The effects of nutrient additions to phytoplankton size structures based on chlorophyll in the Equatorial Pacific Ocean

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Abstract

Phytoplankton, the primary producers of ocean, have produced over half of the oxygen in the atmosphere and use dissolved carbon dioxide to accumulate biomass which is critical to the biological pump. In High-Nutrient, Low-Chlorophyll zones such as the equatorial Pacific, smaller phytoplankton (<10 µm) are more abundant than larger phytoplankton (>10 µm). The composition of size structure of phytoplankton communities changes with the abundance of nutrients, due to larger phytoplankton having greater nutrient needs. The addition of nutrients can give insight into how the biological pump may be affected by changing conditions in the ocean, as larger size classes of phytoplankton have faster sinking rates. In incubation experiments conducted on the TN413 cruise, size-fractionated chlorophyll concentrations were compared between treatments of different nutrients as well as nutrient-rich water from below the mixed layer to simulate upwelling in the region. I hypothesize that an increase in nutrient availability will result in greater abundance of larger phytoplankton due to nutrient co-limitation. The addition of deep water had the largest affect on chlorophyll concentration measured across all size classes in the incubation experiments while the addition of nitrate, silicate, and iron with deep water had little observable effect on chlorophyll concentrations. Results of added nutrients to incubations were contrasting, with phytoplankton larger than 10 µm increasing in abundance in incubations from the equator and smaller phytoplankton less than 10 µm dominating at 5° North, while at 5° South almost no growth was observed. Co-limiting nutrients are strongly implied, although future work at this location without deep water additions to incubations is necessary to determine what nutrients are limiting in this area to better understand how the biological pump may be affected by nutrient availability.

Plain Language Summary

Phytoplankton are microscopic organisms that produce much of the oxygen in the atmosphere. They are extremely important in a process known as the biological pump, where carbon is exported throughout the ocean. Some of this carbon is exchanged into the atmosphere but a portion is also exported to the depths of the ocean, known as carbon sequestration. Understanding how the biological pump works is key to understanding how climate change may affect us in the future. The size of phytoplankton determines how fast it sinks when it dies, which affects how much carbon is exported. This study aims to better understand how phytoplankton sizes change in a community based on nutrient availability. Samples of surface water where phytoplankton are most abundant were added to incubation bottles with different nutrients added and they were allowed to grow. Chlorophyll concentration was used as a proxy for phytoplankton growth and was separated into different size classes. Overall, phytoplankton growth was not limited by a single nutrient alone, and water upwelled from depth contained all the nutrients needed for growth. Changes in the size of phytoplankton with nutrient additions was inconclusive and further work is necessary to understand the role of nutrient availably in the carbon cycle and climate change.

Introduction

Phytoplankton are the autotrophic primary producers of the ocean that use sunlight for energy to fuel cellular functions and grow. Phytoplankton biomass is typically measured by chlorophyll concentrations, the structure responsible for the conversion of energy from sunlight into energy that can be harnessed by the cell. Because phytoplankton are primary producers, they have a pivotal role in the marine food web (Honjo et al., 2014). Using the energy from the sun, phytoplankton convert dissolved inorganic CO2 as well as other inorganic macronutrients such as nitrate to create biomass and release oxygen as a byproduct. The organic matter produced by phytoplankton can then be used up the food web through consumption as well as by bacteria consuming the byproducts or excretions of phytoplankton in the microbial loop (Pomeroy et al., 2007).

The biological pump is the combination of physical, chemical, and biological processes that drive the circulation of carbon throughout the ocean (De La Rocha & Passow, 2007). Carbon dioxide is first brought into the surface ocean from the atmosphere in the form of dissolved inorganic carbon through surface gas exchange. The organic matter produced by phytoplankton is consumed at higher trophic levels. Only 1-3 percent of primary production sinks to depth; most is recycled at different trophic levels and in the microbial loop or respired in the form of dissolved carbon dioxide (De La Rocha & Passow, 2007; Murray et al., 1994). This overall process of the biological pump results in a small amount of carbon being pumped from the atmosphere to depth, effectively sequestering carbon (Honjo et al., 2014).

Phytoplankton can be grouped into different classes by size which can be determined through size-fractionated chlorophyll experiments, where samples of seawater are passed

through different sizes of mesh and the concentrations of chlorophyll can be determined, giving a proxy for phytoplankton abundance. The proportion of size classes of phytoplankton in the community after size fractionation is important for better understanding the biological pump. Different proportions of phytoplankton size classes in the community can affect the overall sinking rates in the water column. The sink rate of organic matter can affect the biological pump because higher sink rates result in less time for organic matter to be reused before it sinks to depth. Sinking rates of phytoplankton or organic matter are determined in large part by size, with larger cells sinking faster than small cells (De La Rocha & Passow, 2007). Higher sinking rates result in less organic matter that stays in the photic zone, the layer of water that light penetrates and there are therefore less nutrients available in the food web. Therefore, when phytoplankton communities are made up of dominantly small phytoplankton, overall sinking rates are low. This means that more organic matter stays in the upper water column to be recycled. Higher sinking rates due to communities of larger phytoplankton can result in more organic matter sinking to the deep ocean, resulting in the sequestration of carbon and organic matter through the biological pump (Maranon et al., 2001).

The equatorial Pacific Ocean is within a high-nutrient, low-chlorophyll zone (subsequently referred to as HNLC) (Edwards et al., 2003). In HNLC zones, phytoplankton growth is limited by the trace nutrient iron despite levels of macronutrients nitrate, phosphate, and silicate high enough to allow greater phytoplankton growth rates (Marchetti et al., 2010). These macronutrient levels are relatively high due to phytoplankton being limited by iron in the area, and thus cannot make use of the macronutrients.

Nutrients are supplied to the equatorial Pacific through upwelling from the easterly Equatorial Under Current (EUC) as well as from the South Pacific Equatorial Current, and the EUC current is the main source of iron to the region (Aufdenkampe and Murray, 2002). The EUC is affected by El Nino/Southern Oscillation (ENSO), a climate pattern characterized by the strengthening or weakening of trade winds and upwelling in the western equatorial Pacific. The EUC is strongest during La Nina and weakens and can halt during El Nino periods (Hayashi et al., 2020). At the time of the cruise, this oscillation was in transition between three consecutive La Nina periods to a strong El Nino (National Oceanographic and Atmospheric Administration, 2023).

In this type of environment, phytoplankton with smaller cell sizes are more abundant due to a higher nutrient uptake efficiency (Marchetti et al., 2010). Smaller phytoplankton are better at bringing in necessary trace nutrients such as iron because they have a higher surface area to volume ratio. This relatively high surface area allows small phytoplankton to bring in iron more easily than larger cells (Pomeroy et al., 2007). Iron is a necessary nutrient for phytoplankton growth because of the central role it has in metabolic reactions within phytoplankton cells to produce energy, most notably as an electron carrier in the photosynthetic transport chain (Schoffman et al., 2016). Because of the iron limitation, the size structure of phytoplankton communities will have a greater proportion of smaller cell sizes (Landry et al., 1996). In iron-deficient zones that are HNLC, small phytoplankton sizes are dominant and restricted in biomass due to zooplankton grazing.

Diatoms dominate the microplankton size fraction (>10 μm) and are distinct from other classes of phytoplankton due to the silica shells they form. They account for one-fifth of global

primary production and are therefore significant contributors to the biological pump in the world's oceans (Armbrust, 2009). Additionally, because of their large size compared to other phytoplankton, diatoms have a high sinking rate and contribute a greater portion of biomass to organic matter sinking to the deep ocean. Because of the silica shells they form, diatoms have different nutrient needs than other types of phytoplankton. These silica shells result in a higher uptake of silicate from seawater than other classes of phytoplankton. Diatoms are less abundant in the equatorial Pacific Ocean due to their large cell size and this area is instead dominated by smaller phytoplankton (Marchetti et al., 2010).

Co-limitation of nutrients, the limitation on primary productivity and phytoplankton growth by more than one nutrient, may affect size-classes of phytoplankton differently due to larger nutrient demands for larger phytoplankton. Specifically, nutrient needs of diatoms may result in the limitation of growth for these organisms from more than just iron. In previous studies, diatoms were found to be limited in growth by both silicate and iron and growth was greatest in incubations with both nutrients added (Brzezinski et al., 2011).

Adding different nutrients to incubations of phytoplankton can help to determine which nutrients are limiting phytoplankton growth based on which nutrient additions result in phytoplankton blooms. Using size-fractionated chlorophyll concentrations before and after incubations can provide insight into the types of phytoplankton that respond to nutrient additions. An incubation with a large increase in phytoplankton greater than 10 µm in size with the addition of silicate and iron could indicate a bloom of diatoms. In experiments measuring nutrient concentrations after the addition of various nutrients to incubations of equatorial Pacific seawater, the largest change in bio nutrient concentrations occurred with the addition

of silicate and iron together (Marchetti et al., 2010). This is significant because a large diatom population affects the biological pump, increasing organic matter sinking through the mixed layer.

Phytoplankton in the equatorial Pacific are dependent on the upwelling of nutrients from below the mixed layer. Adding deep water to incubations provides all the nutrients that the phytoplankton communities collected at the surface would normally receive through upwelling but in much greater quantities. This ensures that phytoplankton communities have enough of all the different nutrients necessary for population growth and can amplify changes to chlorophyll concentrations between different treatments. With the addition of a large quantity of a single nutrient to incubations, high growth indicates a nutrient limitation that upwelling does not provide. Further separating this nutrient dependance by size class can help to determine the sizes of phytoplankton affected by nutrient limitations. The differences in size structures in phytoplankton communities can give insight into the biological pump.

I hypothesize that phytoplankton in the equatorial Pacific are co-limited by multiple nutrients and increasing nutrient availability will result in a shift in size structure towards larger phytoplankton (> 10 μm). In order to test this hypothesis, I compared chlorophyll concentrations after size-fractionation of multiple treatment groups of incubations from three locations in the equatorial Pacific.

Methods

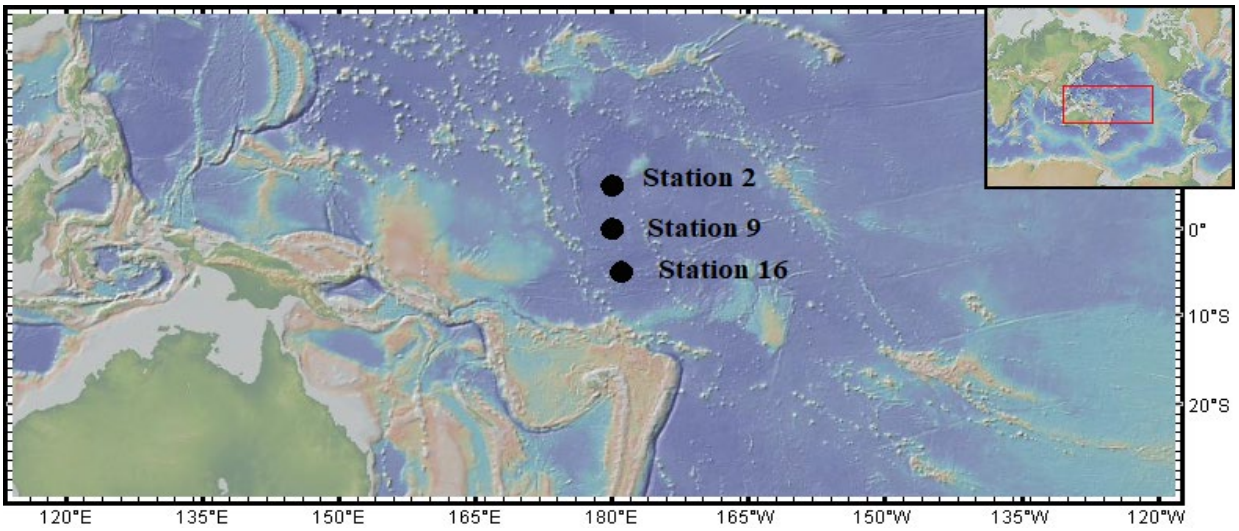

Figure 1 ***Figure 1.*** *TN413 station locations at which water samples were used for incubation experiments.*

All measurements were conducted onboard the *R/V Thomas G. Thompson* between March 3, 2023 and March 7, 2023. Water was collected for nutrient concentration measurements and incubation experiments during the TN413 hydrographic survey between 5 North and 5 South at nominally 180 (Figure 1).

Sea water for the incubation experiments was collected with a STAY Fish diaphragm pump from a depth of 10 meters to avoid trace metal contamination. Water was pumped directly into clear, sterilized 2L bottles through a 200 µm mesh filter at Station 2 and Station 16 while 4L bottles were used at Station 9. 15 bottles for incubations were prepared for each of the three stations.

A negative control treatment was used with no additions of nutrients or water from depth. All additional incubation bottles were treated with 5% high-nutrient seawater from depth to simulate upwelling. This deep water was collected using 10-liter Niskin bottles on a 24-bottle rosette from a depth of 2000 meters for both Station 9 and Station 16. For Station 2,

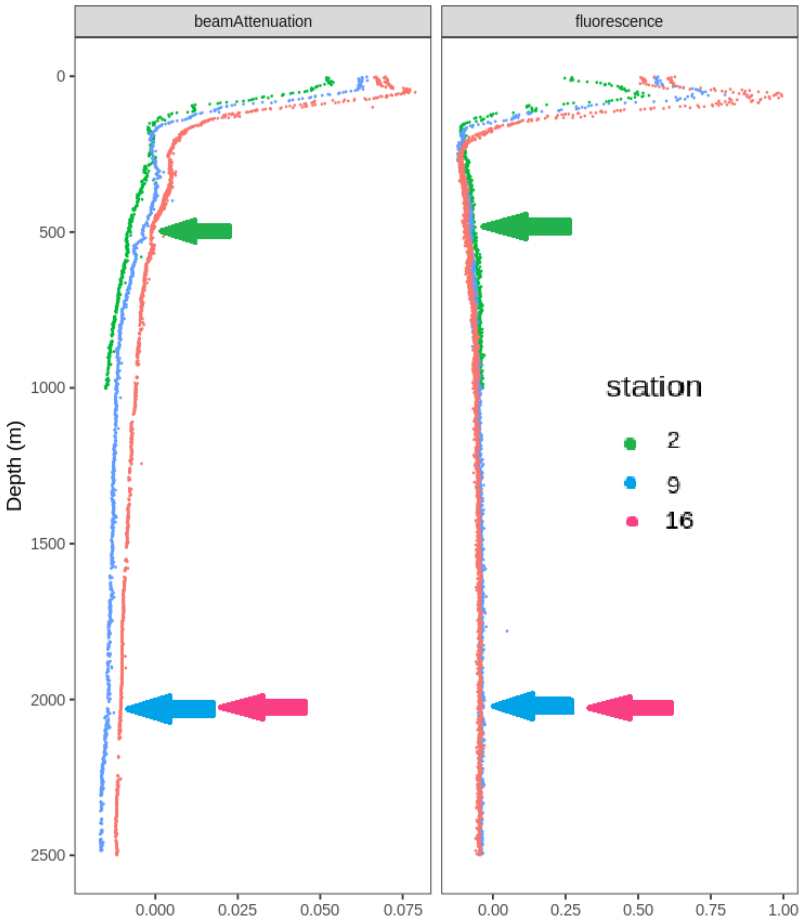

Figure 2. *Depth profiles of beam attenuation and fluorescence indicating the source of deep water used for nutrient additions to incubation treatments.*

water from a depth of 500 meters was used (Figure 2). Water from the same Niskin bottles were used to measure both the silicate and nitrate concentrations added to the incubation bottles for Station 2 and Station 9 but no nutrient data was collected at 2000 meters at Station 16. A positive control treatment contained only the deep water addition, with no other added nutrients. The three nutrient treatments were prepared with 5% deep water and either 2 mL of 5 mM nitrate, 1 mL of 0.03 M silicate, or 0.5 mL of 12 µM iron chloride. Each of the five treatments were prepared in triplicates, for a total of 15 incubations at the three stations.

Treatments were prepared immediately after water was collected from the pump and were then placed in clear incubation containers on the open deck with full sunlight access. Black mesh covered each container to block 70% of sunlight to simulate conditions at roughly

the depth at which incubation samples were taken. Additionally, each incubation container had surface sea water continually circulated into and out of the container to regulate temperature.

Size-fractionated chlorophyll measurements

Initial chlorophyll measurements were not made for each individual incubation bottle due to water volume constraints. Initial size-fractionated chlorophyll measurement for each of the three stations was made using water collected from the STAY fish pump in triplicates. Size-fractionated chlorophyll measurements were taken after incubating for 72 hours for Stations 2 and 16 (t=3). For the incubations from Station 9, three size-fractionated chlorophyll measurements were taken after 58, 78 and 105 hours (t=2, t=3, and t=4).

0.5 liters of water taken directly from each incubation bottle, or from the STAY fish pump for initial measurements, was passed through 10 µm, 3 µm, and 0.2 µm filters. For Station 9 and 16, the final size-fractionated chlorophyll measurements were made using 0.3 liters of water due to a high chlorophyll concentration in the incubation bottles. After filtration, the filters were immediately placed in 90% acetone and placed in freezer for 24 hours. These samples were then vortexed and placed in centrifuge for ten minutes to resuspend the chlorophyll. Samples were then placed in a TD700 Fluorometer (Turner Designs, USA). After the first fluorometer reading, three drops of hydrochloric acid were added to the samples and then a second reading was taken. Equation 1 was used to calculate chlorophyll concentrations for each sample.

Equation 1. *Chl-a = K * (Fm/(Fm-1)) * (F_0 – F_A) * (Extraction volume / filter volume)*

Where K is the response factor from calibration calculations specific to the individual instrument, Fm is the acidification coefficient, F_0 is the reading before acidification and F_A is the reading after acidification.

Average chlorophyll concentrations were calculated for each triplicate incubation condition for each station to generate figures. T-tests were used to calculate significance between treatment groups.

Results

The nitrate concentration of the deep water added to incubation bottles for Station 2 was 54.59 µM. The resulting concentration of nitrate from 5% deepwater for was 2.73 µM. Average silicate concentration was obtained from Scripps Institute of Oceanography for all incubations due to inaccurate shipboard measurements. At 5 North, average silicate concentrations at 500 meters are approximately 40 µM, resulting in a concentration of 2.0 µM silicate for all incubations except the control at Station 2. For the nitrate treatment, the concentration of nitrate was about 8 µM, almost triple the concentration of the 5% deepwater treatment. The silicate nutrient spike resulted in a silicate concentration of just over 15 µM, 7.5 times the silicate added from deep water alone. The concentration of nitrate in deep water added to the incubations at Station 9 was 33.1 µM, resulting in 1.7 µM nitrate in the bottles. The nitrate treatment at this station resulted in a 6.7 µM nitrate concentration, about four times greater than the other treatments. The average concentration of silicate from a depth of 2000 meters at the equator was 138 µM resulting in 6.9 µM silicate in the incubations with 5% deepwater added. The silicate spike treatment resulted in a concentration more than twice the concentration of the deep water additions only.

Initial chlorophyll concentrations for all size classes at each station incubations were performed were below 0.10 µg/L apart from chlorophyll measured at Station 16 in the 0.2-3 µm size range which was 0.16 µg/L (Figure 3). At Station 16, chlorophyll concentrations remained consistent from initial to final concentrations (Figure 3C). The chlorophyll concentrations in the

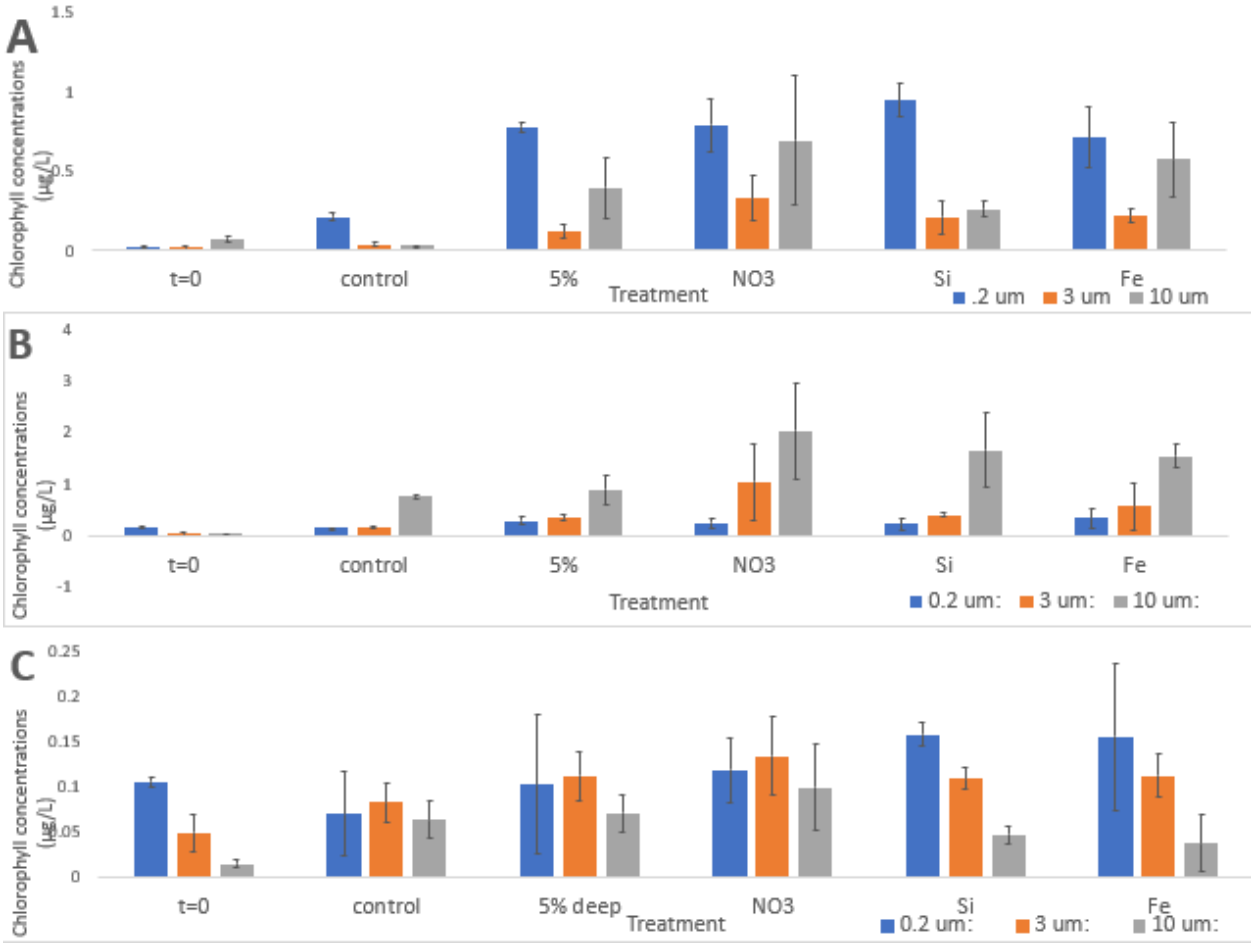

Figure 3. *Chlorophyll concentrations by size class at beginning of incubations (t=0) and for each incubation treatment for Station 2 after 72 hours (A), Station 9 after 105 hours (B) and Station 16 after 72 hours (C). Note the different y-axis scales for each panel.*

0.2-3 µm size class from Station 2 increased significantly for all incubation treatments, although no significant change was observed between the positive control (5% deep water) and the

three nutrient spike incubation treatments that were also prepared with 5% deep water (Figure 3A). Incubations from Station 9 at the equator all had significantly higher chlorophyll concentrations in the greater-than 10 µm size class between both the control treatments and the three incubations with added nitrate, silicate and iron, although there was no statistical difference in chlorophyll concentrations between the three nutrient-spiked incubations.

Incubations with water samples from Station 9 were done in 4 liter bottles, allowing for more size-fractionated chlorophyll concentration measurements to be taken over a period of about 4 days (105 hours total). Between the measurements taken at 58 and 105 hours, the chlorophyll concentration in the 0.2-3 µm size class declined from a maximum of 0.3-0.4 µg/L in each of the five treatment groups (Figure 4A). The control and silicate treatments had a steady decline in chlorophyll concentration while the 5% deep water and nitrate treatments dropped in chlorophyll concentration before rising slightly again, although less

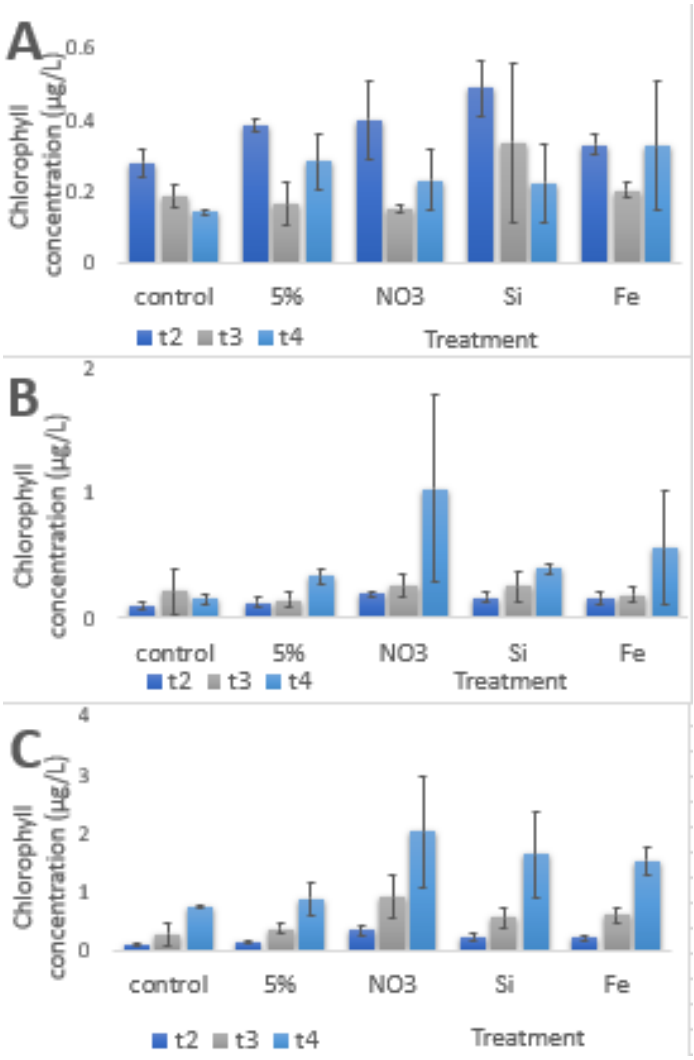

Figure 4. *Size-fractionated chlorophyll concentrations over time from Station 9 incubation for size classes of 0.2-3 µm (A), 3-10 µm (B), and >10 µm (C). Note the different y-axis scales. T2, t3, and t4 measurements were taken after 58, 78, and 105 hours respectively.*

than the concentration measured after 2 days. There was an initial drop of chlorophyll concentration for the iron treatment before increasing at on the final measurement to similar levels from 2 days into the incubation. The chlorophyll concentrations for the 3-10 µm size class increased slightly between the 58- and 78-hour measurements and were similar in value across all five treatments (Figure 4B). This size class spiked in chlorophyll concentration across all treatments except the control, with the largest increase in the nitrate treatment. The silicate and iron treatments both increased more than the 5% deep water on the final size-fractionation chlorophyll concentration measurement. The size class greater than 10 µm uniformly increased in chlorophyll concentration over time, with the nitrate, silicate and iron treatments all increasing significantly more and both the control and deep water treatments (Figure 4C).

The initial proportion of chlorophyll concentration in the size class over 10 µm was three times larger than the proportion of chlorophyll from either of the other two size classes for Station 2 incubations. This ratio of chlorophyll concentrations reversed in all treatments in the

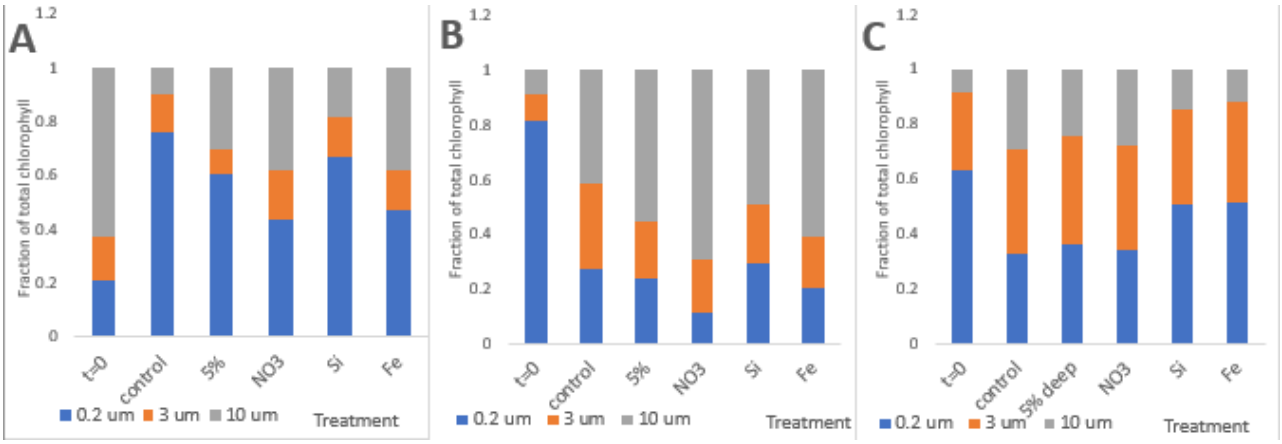

Figure 5. *Fraction of chlorophyll in each size class to the total chlorophyll concentration for each incubation treatment from Station 2 (A), Station 9 (B), and Station 16 (C). Proportions of chlorophyll concentrations reflect measurements made after 72 hours of incubation for Stations 2 and 16, 78 hours for Station 9.*

final size-fractionated chlorophyll measurements (Figure 5A). At Station 9, the reverse was observed: 80% of chlorophyll was initially in the 0.2-3 µm size class. After 78 hours, the size class over 10 µm was dominant for all treatments except the control, making up 50-70% of total chlorophyll concentrations (Figure 5B).

Discussion

Initial chlorophyll concentrations were low across all three stations, consistent with expectations in a HNLC area. Water collected from 5 North at Station 2 initially had a high concentration of chlorophyll from the larger size class class while high initial concentrations of chlorophyll in small size classes followed expected results in water collected from the equator and 5 South. The highest initial concentration of chlorophyll from Station 2 was observed in samples filtered for phytoplankton over 10 µm. This may be because of higher concentrations of nutrients at this location which would favor larger phytoplankton. Compared to the other two incubation experiments, the incubations at Station 9 had much higher chlorophyll concentrations, most likely due to a longer incubation time. Chlorophyll concentrations from the largest size class increased the most across all treatment groups, consistent with expectations in a high nutrient environment, especially with the addition of iron (Schoffman et al., 2016). Low nutrient environments favor smaller phytoplankton that are more efficient at nutrient uptake. Little to no phytoplankton growth occurred in incubations from Station 16 relative to the control group. Initial chlorophyll concentrations were on the same order of magnitude as the other stations. The lack of growth observed in these incubations could be due to low nutrient concentrations in the deepwater added to the treatment groups. It could also be explained by predation, although it is unlikely that every treatment group would be so

similar. The three incubation experiments had three different trends in the size composition of phytoplankton populations over time with the addition of nutrients, making it impossible to draw conclusions about how nutrient availability affects the size composition of phytoplankton. Due to the much higher proportion of chlorophyll (60-80%) in the 0.2-3 µm size fraction in initial measurements from water collected at the equator and 5 South, larger size fractions of phytoplankton are most likely limited by nutrients at both sites. Initial measurements of size fractionated chlorophyll from water collected at 5 North indicate that phytoplankton are not limited as heavily at this site due to the higher proportion of chlorophyll measured in the greater than 10 µm size class.

Nutrient treatments did not result in a significantly higher chlorophyll concentration compared to the deep water treatment. The nutrient spikes added to the nitrate, silicate and iron treatment groups were several times higher than the nutrients added from the deep water; nutrient limitations would result in large chlorophyll concentrations compared with the deep water treatment. The lack of significant increases in chlorophyll concentrations between nutrient treatments and the deep water treatment indicates that the deep water added to the incubations from depth had the greatest impact on phytoplankton growth. This implies that phytoplankton growth is limited in the equatorial Pacific by more than one nutrient. Additionally, a lack of significant increase in chlorophyll concentrations between treatment groups indicates that phytoplankton had not reached a point during incubations where the necessary nutrients had been exhausted. This is supported by the general trend of increasing chlorophyll concentrations over time in incubations from water collected at the equator across all treatments and size classes. If one or more nutrients that limited phytoplankton growth had

been used up during incubations, expected chlorophyll concentrations would level off or crash as the nutrient was depleted. Increased chlorophyll concentrations over time during incubations indicate that the phytoplankton had all the nutrients required for growth. Continuing incubations for a longer period of time until nutrients were used up would likely result in a greater difference in chlorophyll concentrations between treatment groups.

The size class composition of phytoplankton was relatively constant between treatments, including the control, for each incubation experiment in final measurements. Because the addition of various nutrients and deep water caused different reactions at different sites but were consistent between treatments, the initial population composition most likely determines how the size composition of phytoplankton populations changes, rather than the addition of nutrients.

The effects of the availability of any single nutrient on the biological pump cannot be determined from the results of this study due to the high variability of incubation experiments between stations. However, the consistent impact of deep water additions to the incubations on chlorophyll concentrations indicates that the rate of upwelling in the equatorial Pacific could be a controlling factor on the carbon cycle in the area, rather than a single nutrient such as iron. A decline in upwelling in this location may result in a lower phytoplankton abundance as well as abundance of larger phytoplankton, decreasing the effectiveness of carbon sequestration in the biological pump.

Conclusions

Additions of deep water in incubations accounts for all the nutrients needed for phytoplankton growth in the equatorial Pacific. Adding nitrate, silicate or iron separately to

incubations with deep water did not result in any increased chlorophyll concentration. Phytoplankton population size structures changed over time differently with samples from different locations near the equator and could not be attributed to a single factor. The delivery of nutrients through upwelling limits phytoplankton growth more than any single nutrient in the equatorial Pacific and phytoplankton are co-limited by multiple nutrients, although this study could not determine which nutrients are co-limiting.

Changes in the size composition of phytoplankton can impact the efficiency of the biological pump and further research is needed to further understand the factors that contribute changes in size structure. To better understand these factors, more incubations from the equatorial Pacific can be performed with nutrient concentration sampling during incubations. This would give insight into nutrient uptake and combined with size-fractionation experiments, determine how nutrient limitation affects phytoplankton size structures. Additionally, longer incubation times and larger volumes of incubations are suggested. For more clear results, incubations should include some treatment groups that do not include deep water additions as well as some treatments with multiple nutrients added to determine co-limitation.

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