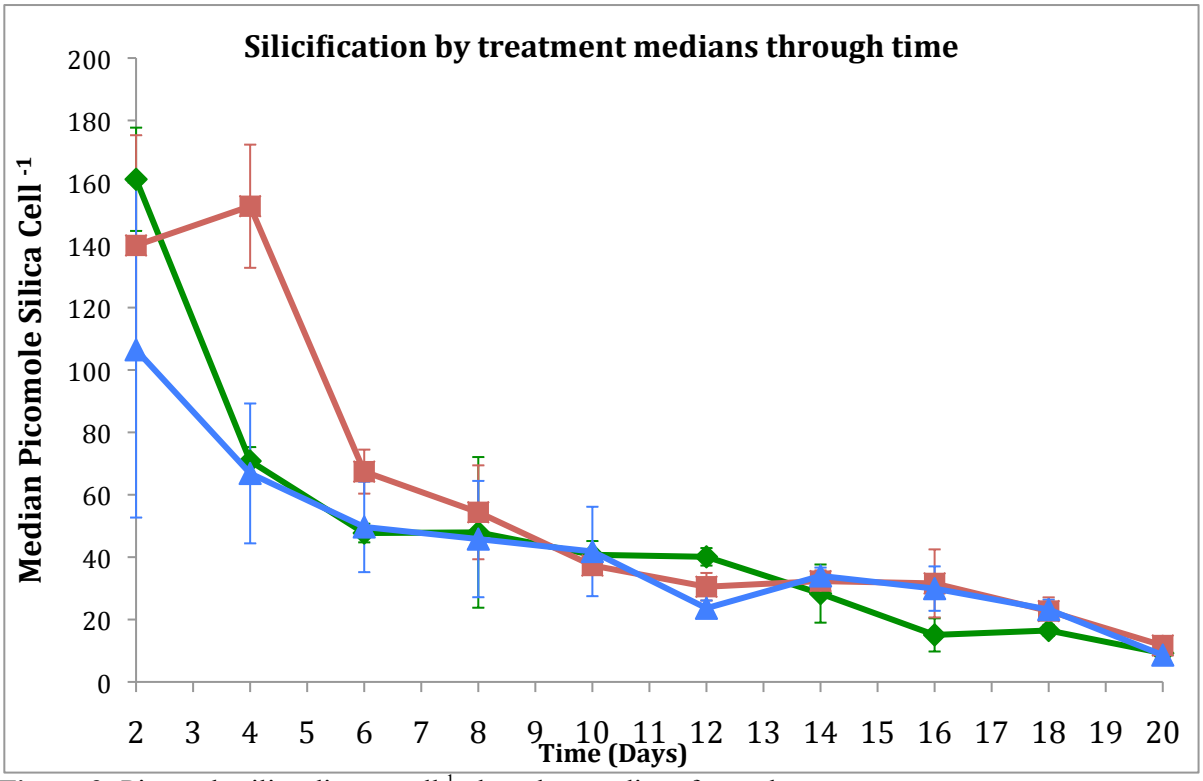

Figure 3: Picomole silica diatom cell^{-1} plotted as medians for each treatment group through time. Green diamonds represent control treatment medians. Red squares show high treatment medians. Blue triangs show drift treatment medians.

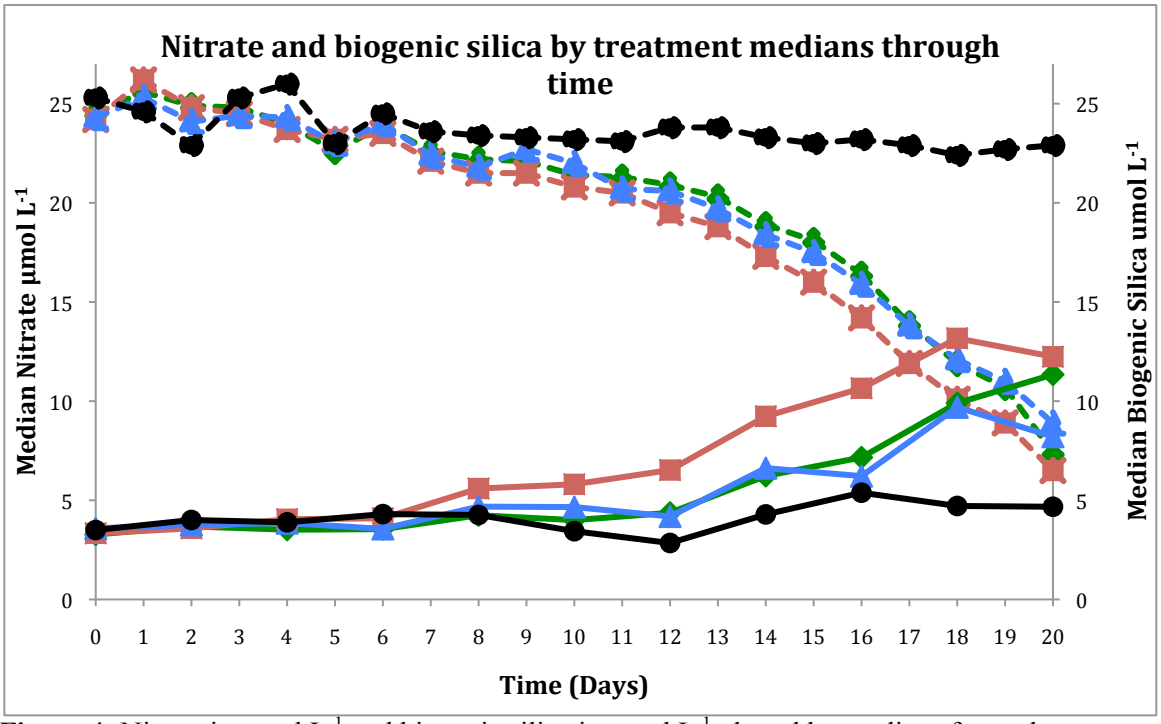

Figure 4: Nitrate in µmol L^{-1} and biogenic silica in µmol L^{-1} plotted by medians for each treatment through time. Dashed lines show nitrate concentrations with black circles being the dock sample, green diamonds showing control treatment, red squares showing high treatment and blue triangles showing drift treatment. Solid lines indicate biogenic silica concentrations, with the same symbols for treatments as nitrate.

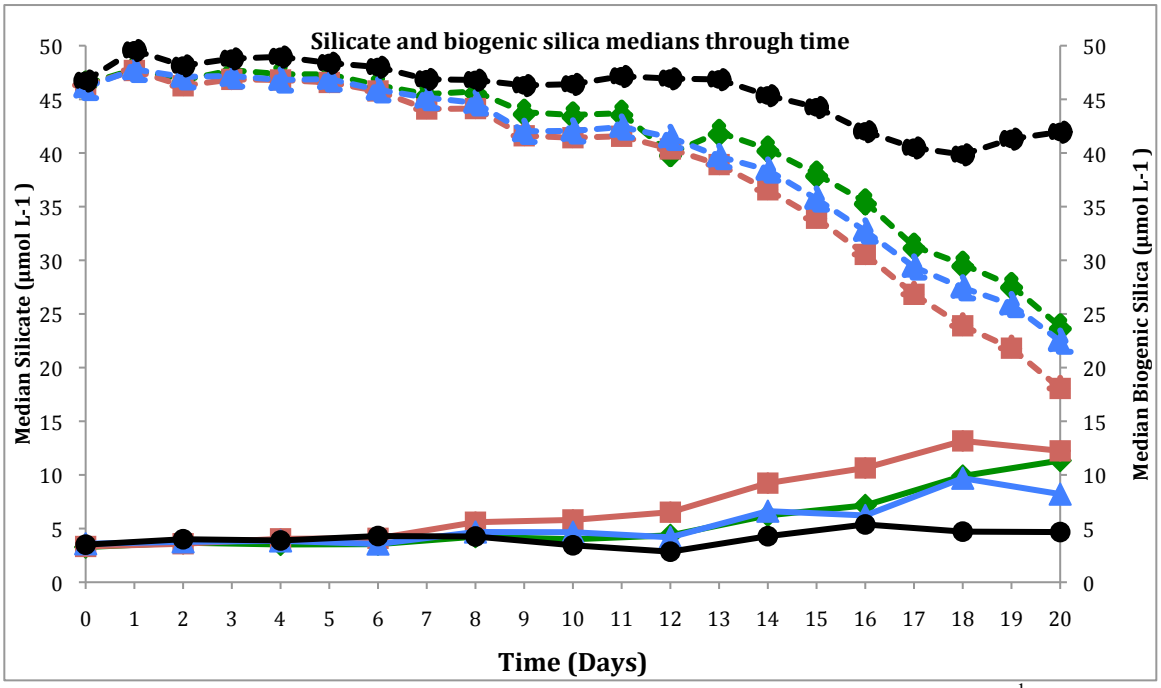

Figure 5: Median silicate concentrations and biogenic silica concentrations in µmol L^{-1} plotted through time. Green diamonds represent control treatment, red squares represent high treatment and blue triangles represent drift treatment. The dashed lines show silicate concentrations and the solid lines show biogenic silica concentrations.

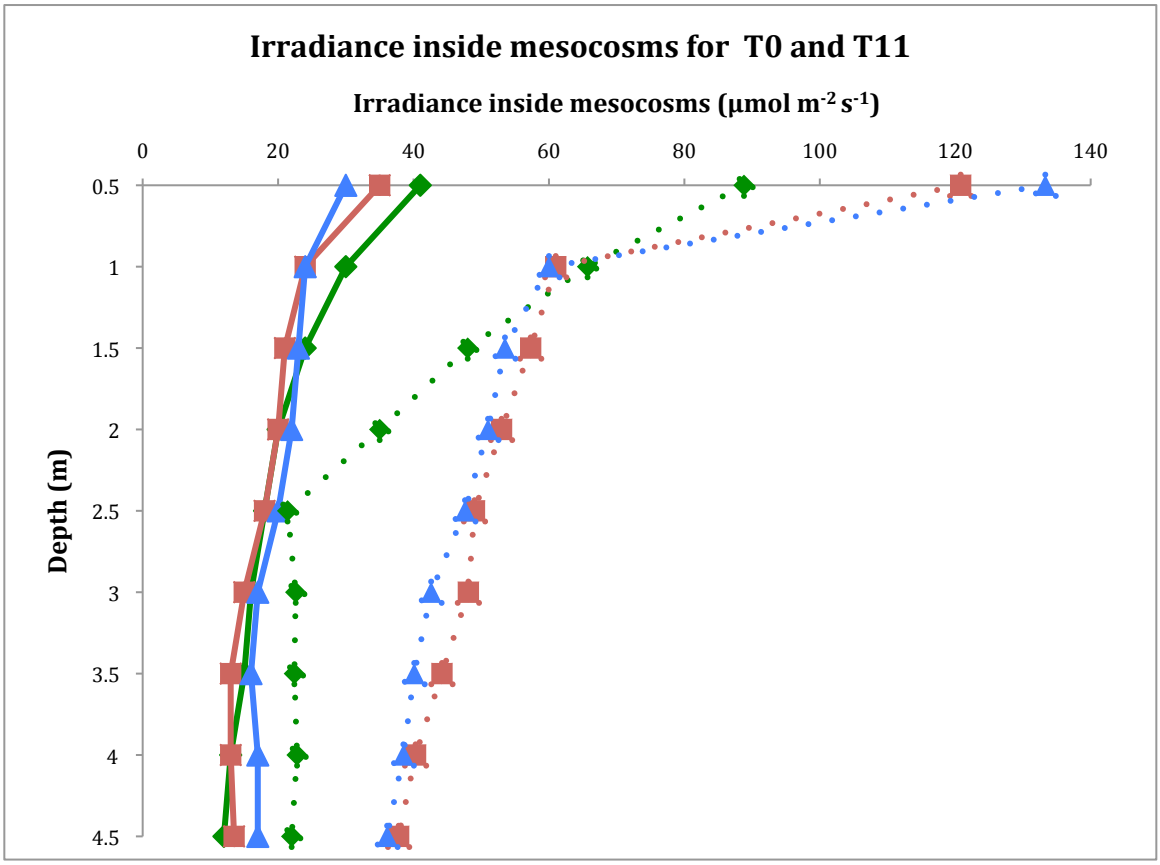

Figure 6: Irradiance for each treatment (averaged) plotted versus depth. Solid lines show light levels inside mesocosms for T0 with mesh shower caps in place. Dashed lines show light levels inside mesocosms for T11 after mesh shower caps have been removed.

Table 1: Shows the results of Spearman correlation coefficients (ρ) by treatment for nitrate and silicate. The closer the number is to -1 or 1, the better the association between variables.

A. Nitrate (Murray et al., 2013) and Biogenic Silica	Control Treatment	High Treatment	Drift Treatment
Correlation Coefficient **(ρ)**	**-.923**	**-.913**	**-.884**
B. Silicate (Murray et al., 2013) and Biogenic Silica	Control Treatment	High Treatment	Drift Treatment
Correlation Coefficient **(ρ)**	**-.939**	**-.897**	**-.854**

Table 2: Shows Spearman correlation coefficients (ρ) for different variables tested by treatment. The closer the number is to -1 or 1, the better the association is between variables.

A. Silicification and Grazing Rate (Govenar 2013)	Control Treatment	High Treatment	Drift Treatment
Correlation Coefficient **(ρ)**	**.382**	**-.054**	**N/A**
B. Biogenic Silica and Phytoplankton cells mL^{-1} (Stephens 2013)	Control Treatment	High Treatment	Drift Treatment
Correlation Coefficient **(ρ)**	**.887**	**.898**	**.786**
C. Biogenic Silica and Chlorophyll (Porcino 2013)	Control Treatment	High Treatment	Drift Treatment
Correlation Coefficient **(ρ)**	**.945**	**.945**	**.889**
D. Silicification and pCO_2 (Murray et al. 2013)	Control Treatment	High Treatment	Drift Treatment
Correlation Coefficient **(ρ)**	**.506**	**.514**	**.592**
E. Biogenic Silica and Bacterial Abundance (Apple 2013)	Control Treatment	High Treatment	Drift Treatment
Correlation Coefficient **(ρ)**	**.780**	**.586**	**.096**

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