The impact of varying nutrient concentrations on the growth and size distributions of phytoplankton populations within the western equatorial Pacific

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Abstract:

The purpose of this research is to identify how the surface water properties and population makeup of phytoplankton communities impact how their growth and size distributions respond to variations in nutrient concentrations. I conducted my research from 5 °N to 5 °S within the western Pacific during the March-February 2023 transition from La Nina to El Nino. Model nutrient data and surface water chlorophyll concentrations were used to provide context on how this transition in conditions impacted phytoplankton populations at different locations and timepoints. North to south trends along the transect displayed lower surface water chlorophyll concentrations and deeper mixed layers which is a clear indicator of changing surface water conditions. To gauge how varying nutrient additions impacted the size distributions and growth of phytoplankton populations I conducted incubation experiments on communities retrieved from the northernmost, equatorial, and southernmost station. The treatment groups I used in these experiments were a control group, a deepwater addition group, a deepwater addition paired with a silica addition group, and a deepwater addition paired with a nitrate addition group. Most of the treatment groups and size classes within the northernmost and equatorial station showed significant growth whereas the southernmost station exhibited far less growth. In contrast to the other stations, the southernmost station displayed a surprising lack of growth when exposed to higher nutrients. For the equatorial and northernmost stations, deepwater additions demonstrated the highest impact on phytoplankton growth within each of the nutrient addition groups. Overall, communities from the three locations of study showed a diverse response to changing nutrient conditions in terms of growth and shifting population size distributions.

Plain Language Summary:

The purpose of this research is to understand how varying nutrient conditions impact the growth and size distribution of phytoplankton populations. Phytoplankton are tiny organisms that lie in surface waters and are responsible for over 50% of our ocean's primary productivity. With this research, I took chlorophyll concentrations at various depths throughout the water column at different stations between 5 °N and 5 °S to quantify phytoplankton abundance. We correlated this chlorophyll data with model nutrient data to provide context on surrounding environment of the phytoplankton populations we sought to study. The results of this data showed a trend of lower surface water chlorophyll concentrations at the southern stations and higher nutrient concentrations closer to the equator. To understand how phytoplankton growth and size distributions responded to various nutrient conditions, I conducted incubation experiments on communities within the northernmost, equatorial, and southernmost station. My incubation set-up consisted of a bottle in which we filled with surface water, added different concentrations/types of nutrients, and let sit for 3-4 days. The treatment groups I used in these experiments were a pure surface water group, a deepwater addition group, a deepwater addition plus silicate addition group, and a deepwater addition plus nitrate addition group. After incubations were complete, we saw significant growth within the northernmost and equatorial station whereas the southernmost station displayed far less growth. Overall, we found that deepwater additions had the most significant impact on the northern and equatorial station whereas the southern station wasn't impacted by nutrient additions at all.

Introduction:

Phytoplankton play the vital role as primary producers within our oceans, accounting for 50% of our oceans' primary production and 50% of our global oxygen production (NOAA,

2023). Any organic matter they produce that isn't consumed at the surface waters will sink and provide sustenance for life at greater depths. If this sinking organic matter reaches the seafloor and is buried, it will serve as a sink for atmospheric carbon dioxide, aiding with climate regulation. As climate change continues to develop, ocean mechanisms will alter, leading to unstable and unpredictable conditions for phytoplankton populations (Bopp, et al., 2005). Current models have predicted that warming of our oceans will result in increased surface layer stratification, leading to decreases in surface water nutrient concentrations (Bopp, et al., 2005). Lower available nutrients will eventually induce lower phytoplankton counts and biomass, negatively affecting the overall health of the surrounding environment (Bopp, et al., 2005). Reductions in phytoplankton primary productivity will lead to elevated atmospheric carbon dioxide concentrations, compounding climate consequences (Dugdale and Wilkerson, 1998). These impacts of climate change are predictable at a global level but it's necessary to conduct further research to understand and predict responses to changing climates at a regional and community level.

Regionally based research on various phytoplankton communities and their responses to changing conditions is sparse but past studies have examined the responses of phytoplankton community growth to varying nutrient conditions. In 1988, a study was conducted within the western equatorial Pacific waters where scientists evaluated if upwelling silica controlled nitrate uptake within phytoplankton populations (Dugdale and Wilkerson, 1998). Through this research, nutrient concentrations were measured within the upper 200 meters of water to determine the role silicic acid played in nitrate uptake as well as the influence silicic acid and nitrate uptake had on primary production (Dugdale and Wilkerson, 1998). Initially the researchers hypothesized that limitations in iron availability may have a tangible impact on phytoplankton

growth, but it was instead concluded that silicic acid played the primary role in limiting the diatoms' productivity and nitrate uptake (Dugdale and Wilkerson, 1998). More recently, research was conducted studying nutrient limitations of diatom populations within the northwest Pacific (Browning, et al., 2022). The findings of this study uncovered that some populations of phytoplankton were exclusively limited by nitrogen while others were co-limited by iron and nitrogen (Browning, et al., 2022). Along the equator between 110º-140ºW, research was conducted, examining multiple nutrient concentrations and their correlation to the concentrations of chlorophyll and particulate inorganic matter (Brzezinski, et al., 2011). These correlations were used to assess the relationship between each nutrient and the primary productivity within the region (Brzezinski, et al., 2011). Later in this study, microcosm experiments were conducted using separate additions and co-additions of iron, silica, and germanium in a set of six experimental groups (Brzezinski, et al., 2011). The findings concluded that the phytoplankton populations grew the most when Fe-Si and Si additions occurred (Brzezinski, et al., 2011).

The main takeaway from these experiments is that there isn't a catch-all when it comes to how phytoplankton populations respond to changing conditions in our oceans. Different ecosystems and communities will express unique responses to shifting environmental conditions not only because they are diverse complex systems but because they are subject to a wide spectrum of physical processes and circulatory patterns. The lack of regional research focusing on the responses of phytoplankton populations and their responses to changing environmental conditions adds value to future research in this area. The western equatorial Pacific, a region that has been subject to limited research, is the focal point of this study. With this research, I examined how nutrient limitations in the future may impact the growth and size distributions of phytoplankton populations within this region. Research was conducted under changing La Niña

to El Niño conditions in which surface water nutrient concentrations become scarcer (Castro, et al., 2002). This phenomenon helped to simulate climate change scenarios where nutrient concentrations become less accessible for primary producers, due to factors like increased stratification of the oceans' surface layer (Intergovernmental Panel on Climate Change, 2022). To understand which nutrients have the most significant impact on phytoplankton concentrations, I conducted incubation experiments involving the analysis of phytoplankton communities within the northernmost (Station 2), equatorial (Station 9), and southernmost (Station 16) station along our equatorial transect (Fig. 1) and how they respond to varying nutrient conditions.

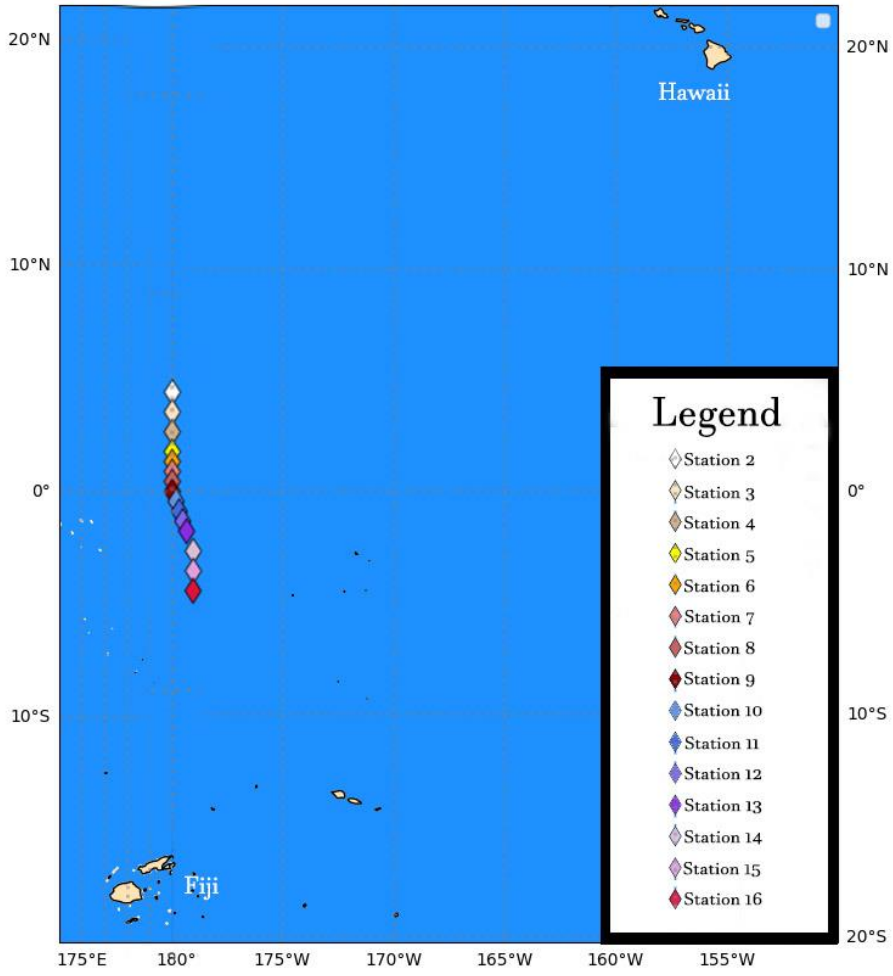

Fig. 1. A mapped out plan for the cruise that took place February 23rd, 2023, to March 11th, 2023.

A question I sought to answer through this research was, how do varying nutrient concentrations impact the concentrations and size distributions of phytoplankton communities within western equatorial

surface waters? To assess these impacts, I hypothesized that the equatorial station would host communities in which larger phytoplankton dominate due to increased upwelling of high nutrient deep water. In contrast to the equatorial station, I predicted that the northernmost and southernmost station would display smaller phytoplankton size distributions due to lower nutrient concentrations. I then hypothesized that when nutrients are added to said populations the size distribution will shift to be larger, as larger phytoplankton tend to thrive at nutrient rich environments (Pomati, et al., 2020). The nutrient I believed would have the most impact on growth was nitrate because past research in similar equatorial environments has shown nitrate to be the most significant factor when inducing biomass production (Thomas, 1969). Through my research, I was able to discern which additions had the largest impact on the phytoplankton growth of various size classes. Unfortunately, I failed to retrieve measurements of changing nutrient concentrations within the incubations, which limited my scope on which nutrients were limiting growth among each of the treatment groups. Regardless, I believe the information I retrieved is valuable and helps us to better understand the complexities of phytoplankton populations within the western equatorial Pacific.

Methods:

This study was conducted on a cruise that spanned from Honolulu, Hawaii, U.S.A. to Suva, Fiji from February 23rd – March 11th, 2023 (Fig. 1). All data collection, laboratory analysis, and incubation experiments occurred onboard the R/V Thomas G. Thompson. To obtain chlorophyll data, conductivity, temperature, and depth (CTD) casts were conducted at each station along the equatorial transect (Fig. 1). Each CTD cast retrieved physical water samples from 5-10 depths that ranged from surface waters to 500-5250 meters depth. For each one of these samples, 1-liter of water was run through a 25 mm diameter Whatman GF/F 0.7 µm filter

attached to a diaphragm pump which was engineered by the University of Washington. After filtration, I rinsed off the filtration container with milli-q water. Each filter then underwent the process of fluorometric chlorophyll analysis. The first step of this process involved placing the filter in an enclosed plastic tube filled with a 10 mL of acetone solution, then placed in a dark freezer for 24 hours. After 24 hours, the test tube was removed from the freezer and mixed with a Waters & Rogers Vari-Whirl for 15 seconds. Chlorophyll concentrations were determined with a TD-700 Fluorometer (Turner Designs). Prior an acetone only blank was run and the instrument was zeroed out. The liquid from the mixed chlorophyll/acetone solution was then poured into a clear test tube and placed into the fluorometer. Once the displayed values leveled out, I recorded the F0 value. I then added 3 drops of iodine solution to the chlorophyll/acetone solution in order to retrieve the Fa values. These values were then used to calculate the chlorophyll concentrations for the given sample.

Model data displaying depth profiles of silicate and nitrate concentrations at the specific location and day of each station was collected from CMEMS - Global Monitoring and Forecasting Centre. This data was used to correlate chlorophyll depth profiles to nutrient depth profiles.

The incubation experiments occurred at the northernmost (Station 2), equatorial (Station 9), and southernmost (Station 16) stations. The surface water retrieved for these incubations was pumped from a ~ 6 meter depth through a STAYFISH pump to prevent iron contamination from the ship. The water was pumped through a 20 μm filter into 12 2-L polycarbonate carboys for the northernmost (Stations 2) and southernmost (Station 16) stations and 12 4-L polycarbonate carboys for the equatorial station (Station 9). The 12 polycarbonate carboys were equally separated into a control group that only held STAYFISH water; a positive control group that was

made up of a 19:1 STAYFISH water to deepwater ratio; a nitrate addition group that was made up of a 19:1 STAYFISH water to deepwater ratio plus nitrate additions; and a silicate addition group that was made up of a 19:1 STAYFISH water to deepwater ratio plus silicate additions. For the nitrate addition group, I added 2 mL of a 5 mM nitrate solution to the incubations for the northernmost (Station 2) and southernmost (Station 16) stations and added 4 mL of the 5mM nitrate solution to the incubations for the equatorial (Station 9) station. These additions ended up adding 5 umol/liter of nitrate to each of the nitrate addition groups. For the silicate addition group, I added 1 mL of a 0.03 M silicate solution to the incubations for the northernmost (Station 2) and southernmost (Station 16) stations and added 2 mL of the 0.03 M silicate solution to the incubations for the equatorial (Station 9) station. These additions ended up adding 15 umol/liter of silicate to each of the silicate addition groups. The reasoning behind adding these specific concentrations was to match the silicate and nitrate concentrations found within the deepwater of this region. The deepwater additions for the incubations were retrieved from a CTD cast at each station. The depth of retrieval for deepwater additions of the northernmost (Station 2) and southernmost (Station 16) stations was 500 meters while deepwater additions for the equatorial station (Station 9) were retrieved from a depth of 2000 meters. Once all additions occurred, each polycarbonate carboy was placed into clear containers in which surface seawater circulated to keep phytoplankton at a surface water temperature. To prevent overexposure to light, a 70% light filter was placed over each container. Once the incubations were set, to obtain the initial chlorophyll concentrations of the 0.2 um, 3.0 um, and 10 µm size classes for each incubation, three separate 0.5 liter water parcels of STAYFISH water ran through three replicate sets of three filters attached to a diaphragm pump. These filters consisted of a 10 µm filter that the water ran through first; a 3.0 µm filter that the water ran through second; and a 0.2 µm filter that the water

ran through last. These were then processed through fluorometric chlorophyll analysis. After 72 hours (t=3 days) of incubating, each of the 12 incubations ran through the same steps that the initial STAYFISH water ran through in order to obtain the chlorophyll measurements and size distributions for the northernmost (Station 2) and equatorial (Station 16) stations. The equatorial station (Station 9) incubations underwent the same procedure as the northernmost (Station 2) and southernmost (Station 16) station, except measurements were taken at 58 hours (t-2) at 78 hours (t-3) and at 105 hours (t-4). To analyze the significance of the data, t-tests were conducted to understand if there were significant differences between data points representing chlorophyll concentrations at specific time points, stations, treatments, and size classes. I decided significant p-values lay below a value of 0.05. Measurement errors were not included in these calculations, which should be considered.

Results:

From station to station, the deep chlorophyll maxima varied in concentration and depth, overall showing a decline in surface water chlorophyll concentrations from north to south (Fig. 2). Model data indicated steady decreases in deepwater nitrate and silicate concentrations between the northernmost (Station 2) to the southernmost station (Station 16) (Fig. 3). Silicate and nitrate were the primary nutrients of interest as they represent two of the nutrient additions that were used in the treatment groups. Deepwater profiles were taken to better understand the nutrient concentrations that were being added through the deepwater additions. Throughout the transect, nitrate reaches its highest concentrations at >800 meters depth while the highest concentration of silicate lies at around 2500 meters depth (Fig. 3). Surface water nitrate and silicate concentrations reached their highest point at the equator (Station 9) and steadily decreased as the distance from the equator increased (Fig. 3). At the southernmost (Station 16)

and northernmost (Station 2) station, surface water nitrate concentrations were close to zero (Fig. 3).

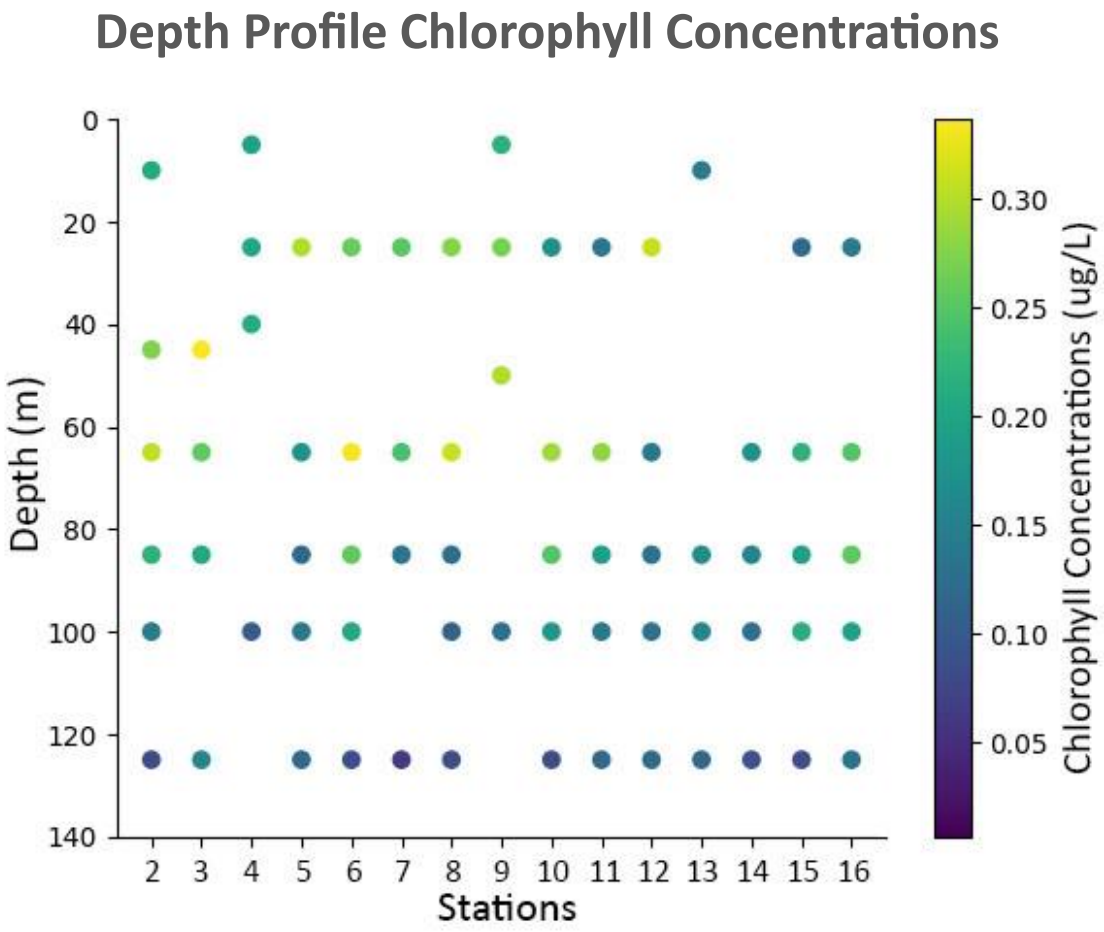

Fig. 2. Depth profiles of chlorophyll concentrations throughout the water columns of Stations 2-16. Station 2 represents the northernmost station at 5 degrees north while Station 16 represents the southernmost station at 5 degrees south.

To determine how phytoplankton populations responded to variations in nutrient concentrations at the northernmost station (Station 2), multiple phytoplankton communities exposed to varying nutrient conditions were incubated for 3 days. Differences between treatments and/or size classes were determined through a t-test for all following incubation results, where a p-value of less than 0.05 was deemed significant. Within these incubations, the 10 µm size class exhibited significantly higher (t-test: p-value < 0.05) chlorophyll concentrations

Model Profile Data

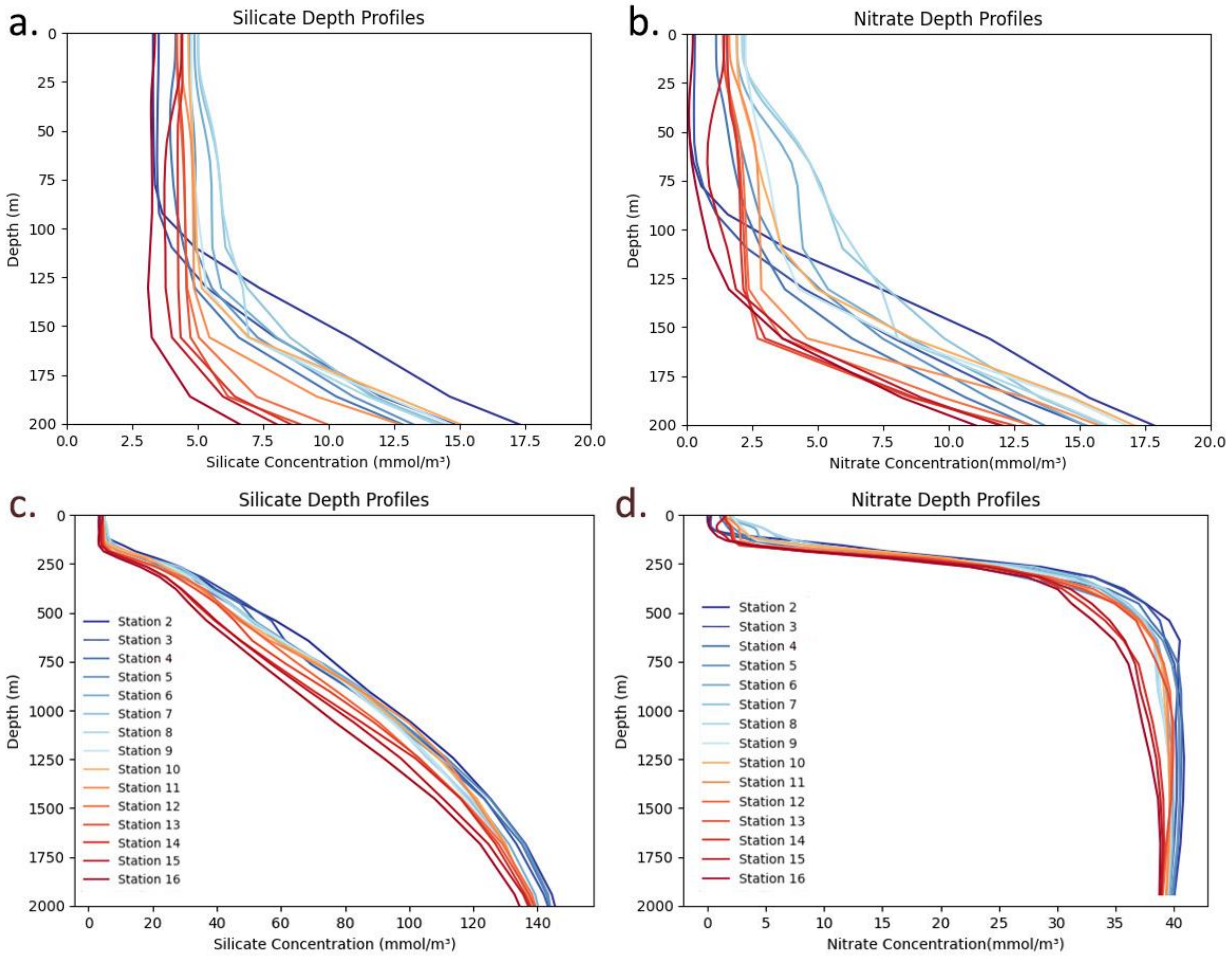

Fig. 3. Model silicate and nitrate profiles along stations 2-16 of the equatorial transect. Profile (a) was used to display silicate profiles with a higher resolution at the surface whereas profile (c) presented surface concentrations further down in the water profile in which deepwater additions were retrieved from. The same reasoning was used for profiles (b) and (d) in which the graphs were used to portray high resolution surface water and deepwater nitrate concentrations. Surface water nitrate concentrations were highest in closer proximity to Station 9 (the equator). Retrieved from the CMEMS - Global Monitoring and Forecasting Centre.

than the smaller size classes (Fig. 4). Within the 0.2 µm size class, each treatment group observed significant growth, while the 3.0 µm populations showed significant growth in every treatment group besides the control group (Fig. 4). Unlike the smaller size classes, the 10 µm populations exhibited a significant decrease in chlorophyll concentration within the control incubations and no significant change within the nitrate additions (Fig. 4). Like the other populations, the positive control and silicate additions within the 10 µm displayed significant increases in chlorophyll concentrations (Fig. 4). Compared to the control group, the 0.2 µm and 10 µm populations that were exposed to nutrient additions exhibited significantly higher chlorophyll concentrations (Fig. 4). Within the 3.0 µm size class, the positive control and nitrate addition group displayed significant increases in chlorophyll concentrations in comparison to the control group whereas the silicate addition group showed no significant difference (Fig. 4). When comparing the positive control to both the silicate and nitrate additions, there were no significant differences among any of the size classes (Fig. 4). Overall, the 0.2 µm size class displayed the highest chlorophyll concentrations after incubations occurred whereas the 3.0 µm populations showed the lowest concentrations (Fig. 4).

Unlike initial size distributions for the northernmost station (Station 2), the equatorial station (Station 9) incubations showed significantly higher levels of chlorophyll within the 0.2 µm populations than the larger size classes (Fig. 5). Within these 0.2 µm size classes, each treatment group displayed significant growth between t-0 and t-2 but showed no significant differences in concentration between t-0 and t-4 (Fig. 5). Rather than increasing after t-2, 0.2 µm size groups that experienced silicate additions decreased in chlorophyll concentration between t-2 and t-3 (Fig. 5). Likewise, nitrate addition treatments experienced decreases in chlorophyll concentrations between t-2 and t-4 (Fig. 5). For the 3.0 µm size class, the control, positive

control, and silicate additions exhibited significant growth in chlorophyll between t-0 and t-4, whereas the nitrate additions showed no significant growth (Fig. 5). Within the 10 µm size populations, every treatment group displayed significant growth between t-0 and t-4 (Fig. 5). When comparing the 0.2 µm size class chlorophyll concentrations of the control group to the other treatments, the positive control is the only treatment that exhibits significantly higher chlorophyll concentrations at t-4 (Fig. 5). The 3.0 µm size class's chlorophyll concentrations for the nitrate additions had no significant difference in concentration when comparing to the control group at t-4 but the silicate and positive control concentrations were significantly higher (Fig. 5). No treatment within the 10 µm size class had a significant difference in chlorophyll concentration in comparison to the control group (Fig. 5). There was no significant difference in chlorophyll concentrations when comparing the t-4 values of the positive control to the t-4 values of the silicate and nitrate additions within each size class (Fig. 5). Out of each size class, the 10 µm populations exhibited the highest concentrations at t-4 (Fig. 5).

Within the southernmost station (Station 16) incubations, the initial chlorophyll concentrations for the 0.2 µm size class were significantly higher than initial concentrations for the other size classes while the 10 µm size class held significantly lower concentrations than the other two size classes (Fig. 6). For the 0.2 µm populations, silicate was the only treatment group that displayed any significant growth (Fig. 6). 3.0 µm size classes showed significant increases in chlorophyll concentrations for all but the control group while the 10 µm size classes showed significant growth within every treatment group (Fig. 6). Within the 0.2 µm size class, silicate was the only treatment group to show significantly higher chlorophyll concentrations than the control group (Fig. 6). For the 3.0 and 10 µm size classes, the positive control, nitrate, and silicate additions displayed no significant differences in chlorophyll concentrations compared to

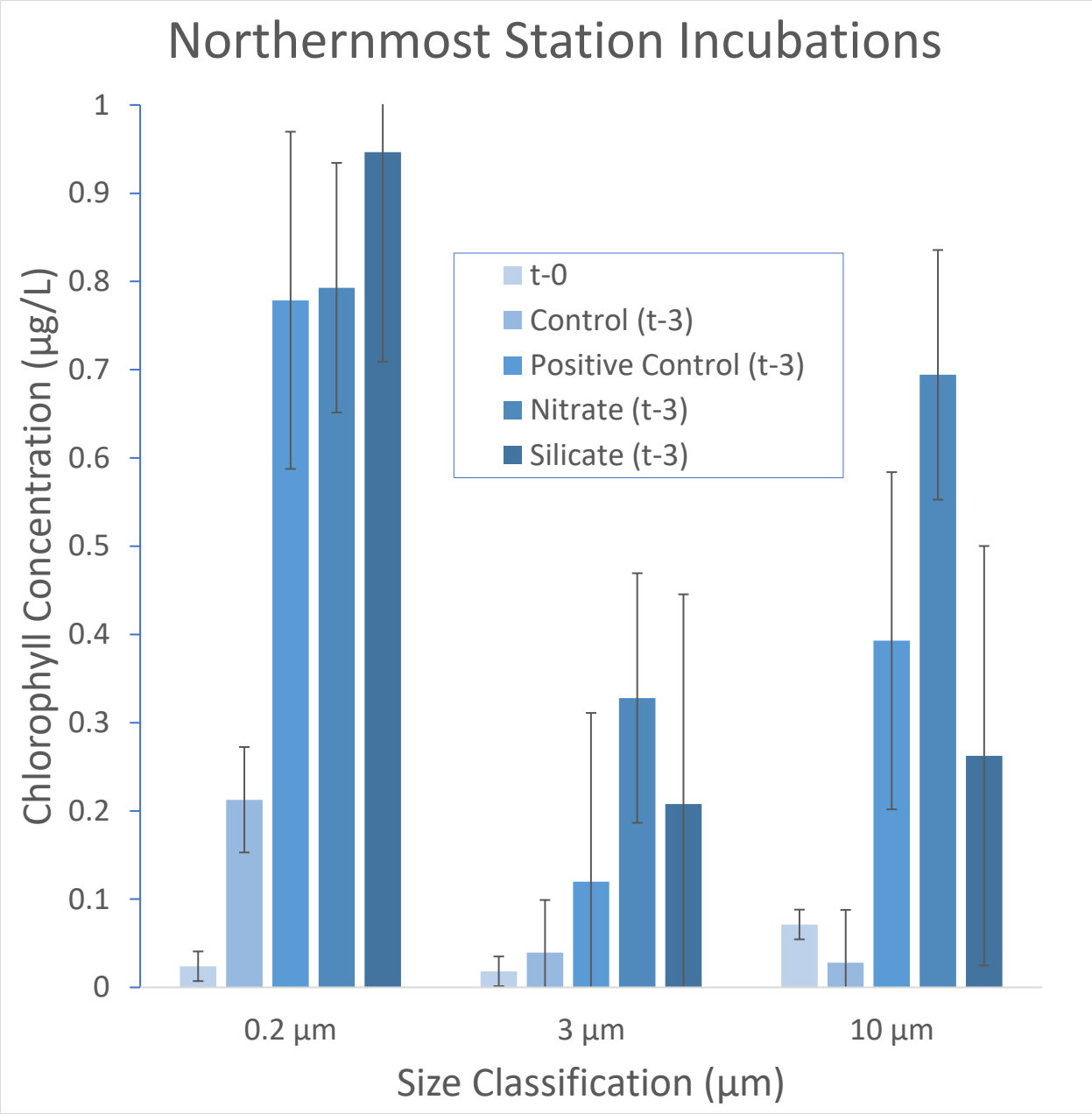

Fig. 4. Averaged out chlorophyll concentrations retrieved from station 2 incubation experiments. Incubation test groups consisted of control groups that comprised of STAYFISH water retrieved from 20 meters depth; positive control groups consisting of a 1:19 ratio of deepwater, retrieved from 500 meters depth, to STAYFISH water; nitrate addition groups consisting of a 1:19 ratio of deepwater, retrieved from 500 meters depth, to STAYFISH water as well as nitrate additions amounting to nitrate concentrations of 5 micromoles per liter of incubation water; and silicate addition groups consisting of a 1:19 ratio of deepwater, retrieved from 500 meters depth, to STAYFISH water as well as silicate additions amounting to silicate concentrations of 15 micromoles per liter of incubation water. “t-0” represents the initial chlorophyll concentrations of the STAYFISH water. The data is separated into three separate phytoplankton size classes, each color representing a separate incubation group.

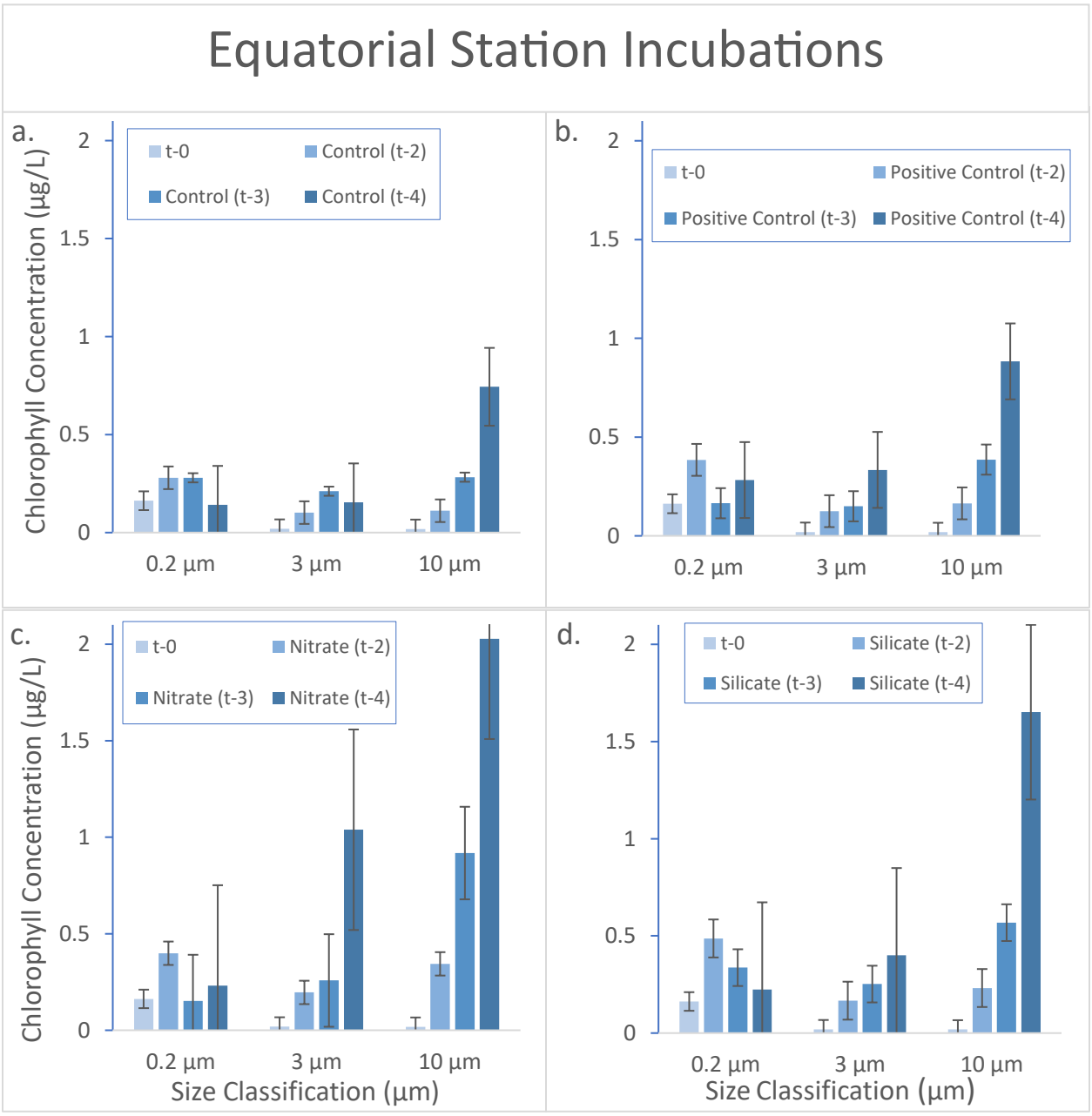

Fig. 5. Averaged chlorophyll concentrations retrieved from Station 9 incubation: (a) control groups that comprised of STAYFISH water retrieved from 20 meters depth; (b) positive control groups consisting of a 1:19 ratio of deepwater, retrieved from 2000 meters depth, to STAYFISH water; (c) nitrate addition groups consisting of a 1:19 ratio of deepwater, retrieved from 2000 meters depth, to STAYFISH water as well as nitrate additions amounting to nitrate concentrations of 5 micromoles per liter of incubation water; and (d) silicate addition groups consisting of a 1:19 ratio of deepwater, retrieved from 2000 meters depth, to STAYFISH water as well as silicate additions amounting to silicate concentrations of 15 micromoles per liter of incubation water. “t-0” represents the chlorophyll concentrations of the STAYFISH water. The data is separated into three separate size classes, each color representing a separate time point of chlorophyll measurement.

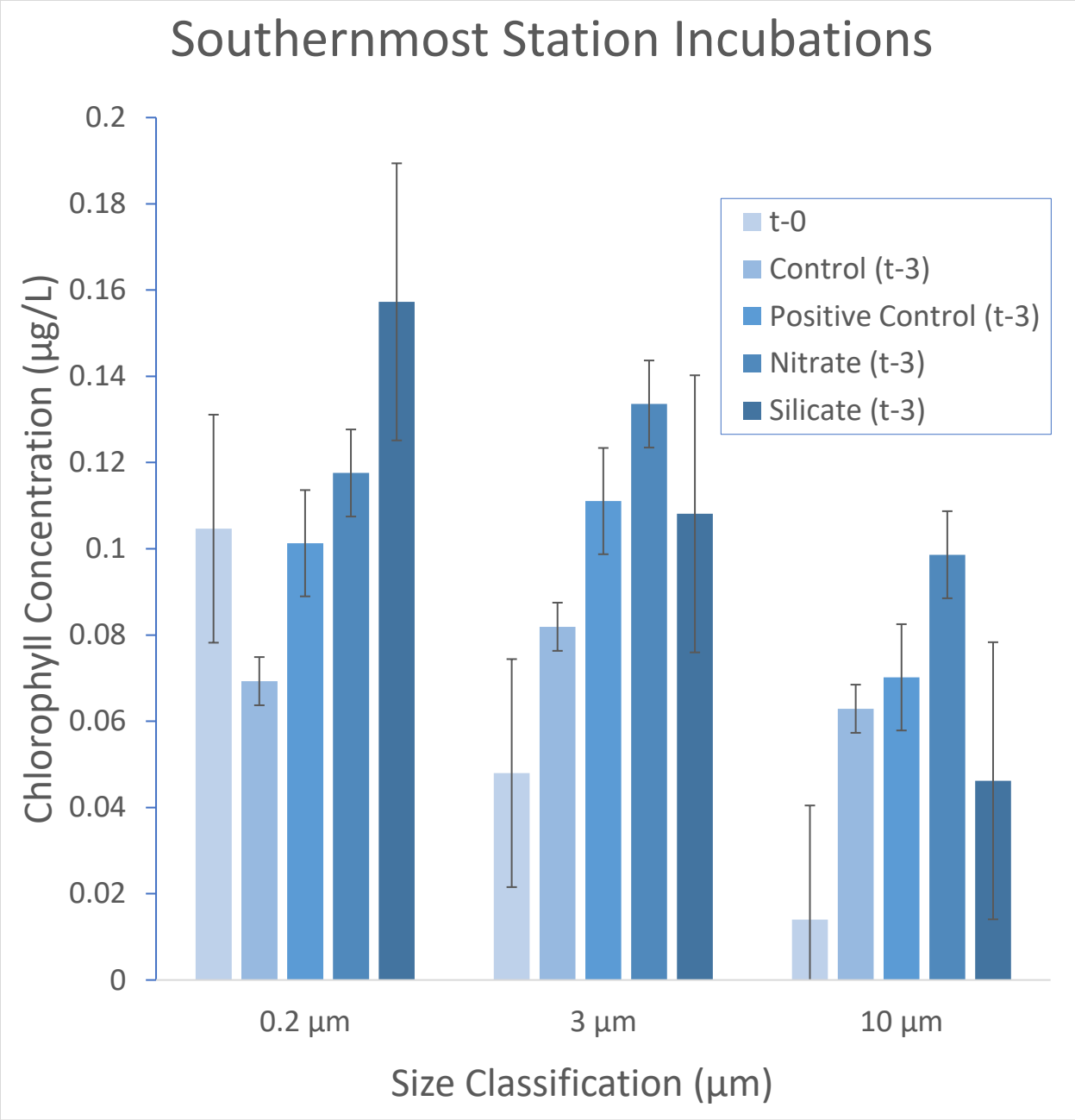

Fig. 4. Averaged out chlorophyll concentrations retrieved from station 16 incubation experiments. Incubation test groups consisted of control groups that comprised of STAYFISH water retrieved from 20 meters depth; positive control groups consisting of a 1:19 ratio of deepwater, retrieved from 500 meters depth, to STAYFISH water; nitrate addition groups consisting of a 1:19 ratio of deepwater, retrieved from 500 meters depth, to STAYFISH water as well as nitrate additions amounting to nitrate concentrations of 5 micromoles per liter of incubation water; and silicate addition groups consisting of a 1:19 ratio of deepwater, retrieved from 500 meters depth, to STAYFISH water as well as silicate additions amounting to silicate concentrations of 15 micromoles per liter of incubation water. “t-0” represents the initial chlorophyll concentrations of the STAYFISH water. The data is separated into three separate phytoplankton size classes, each color representing a separate incubation group.

the control group (Fig. 6). In all three size classes, there are no significant differences in chlorophyll concentrations between nitrate, silicate, and the positive control group (Fig. 6). Overall, the 10 µm size class had a significantly lower chlorophyll concentration than the other size classes while the 0.2 µm and 3.0 µm size classes showed no significant difference in chlorophyll concentrations (Fig. 6).

When comparing the size distributions of each station, I observed that within the 0.2 µm size class, the northernmost station (Station 2) exhibited the highest concentrations of chlorophyll while the southernmost station (Station 16) showed the lowest chlorophyll concentrations (Figs. 4, 5, 6). For the 3.0 µm size class, the equatorial station (Station 9) exhibited a significantly higher chlorophyll concentration than the other two stations (Figs. 4, 5, 6). With the 10 µm size class, the equatorial station (Station 9) exhibited the highest chlorophyll concentrations while northernmost station (Station 2) exhibited the lowest concentrations (Figs. 4, 5, 6).

Discussion:

The focal point of this research was the impact of varying nutrient conditions on phytoplankton populations and their respective size classes. The experiments and data collection took advantage of the shift of La Nina to El Nino that occurred in February-March 2023. This transition gave us the opportunities asses the changes in nutrient conditions at different locations along the equatorial transect and correlate said changes to phytoplankton concentrations. To collect data on the silicate and nitrate concentrations within the water column of each station, I tried to collect physical samples to be sent to the University of Washington for analysis. Unfortunately, the samples thawed during transit, so I instead chose to obtain model data for

each location and time point from the CMEMS – Global Monitoring and Forecasting Centre (Fig. 3).

Surface water nutrients decreased as the distance from the equator increased, which is likely caused by increased upwelling of nutrient rich deepwater along the equator (Castro, et al.) (Fig. 3). While silicate concentrations clearly decreased as they neared surface waters, they plateaued at a minimum concentration of around 2.5 mmol/m^3. At its lowest concentrations, surface water nitrate neared complete depletion at the northernmost (Station 2) and southernmost (Station 16) stations. The variation in concentration between silicate and nitrate lead me to believe that nitrate was the limiting nutrient within the phytoplankton populations. Traveling north to south, the model data clearly indicates that the mixed layer depth or depth at which nutrients begins to increase gets deeper and deeper (Fig. 3). As mixed layers deepen, proximity of phytoplankton communities to nutrient rich deepwater begins to decrease, leading to lower growth. Overall, the chlorophyll depth profiles for each station displayed a trend of lower surface water chlorophyll concentrations from northern to southern stations (Fig. 4). The switch from shallower to deeper mixed layers and lower chlorophyll concentrations are clear indicators that a transition from La Nina to El Nino is occurring (Fig. 3). While I mainly focused on these surface waters when determining environmental impacts on phytoplankton, deepwater nutrient data was important for understanding the deepwater additions to the incubation experiments.

Deepwater nutrient profiles followed a slightly different trend where deepwater nutrients concentrations decreased as the latitude moved south (Fig. 3). The variation in deepwater nutrients from north to south may result from the difference in ages of the water parcels. North Pacific waters tend to be older than water within the South Pacific (Matsumoto, 2007), which allots more time for deepwater nutrient regeneration to occur. It’s important to note these

differences because these variations in deepwater nutrients most likely had an impact on deepwater additions for the incubation experiments. At 500 meters depth, the northernmost station (Station 2) held nitrate concentrations of 38.5 mmol/m^3 whereas the southernmost station (Station 16) held nitrate concentrations of 32.0 mmol/m^3 (Fig. 3). For silicate, the northernmost station (Station 2) displayed a concentration of 53.0 mmol/m^3 at 500 meters while the southernmost station (Station 16) displayed a concentration of 34.0 mmol/m^3 at 500 meters. In contrast to the northernmost (Station 2) and southernmost (Station 16) stations, deepwater additions for the equatorial station (Station 9) were retrieved from a 2000 meter depth where the silicate concentrations were 140 mmol//m^3 and the nitrate concentrations were 39.5 mmol/m^3. This difference in depth had little impact on nitrate additions but in terms of silicate, almost tripled the concentration of the northernmost station's (Station 2) deepwater additions (Fig. 3). Variance in these deepwater additions will have impacts on the growth rate and overall biomass obtained in each treatment group, so it's necessary to note the differences between each stations' incubations. It's also important to consider that these deepwater additions add more than just nitrate and silicate. It will alter the concentrations of multiple nutrients, DOC, POC, DIC, add microbial populations, and other variables not noted to the existing surface water sample.

Before nutrient additions occurred, I measured and analyzed the size class distribution within the initial water samples for each station's incubations. Larger size classes (3.0 and 10 um) ended up displaying higher concentrations within the northernmost station (Station 2) whereas the southernmost (Station 16) and equatorial station (Station 9) displayed higher concentrations in the 0.2 μm size class category (Fig. 4 and 5). These results were puzzling, since typically, larger phytoplankton thrive in resource rich environments where their growth

outmatches the pressures of predation (Pomati, et al., 2020). Since the equatorial station (Station 9) was shown to be a nutrient rich environment, I assumed the larger phytoplankton would thrive in comparison to their smaller counterparts. It is possible that there was an influx in larger phytoplankton predation before I gathered the water for incubations at the equatorial station (Station 9) but that wouldn't explain the fact that the northernmost station (Station 2) displayed higher concentrations of larger size classes than smaller size classes. Regardless, these were the initial size class distributions for each station.

The northernmost station (Station 2) exhibited the highest deepwater nitrate concentrations, the second highest deepwater silicate concentrations, low surface water nutrients, and high concentrations of 10 μm size classes. Surprisingly, after 3 days, the smaller size class outperformed the larger size classes in every treatment. The 3.0 μm size class didn't display any significant growth and the larger 10 μm size class ended up decreasing in chlorophyll concentrations within the control group. I believe the reason for this decline in population was due to a lack of nitrate in the northernmost station (Station 2) surface waters. In contrast to the larger, 10 μm size class, the 0.2 μm size class ended up growing without any nutrient additions and overall outperformed the larger size classes. This growth within the 0.2 μm size class could be a result of less predation, although the filter I used wouldn't have filtered out microzooplankton. Regardless, how could they have grown without nitrate? Without the introduction of new, nutrient rich deepwater or nitrate additions, some form of nitrogen would need to be regenerated to sustain growth within the incubations. Bacterial measurements through the process of SYBER showed significant (p-value <0.05) increases in bacterial populations between the beginning and end the northernmost station's (Station 2) incubations (Lim, 2023), which could lead to ammonia regeneration that small phytoplankton can use as a nitrogen source.

This ammonia regeneration could explain the high growth within phytoplankton populations at this station. The deepwater additions displayed the highest impacts on growth among every size class as there wasn't a single treatment that garnered significantly higher chlorophyll concentrations than the positive control. In hindsight, this shouldn't have been surprising. Adding an influx of deepwater that holds high levels of nutrients ultimately tells us what we already know: nutrient rich deepwater allows surface water phytoplankton to grow. Fortunately, Station 2 gave us interesting, valuable information regardless.

In comparison to the northernmost station (Station 2), the equatorial station's (Station 9) deepwater additions contained lower levels of nitrate whereas silicate concentrations far exceeded that of the previous station by three times the amount. This difference in silicate input was caused by differences in depth of retrieval where the northern (Station 2) and southern (Station 16) stations' samples originated at a 500 meter depth while the equatorial station (Station 9) came from a 2000 meter depth. 2000 meters marks the peak in silicate concentrations among each of these water columns, which explains the difference. In terms of initial phytoplankton conditions, the equatorial station (Station 9) showed low but not exhausted nutrients concentrations, where surface water nitrate had a concentration of 2.5 mmol/m^3 while the surface water silicate had a concentration of 5.5 mmol/m^3. This higher magnitude of surface water nutrients most likely was a result of the increased levels of upwelling of nutrient rich deepwater that occurs at the equator. The initial size class distribution for these incubations showed that there were higher concentrations of the 0.2 μm size class than the larger 3.0 and 10 μm size classes. Between t=0 days and t=2 days, the 0.2 μm size class experienced growth within each of the treatment groups however, overall, between t=0 days and t=4 days, there was no significant growth. Between t=2 days and t=3 days for silicate additions and t=3 days and t=4

days for nitrate additions, there were significant decreases in chlorophyll concentration within the smaller 0.2 µm size class. Furthermore, every one of the larger size classes and treatments, besides the 3.0 µm population within the nitrate additions, displayed consistent growth between t=0 days and t=4 days. The fact that these larger size classes grew in the control treatments suggests that surface waters held high enough nutrient concentrations to sustain life. I believe that the most likely reason for the influx of growth within the control variables for the larger phytoplankton was the lack of mesozooplankton. When filtering out the water for the incubation I was hoping to get rid of or curb the impacts of predation within the experiments to get a better understanding of what nutrients were limiting the phytoplankton growth. Since the larger 10.0 µm size class was so low in concentrations initially but grew to exhibit the highest concentrations in chlorophyll after 4 days, I infer that predation in surface waters had a significant impact on these larger phytoplankton than it did on the smaller phytoplankton. It seems that an inverse situation occurred within the smaller 0.2 µm size class, where growth occurred early on but eventually tapered off and decreased within some treatments. I believe that this decrease in chlorophyll was caused by the predation of microzooplankton that weren't filtered out of the incubation water. Like with the northernmost station (Station 2), the positive control had the greatest impact on the 0.2 µm and 3.0 µm populations' growth whereas there was no significant difference between the growth within the control and growth within any of the incubations with nutrient additions for the 10 µm size class. This result gives evidence that nutrients within the surface waters at the equatorial station (Station 9) aren't limiting the growth of the larger 10 µm size classes. Since the positive control showed significant growth in comparison to the control for the 0.2 µm and 3.0 µm size class, there is evidence that points to nutrient limitations within the surface waters, however, the extent of grazing that was occurring

in each incubation is unknown, so I can't come to any concrete conclusions with the given information.

The surface waters of the southernmost station (Station 16) exhibited the most similarities to El Nino conditions with the deepest mixed layer depth, low surface water silicate and nitrate concentrations, and a size distribution exhibiting high levels of small 0.2 μm phytoplankton. Overall, the deepwater additions contained the lowest nutrient concentrations. The results of these experiments showed minimal growth in the smaller 0.2 μm size class besides the silicate additions incubations. The 10 μm size class exhibited growth within every treatment, which leads me to believe that like with the equatorial station (Station 9), nutrients weren't a limiting factor for growth within these larger phytoplankton and that instead mesozooplankton grazing played a significant role in quelling the populations. Again, like with the other incubation stations, there was no significant difference in chlorophyll concentrations between the positive control and other nutrient additions. Overall, the southernmost station (Station 16) exhibited the lowest chlorophyll concentrations among the three incubations stations.

When we look at the differences in growth and chlorophyll concentrations between the three incubation stations, we see a few trends. At the end of nutrient addition incubations, the northernmost station (Station 2) exhibited the highest concentrations of 0.2 μm size classes while also showing the lowest concentrations of the 10 μm size class. On the other hand, the equatorial station (Station 9) showed the highest levels of the larger 3.0 μm and 10 μm size classes, which makes sense since the highest silicate concentrations were added within the deepwater additions. It's also important to note that equatorial station (Station 9) exhibited the largest surface water nutrient concentrations of the three stations. The location that exhibited characteristics most like

El Nino, the southernmost station (Station 16), showed the lowest levels of chlorophyll within the 0.2 μm size class, 3.0 μm size class, and overall.

When comparing my results with the findings of past research there is a trend I see popping up. Some research has shown that the phytoplankton in its area of research displays the highest responses in growth with silicic acid (Dugdale and Wilkerson, 1998). Others showed that instead of silicic acid, various phytoplankton communities responded most positively to a multitude of additions, be it nitrate or a co-limitation of iron and nitrogen (Browning, et al., 2022). Even though my study didn't come up with conclusive evidence on a limiting nutrient for phytoplankton populations within its regions of research, it did show, like the research before it, that the responses of phytoplankton to varying nutrient conditions will differ from community to community and region to region.

While I believe I gathered valuable data with my research, in hindsight, I would make changes to the procedure for more concrete results. A lot of my evaluation of the data was limited by the lack of physical samples of nutrient data for each incubation. With this additional nutrient data, I could garner a better understanding of the nutrient limitations within each of the incubations. It also would have been interesting to see how the chlorophyll concentrations changed between t=0 days and t=3 days for the northernmost (Station 2) and southernmost (Station 16) stations in case I missed spikes or decreases in chlorophyll growth. The main change I would make looking back would be the elimination of the deepwater additions to the silicate and nitrate additions. These additions seemed to overwhelm the phytoplankton populations with more than enough nutrients for the extent of the incubations. With this influx of deepwater nutrients, I couldn't see the impacts that silicate or nitrate had on the phytoplankton populations

within each size class and station. Overall, the data is still useful but in hindsight, changes would be made to the process.

Conclusion:

Phytoplankton make up the foundation in which the entire food web sits upon, providing oxygen and food to their communities of residence. I'm emphasizing their importance because the climate is changing. As with a changing climate comes shifting physical and biological processes surrounding marine environments and communities. The reliance marine ecosystems have on the well-being of phytoplankton creates a necessity for us to better understand how changing environmental conditions will affect phytoplankton communities. While it didn't encompass all the variables that may have impacted the studied phytoplankton communities, my research did seek to understand the role of nutrient limitations on population growth. Before conducting my research, it was difficult to predict or hypothesize the outcomes. There has been past research that concluded nitrate was the most influential nutrient for phytoplankton growth in their region of research while the results of other research concluded that silicic acid was the primary nutrient limiting growth (Thomas, 1969) (Dugdale and Wilkerson, 1998). Others realized that their phytoplankton populations displayed iron-nitrate or iron-silicate co-limitations (Browning, et al., 2021) (Brzezinski, et al., 2011). Other research uncovered regions and communities in which nutrients weren't limiting growth at all (Thomas, 1969). The huge variance in the results of past research paints a picture on how complex each of these ecosystems in question are.

Eventually, I predicted that nitrate was going to be the limiting nutrient when it comes to total phytoplankton growth, as past research in similar regions and environments have shown that to be the case (Thomas, 1969). Additionally, the model data displayed surface water nitrate

values as almost completely depleted along the northernmost (Station 2) and southernmost (Station 16) stations. In the end, my results didn't match my hypothesis. Surprisingly despite the nutrient additions, the southernmost station (Station 16) incubations showed no significant changes in phytoplankton growth when adding nutrients. This result could suggest many things. Higher grazing could have occurred within these treatment groups in comparison to the incubations at different locations. We could also have been interacting with a community that requires a nutrient/nutrients that aren't available within the deepwater or silicate/nitrate additions. The deepwater additions had by far the largest impacts on phytoplankton growth within the incubations at the northernmost (Station 2) and equatorial (Station 9) stations, so much so that there was no significant difference between chlorophyll concentrations within the positive control and the silicate or nitrate additions. I concluded that with these incubations, deepwater additions overwhelmed the phytoplankton with far too much nutrients to show nutrient depletion within the allotted time period. Even though I couldn't conclude exactly what was limiting the growth within each station, we now better understand how deepwater additions impact the size distribution of the populations at each of the locations. At the northernmost station (Station 2), I observed that while the initial chlorophyll concentrations of larger phytoplankton were more prominent, after incubations smaller size classes began to outperform the larger ones, even in the control group. Growth within these control groups suggested that either surface water nitrate wasn't completely depleted or that nitrate fixation was occurring. The exact opposite phenomenon occurred at the equatorial station (Station 9), where smaller phytoplankton had higher initial concentrations but began to be outperformed by larger phytoplankton as the incubations occurred. These results were surprising but the systems I was researching are so complex that I shouldn't expect to see the same results permeate the

communities of every location. Could smaller phytoplankton have been consumed by zooplankton at a higher rate at the northernmost station (Station 2) than at the equatorial station (Station 9)? Could the specific nutrients I added to the incubations favor the larger phytoplankton at the equatorial station (Station 9) over the northernmost station (Station 2)? Why didn't nutrients impact the growth of phytoplankton at the southernmost station (Station 16)? These unanswered questions are why further research is necessary.

To get a better understanding of what is impacting the growth and size distribution of the phytoplankton within this region, I would change a few ways in which I conducted the research as well as provide additional research in areas surrounding phytoplankton. To better observe which particular nutrient is limiting the growth of phytoplankton at each location, I would conduct similar incubation experiments in which I forfeit the use of deepwater additions and instead add specific nutrients separately. This way, we can better see what is having the largest impact on each size class at each station. I would also take samples in order to measure nutrient concentrations alongside chlorophyll concentrations to see what is being used up and depleted. Finally, I think better understanding how predation is impacting the growth of separate phytoplankton size classes in these incubations is integral when trying to understand why populations are decreasing or lagging in growth. It's easy to conflate a lack of growth with a lack of nutrients but it's important to account for variables like predation when quantifying the impacts of said nutrients. These systems are complex and diverse. This complexity makes predicting changes more difficult but if we continue to unearth the mysteries behind our oceans, we'll be better equipped to address climate change in the future.

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