# Phytoplankton Community Composition Along Environmental Gradients in the North Pacific Ocean

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**Abstract**

*Prochlorococcus* is a cyanobacterium smaller than 1 µm that accounts for much of the primary production in nutrient-poor areas such as the North Pacific Subtropical Gyre (NPSG). In the transition from the NPSG to more productive coastal regions, there are fronts that have sharp changes in chemical, physical, and biological properties. In more coastal, nutrient-rich conditions, larger phytoplankton are more abundant, including *Synechococcus* and picoeukaryotes. Data collected on cruises going north from the NPSG (the Gradients cruises) were compared to data collected on the TN398 cruise going east from the NPSG to the California coast. A SeaFlow flow cytometer measured small phytoplankton, including *Prochlorococcus*, *Synechococcus*, and picoeukaryotes. *Prochlorococcus* was most abundant in nutrient-poor conditions in the NPSG, reaching a concentration of 300 cells/µL, and larger phytoplankton, including *Synechococcus* and picoeukaryotes, were most abundant in the coastal ocean and subpolar region. The diameters of *Prochlorococcus*, *Synechococcus,* and picoeukaryotes varied on a diel cycle that was most strongly observed in the gyre. The average diameter of *Prochlorococcus* and *Synechococcus* increased by about 0.2 µm outside the NPSG, while the diameter of picoeukaryotes observed by SeaFlow decreased by about 0.4 µm. *Prochlorococcus* abundance was negatively correlated with nitrate and nitrite. In the future, these variables could be compared seasonally, annually, and across ocean basins to better understand how these populations are responding to climate change.

**Plain Language Summary**

*Prochlorococcus*, a small phytoplankton, is highly abundant in nutrient-poor conditions. This area includes the North Pacific Subtropical Gyre (NPSG), a region low in nutrients. In more

nutrient-rich conditions, larger phytoplankton are more abundant, including *Synechococcus* and a phytoplankton group called picoeukaryotes. Data collected on cruises going north from Honolulu (the Gradients cruises) was compared to data collected on the TN398 cruise going east from Honolulu to the southern California coast. *Prochlorococcus* was most abundant in nutrient-poor conditions and larger phytoplankton were most abundant in nutrient-rich conditions. The size of phytoplankton cells changed on a daily cycle. This cycle was most strongly observed in nutrient-poor conditions. The size of *Prochlorococcus* and *Synechococcus* increased in nutrient-rich conditions, while the size of picoeukaryotes decreased. *Prochlorococcus* abundance decreased as nutrient concentrations increased. In the future, these variables could be compared across ocean basins to better understand where phytoplankton are and help predict how these populations may change in the future.

**Introduction**

Phytoplankton are one of the key producers of oxygen for our planet, as well as the base of oceanic food webs and crucial for nutrient cycling (Karl 1999). Therefore, it is critical to understand the processes that lead to their distribution in our oceans. This research is also important for predicting and observing how climate change affects our oceans. Determining how environmental conditions shape the spatial distribution of phytoplankton can help scientists predict how anthropogenic effects will influence these populations in the future.

One region being studied for long-term oceanic change is the largest gyre in the ocean, the North Pacific Subtropical Gyre (NPSG). This is an oligotrophic (nutrient-poor) region with low biomass and low nitrate concentrations (Karl and Church, 2014). The Hawaii Ocean Time series (HOT) program located within the NPSG at station ALOHA (A Long-term Oligotrophic

Habitat Assessment) north of Hawaii has collected over 30 years of oceanographic data for the region (Karl and Church 2014). Other research in the area includes the Gradients cruises, which traveled north from station ALOHA to the subpolar region of the North Pacific (Fig. 1). These cruises collected physical, chemical, and biological data. The cruises crossed the gyre boundary to the north. Gradients 1 occurred April 20- May 4, 2016, Gradients 2 was May 26-June 13, 2017, and Gradients 3 was April 10-29, 2019. The University of Washington senior thesis cruise (TN398) also occurred in the NPSG from December 18-30, 2021 (Fig. 1). Even with these programs, the NPSG is still undersampled due to its large area (Karl and Church 2014).

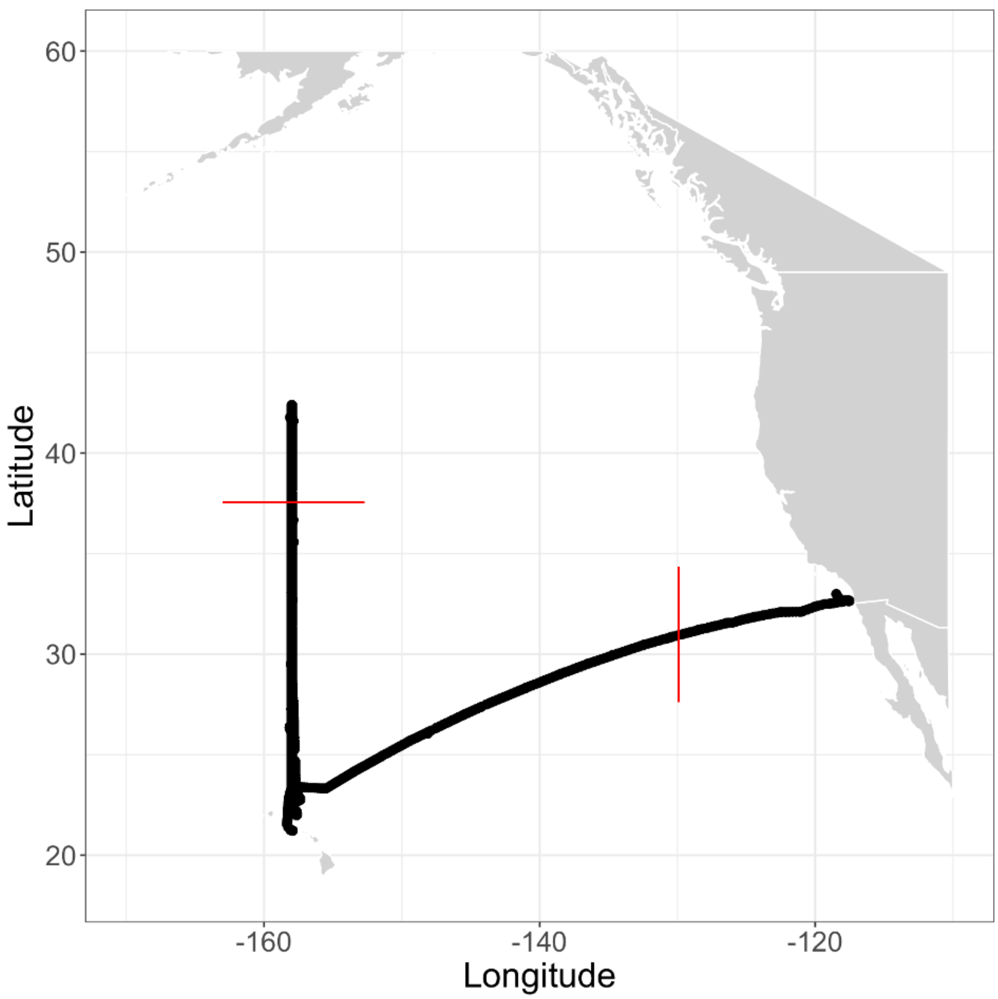

*Figure 1. Cruise track for SCOPE-Gradients 3 conducted from April 10 to April 29, 2019 (traveling north-south) and TN398 conducted from December 18 to December 30, 2021 (traveling east-west). The red lines represent the boundaries of the NPSG based on salinity.*

Nutrient concentrations and phytoplankton distributions are strongly linked. Nutrient uptake in phytoplankton depends on surface area and volume. Smaller cells have a high surface area to volume ratio, making them efficient at taking up nutrients while larger cells are limited by their surface area (Dao 2013). This makes smaller phytoplankton volume-limited rather than surface area-limited and gives them their spherical shape. Therefore, it follows that *Prochlorococcus*, which is smaller than 1 µm (Juranek et al. 2020), is most abundant in oligotrophic conditions.

*Prochlorococcus* is the most abundant phytoplankton in the NPSG (Ribalet et al. 2015), accounting for 63-86% of the primary production occurring from 0-125 m at station ALOHA (Rii et al. 2016). *Prochlorococcus* is separated into clades that represent different evolutionary adaptations of the organism. These clades are often labeled as ecotypes because they are found in different environments (Moore et al. 1998, Larkin et al. 2016). Under high light intensity conditions in the surface waters of the NPSG, there are two main ecotypes found, eHL-I and eHL-II (Larkin et al. 2016). eHL-II is found at higher temperatures whereas eHL-I is found at temperatures below about 17.5°C (Larkin et al. 2016). Overall, *Prochlorococcus* has a higher division rate in warmer waters than cooler waters. Additionally, *Prochlorococcus* biomass has a strong diel cycle, varying in abundance and diameter from day to night (Ribalet et al. 2015). *Prochlorococcus* divides at its highest rate near the equator which indicates it is not nutrient limited in that region (Vaulot et al. 1995). *Prochlorococcus* abundances and cell production decrease in colder water, suggesting that temperature may also limit growth (Ribalet et al. 2015).

However, more information is needed to determine if these properties are seasonal or found year-round.

Unlike *Prochlorococcus, Synechococcus* is an abundant picophytoplankton near the coast where they create phytoplankton blooms. They are critical organisms for their contribution to the carbon cycle in these ecosystems (Tai and Palenik 2009). There are multiple clades of *Synechococcus* that can be found at the surface in different areas of the ocean. Clade II is most abundant in oligotrophic conditions. Clades I and IV are most abundant near the coast (Tai and Palenik 2009). Off Scripps pier in La Jolla, CA, a May bloom of clade I was weakly correlated with an increase in temperature and decrease in phosphate. The rest of the year, clade IV was most abundant (Tai and Palenik 2009). Although *Synechococcus* is abundant near the coast, the overall chlorophyll concentration is often controlled by larger phytoplankton (Tai and Palenik 2009).

Picoeukaryotes are composed of an assemblage of various phytoplankton species, responsible for more primary production than *Synechococcus* and *Prochlorococcus* in the coastal ocean (Worden 2006), but only 10% of the primary productivity in the NPSG (Rii et al. 2016). There is a high species diversity of picoeukaryotes in the NPSG. Twenty-six different taxa of picoeukaryotes have been reported, occupying different niches in the water column with strong seasonal changes in the upper euphotic zone (Rii et al. 2021).

Between the NPSG and coastal waters, there are transition zones that indicate a change in physical, chemical, and biological properties. The transition zone chlorophyll front (TZCF) is a region between the NPSG, subarctic, and eastern Pacific that has a sharp surface chlorophyll gradient. It is defined as the area with a chlorophyll concentration of 0.15 mg $C/m^3$ (Juranek et al. 2020). This location of the TZCF is not stable spatially and can vary seasonally by 1000 km due to wind stress and storms (Polovina et al. 2017). This zone has a net community production 1.5-4x higher than that of the NPSG and is a shift away from *Prochlorococcus*-dominated ecosystems found at lower latitudes (Polovina et al. 2017).

I hypothesized that *Prochlorococcus* would be most abundant in oligotrophic conditions where a high surface area to volume ratio and high nutrient uptake efficiency is critical for growth, whereas larger phytoplankton like *Synechococcus* and picoeukaryotes would dominate in regions with higher nutrient availability. To test this hypothesis, I compared transects that traveled both east and north from the oligotrophic North Pacific Subtropical Gyre through the transition zone chlorophyll front using phytoplankton composition data and nutrient concentrations.

**Methods**

*Nutrient sampling*

Sampling for TN398 took place onboard the *R/V Thomas G. Thompson* from December 18-30, 2021, using the flow cytometer SeaFlow (Ribalet et al. 2019), a thermosalinograph for surface salinity and sea surface temperature, and a rosette of Niskin bottles for nutrient concentrations. Nutrient sampling was conducted every 4 degrees longitude for the first 20 degrees traveled along the cruise track starting at station ALOHA. Then, every 2 degrees longitude was sampled for the final 20 degrees (Fig. 2). This resulted in 15 nutrient samples total between Honolulu and San Diego. Nitrate and phosphate concentrations were analyzed at the University of Washington.

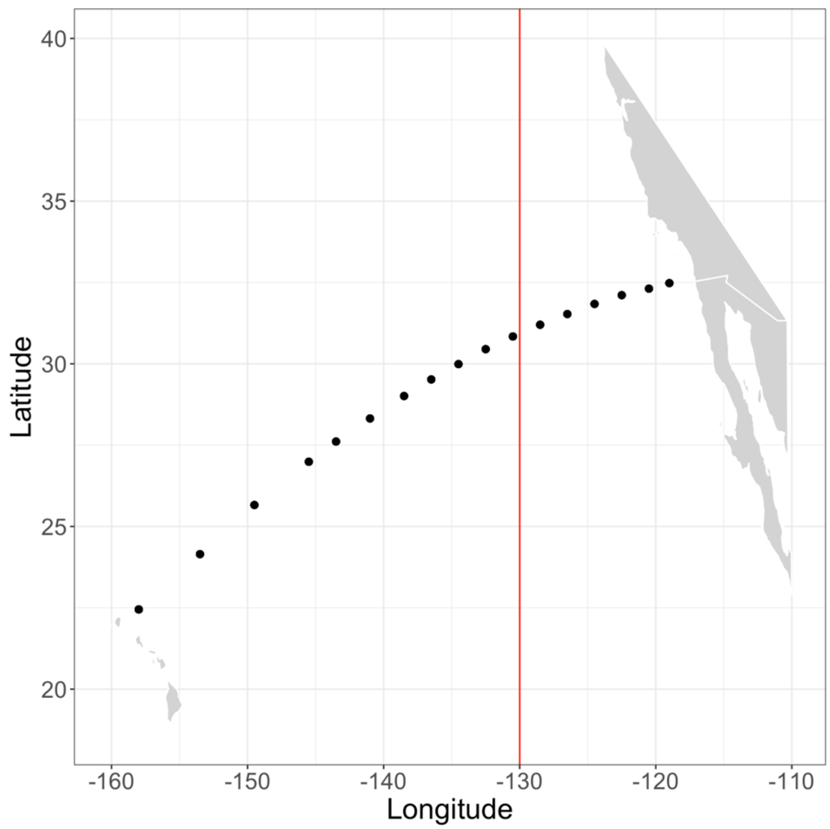


*Figure 2. Cruise track with sampling locations for TN398 from December 18 to December 30, 2021. The red line represents the TZCF based on salinity.*

*Size-fractionated chlorophyll measurements*

Size-fractionated chlorophyll samples were collected at a depth of 5m from Niskin bottles mounted on the rosette sampler. 0.5L of seawater was filtered through both 10 µm and 2 µm Isopore membrane filters (Merck Millipore Ltd., IRL). Separately, 0.5L of seawater was filtered through a 0.2 µm Nuclepore etch-membrane filter (Whatman, USA). The amount of chlorophyll for the 0.2-2 µm size class was calculated by subtracting the chlorophyll on the 10 µm and 2 µm filters from the total chlorophyll on the 0.2 µm filter. The filters were placed in 90% acetone and frozen at -20 degrees C for 24 hours. Samples were thawed, vortexed, and centrifuged before being placed in a TD700 Fluorometer (Turner Designs, USA). Three drops of HCl were added after measurement on the fluorometer and samples were re-run. Chlorophyll-a concentration was calculated using the following equation:

*[Chla] = K * (Fm/(Fm-1)) * (Fo - Fa) * (Extraction volume/ filter volume)*

Where K is specific to the fluorometer based on calibration, Fm is the acidification coefficient for pure chlorophyll, Fo is the fluorescence reading before acidification, and Fa is the fluorescence reading after acidification.

*SeaFlow measurements*

Data was collected during the TN398 student cruise using the flow cytometer SeaFlow (Ribalet et al. 2019) at a depth of 5m using the seawater intake system. *Prochlorococcus*, *Synechococcus*, picoeukaryotes, and 1-µm calibration beads were distinguished by sequential manual gating based on fluorescence and forward scatter (Ribalet et al. 2019). Abundance was calculated using the amount of optimally positioned particles per population divided by the sample volume (Ribalet et al. 2019). Diameters of individual cells were estimated from light scatter by the application of Mie theory and converted to carbon (Ribalet et al. 2019). Results were processed using R code.

Phytoplankton, nutrient, and environmental data from the SCOPE-Gradients cruises were retrieved from Simons CMAP via http://scope.soest.hawaii.edu/data/gradients/gradients.html (Ashkezari et al. 2021). Phytoplankton abundance, chlorophyll concentration, and nutrients were compared, as well as CTD data such as temperature and salinity.

*TZCF definition*

To identify the front(s) between the NPSG, subarctic, and coastal regions, I compared sea surface salinity to the latitude and longitude of the cruise track. For the Gradients cruises going north, this entailed finding the 34.82 PSU boundary between the gyre and TZCF (Juranek et al.

2020). To find the TZCF to the east, I searched for the greatest change in salinity by finding the largest value of the first derivative of the salinity over time graph (Follett et al. 2021). I used this method because this salinity front is correlated with the chlorophyll front (Follett et al. 2021).

**Results**

*Abundance*

Picoeukaryotes increased near the California coast and at northern latitudes, reaching concentrations of 30-80 cells/µL (Fig. 3a and 4a). *Prochlorococcus* was most abundant in the gyre at a concentration of 150-300 cells/µL and sharply decreased spatially at the TZCF to the north (Fig. 3b). The decrease in abundance spatially was more gradual to the east (Fig. 4b). *Synechococcus* was most abundant near the California coast and around the TZCF to the north with a concentration of 50-150 cells/µL. However, *Synechococcus* abundance decreased in northern latitudes above 40 degrees to 30 cells/µL and was at a concentration of 10-50 cells/µL in the gyre (Fig. 3c).

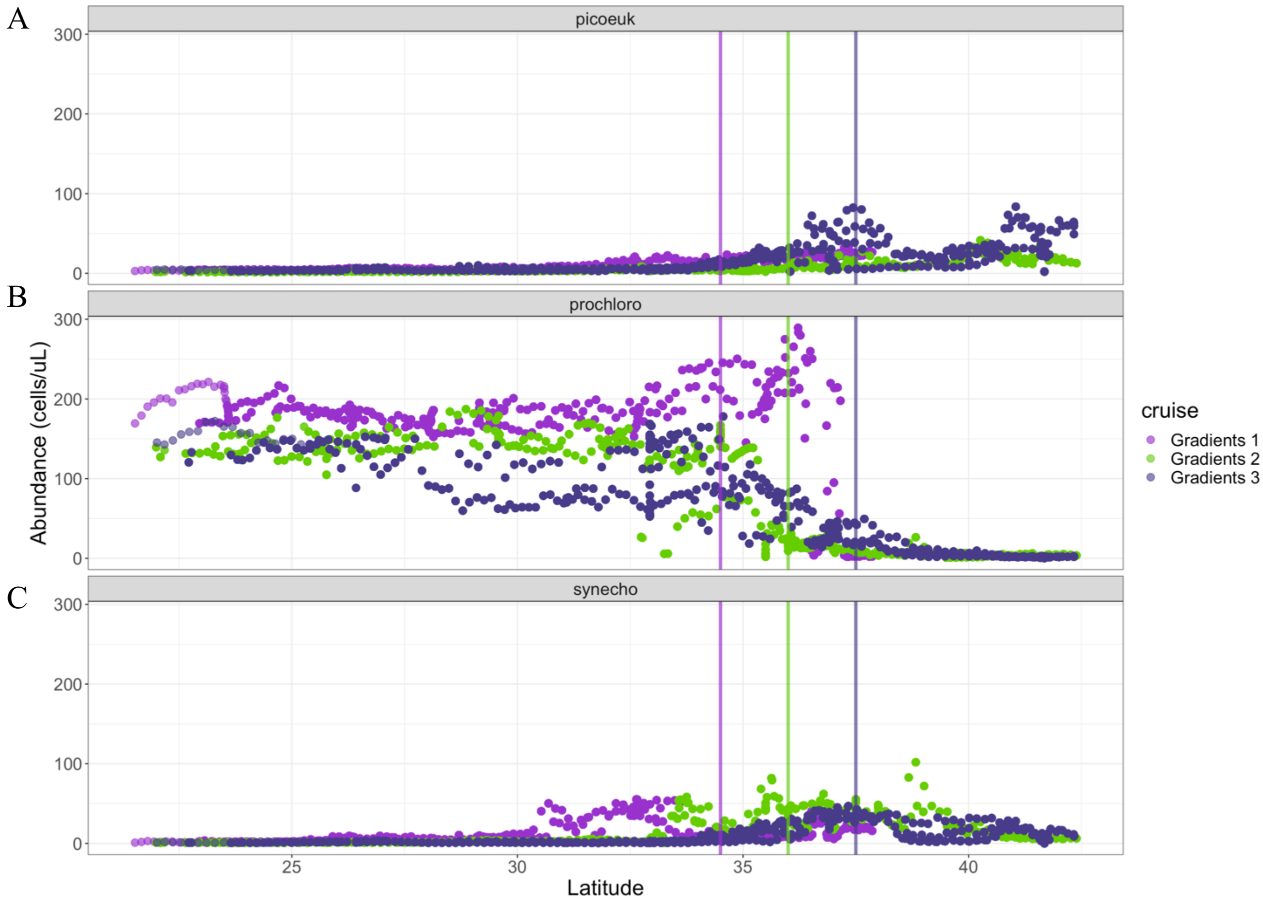


*Figure 3. Latitude vs (a) picoeukaryotes, (b)* Prochlorococcus, *and (c)* Synechococcus *abundance for Gradients 1, 2, and 3. The transition zones for each cruise are represented by the color-coded vertical lines.*

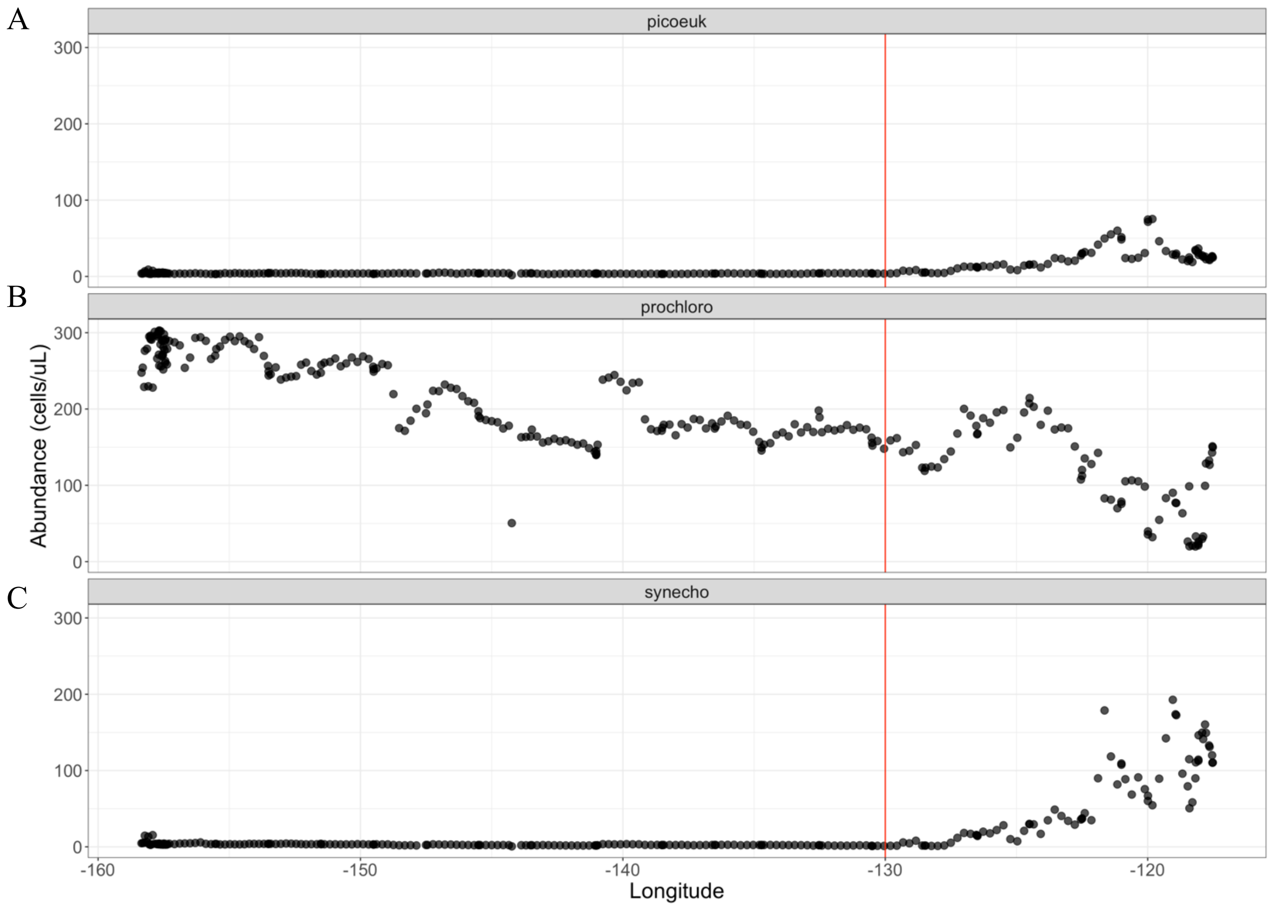


*Figure 4. Longitude vs (a) picoeukaryotes, (b)* Prochlorococcus, *and (c)* Synechococcus *abundance for TN398. The red line indicates the TZCF.*

The concentrations of nitrate plus nitrite and phosphate increased outside of the gyre. Nitrate plus nitrite concentrations increased starting at 37.5 degrees latitude on the Gradients cruises while phosphate started increasing at about 35 degrees latitude. Nitrate plus nitrite reached concentrations of around 6 µM/L for Gradients 3 at 42 degrees latitude. For Gradients 2, 3, and TN398, *Prochlorococcus* abundance decreased with increasing nitrate plus nitrite (Fig. 5b). Gradients 1 had consistent *Prochlorococcus* abundances until the nitrate concentration reached about 6 µM/L. *Synechococcus* and picoeukaryotes had either very little change or a slight increase in abundance compared to nutrient concentrations during the Gradients cruises (Fig. 5). In contrast, *Synechococcus* abundance started increasing rapidly during TN398 at a nitrate concentration of around 0.2 µM/L (Fig. 5c). *Prochlorococcus* abundance decreased as the nitrate plus nitrite to phosphate ratio increased, with some outliers during Gradients 1 (Fig. 6). I also compared the abundance values with size fractionated chlorophyll concentration to assess whether the majority of phytoplankton were in the picophytoplankton size range (0.5-2 µm).

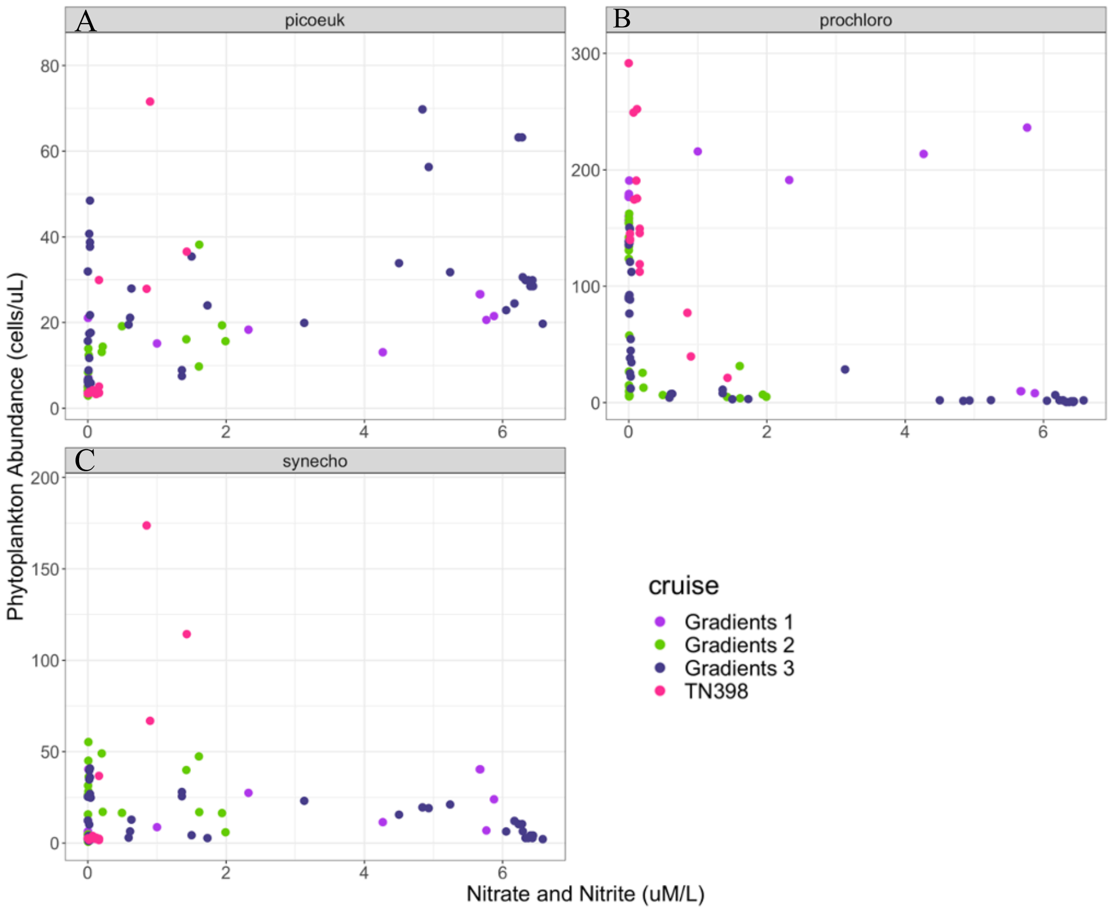


*Figure 5. Nitrate plus nitrite concentration plotted against phytoplankton abundance for (a) picoeukaryotes, (b)* Prochlorococcus*, and (c)* Synechococcus*.*

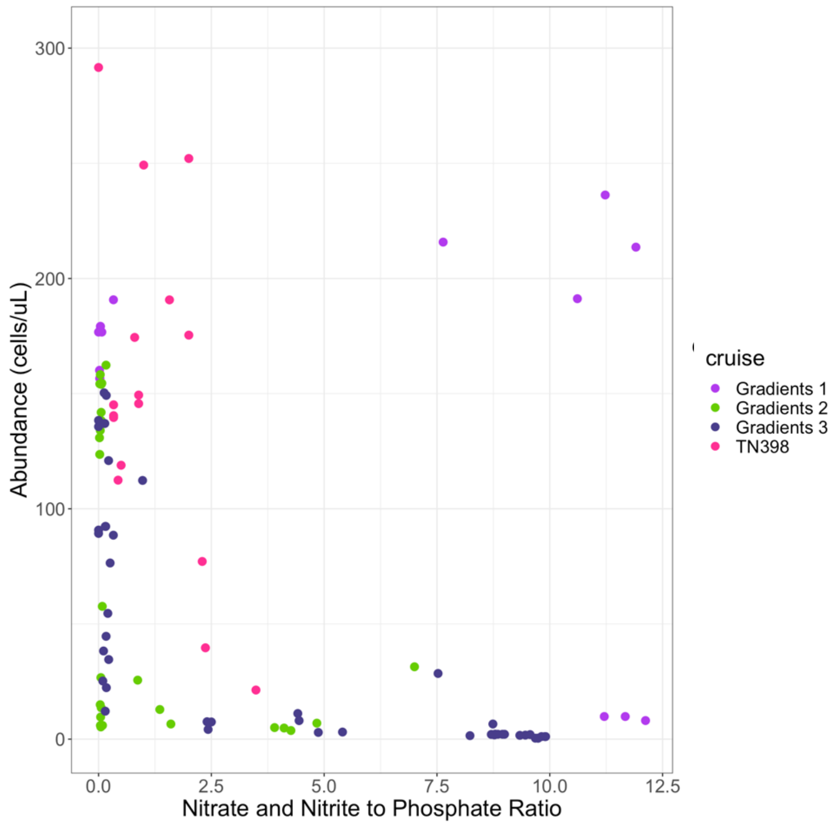


*Figure 6. N:P ratio versus* Prochlorococcus *abundance.*

*Size-fractionated chlorophyll*

The overall chlorophyll concentration for the size class 0.2-2 µm was the greatest and increased the fastest along the TN398 cruise track. Both the size classes 2-10 µm and greater than 10 µm remained constant at around 0.01 µg/L until a longitude of about -125 degrees, increasing rapidly until all three size classes were at a concentration of about 0.3 µg/L near California (Fig. 7). The specific sizes of *Prochlorococcus*, *Synechococcus*, and picoeukaryotes were also compared using SeaFlow measurements.

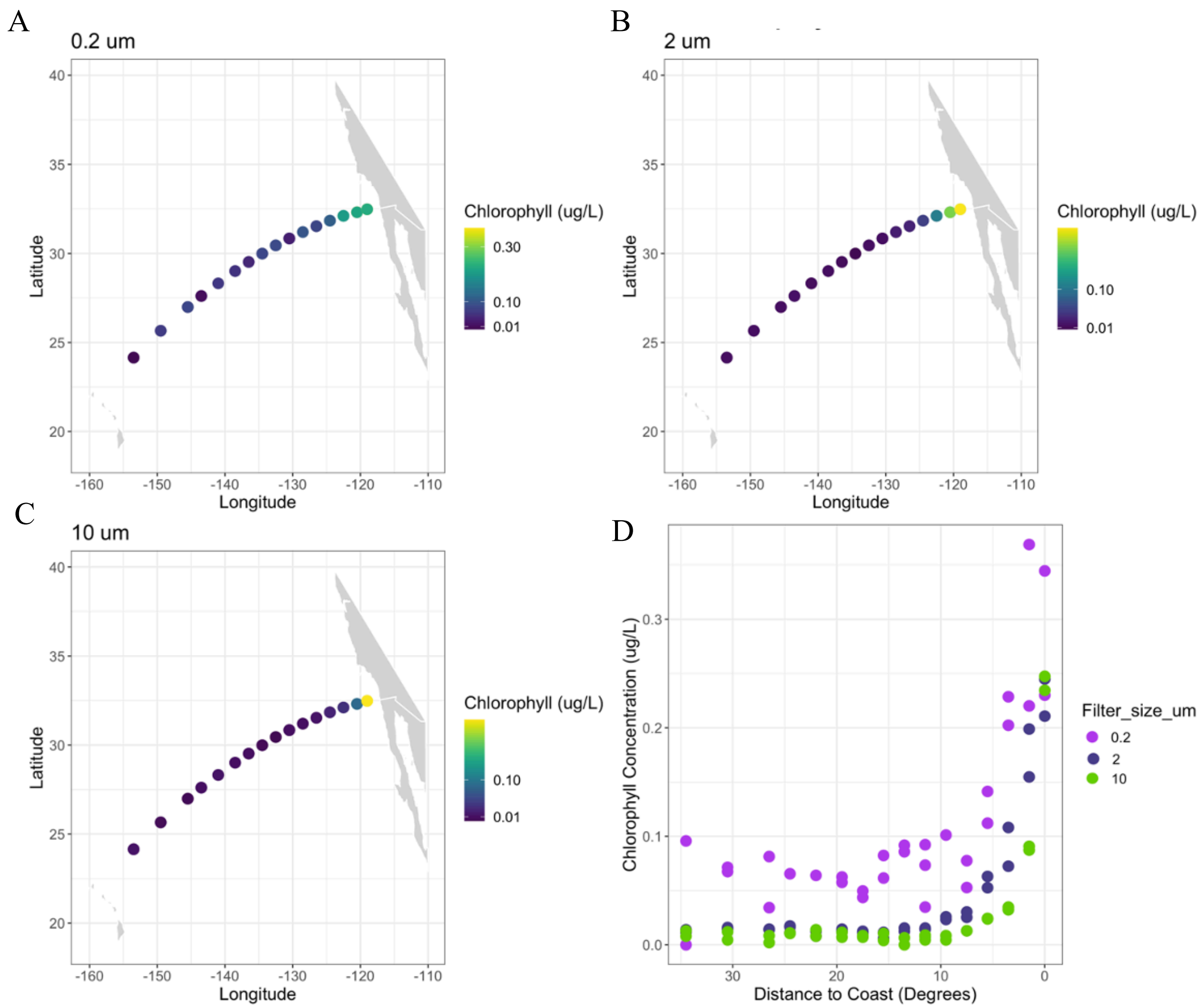


*Figure 7. Size-fractionated chlorophyll during TN398, indicating (a) 0.2-2 µm, (b) 2-10 µm, and (c) greater than 10 µm. (d) compares the distance to the coast with the chlorophyll concentration for all three size classes.*

*Diameter*

The diameter of *Prochlorococcus* increased with an increase in the nitrate plus nitrite to phosphate ratio (Fig. 8). As the N:P ratio reached around 0.35, the diameter of *Prochlorococcus* followed two trends, either staying at around 0.6 µm or increasing to about 0.8 µm (Fig. 8). The average diameter of picophytoplankton changed on a diel cycle for both the Gradients cruises and TN398. This pattern became harder to observe at higher latitudes and close to the California coast. The diel cycle was most strongly defined on TN398. The diameters of *Prochlorococcus* and *Synechococcus* varied with PAR, and after reaching the TZCF, their diameters increased (Fig. 9). For the picoeukaryotes detected by the SeaFlow, the diameter decreased after reaching the transition zone (Fig. 9c).

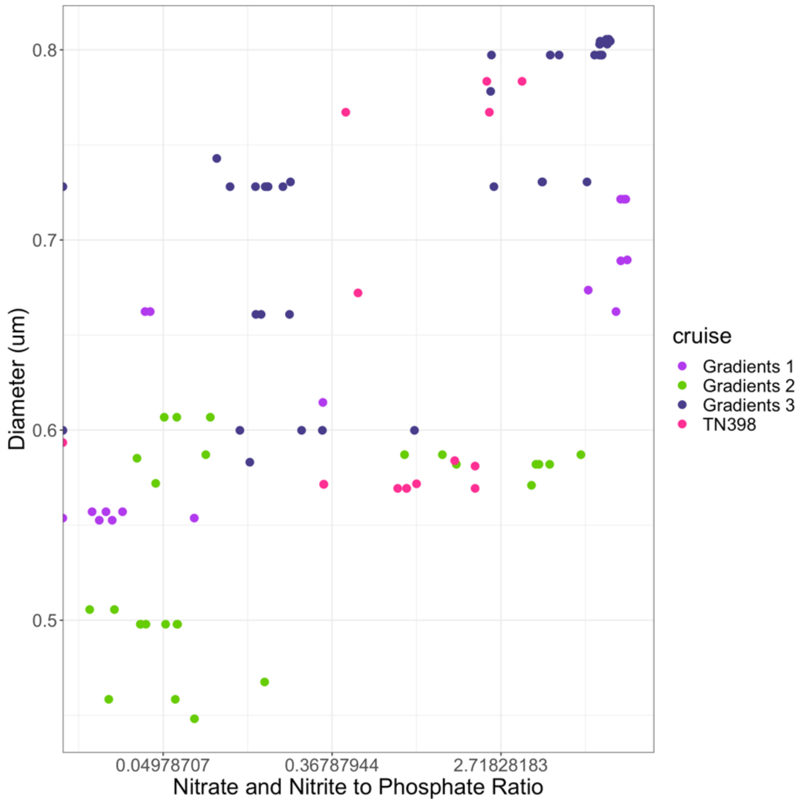


*Figure 8. N:P ratio versus* Prochlorococcus *diameter averaged per day with a logarithmic scale x axis.*

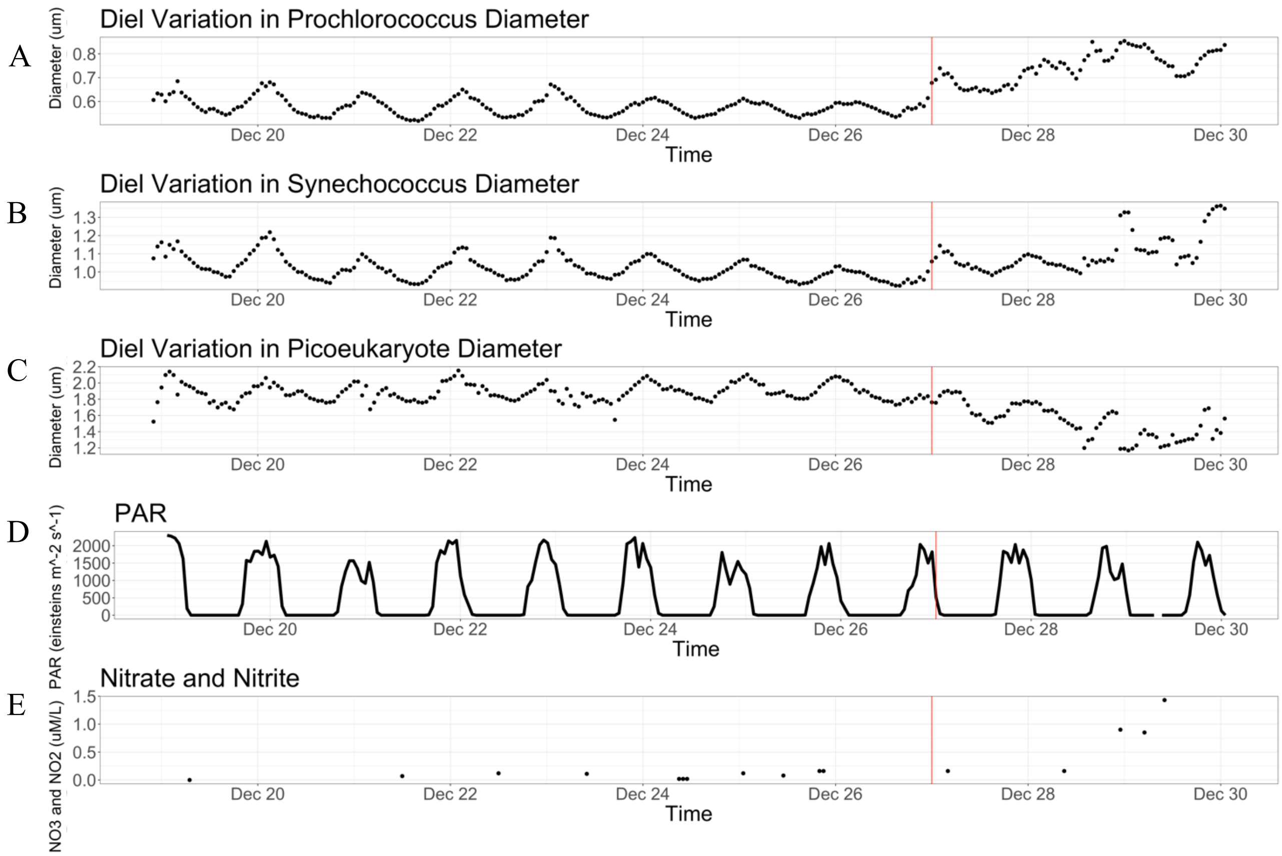


*Figure 9. Average phytoplankton diameter for (a)* Prochlorococcus*, (b)* Synechococcus, *and (c) picoeukaryotes compared to (d) PAR and (e) nitrate plus nitrite concentration. The red line denotes the TZCF.*

**Discussion**

*Abundance and size-fractionated chlorophyll*

*Prochlorococcus* abundance decreased around the TZCF, where the temperature and salinity decreased, and the nutrients increased. This is consistent with previous findings (Polovina et al. 2017) and coincides with the higher abundance of *Synechococcus* and picoeukaryotes. This indicates that *Prochlorococcus* abundance is correlated with temperature, salinity, and nutrients. The large increase in abundance of *Synechococcus* for TN398 with just a small increase in nitrate and nitrite may indicate the flux of nitrate is shifting along the cruise track. The lack of clear trends for *Synechococcus* and picoeukaryote abundances for the Gradients cruises may be due to the small change in nutrient concentrations, they may be utilizing different forms of the nutrients, or there may be shifts in communities.

*Prochlorococcus* abundance followed similar trends across the Gradients cruises and TN398, even though these cruises were at different times of year. Additionally, smaller phytoplankton seem to be responding to smaller changes in environmental conditions than larger phytoplankton because the chlorophyll values increased the most for smaller size classes before the larger size classes. It appears that nutrients do have an effect on *Prochlorococcus* abundance, but do not fully explain the variation seen from cruise to cruise and along the cruise track. However, nutrient fluxes were not measured, so nutrients could be utilized more quickly in the gyre or during a certain year or season, accounting for some of the variation between cruises.

*Diameter*

Light is a primary factor controlling phytoplankton diameter over the diel cycle (Ribalet et al. 2015). *Prochlorococcus*, *Synechococcus*, and the picoeukaryotes are growing during the day and then dividing around dusk. This cycle is most clear in the gyre. There are several possibilities for this change in the diel patterns in cell size near the coast and far north. The abundance of *Prochlorococcus* is low in these regions, which makes it challenging to calculate a mean diameter. The diameters of *Prochlorococcus* and *Synechococcus* are also getting larger, which may be masking some of the diel signal. There may be multiple strains or ecotypes of *Prochlorococcus* and *Synechococcus* that have different diameters. In the context of other environmental changes like nutrient availability, temperature, and salinity, the changing ecotype hypothesis is strong because different strains thrive in different environments.

Additionally, *Prochlorococcus* is getting larger as the nutrients are increasing and the nitrate plus nitrite to phosphate ratio is increasing, suggesting the surface area to volume ratio may be most relevant in areas of very low nutrient concentrations. There also appears to be two different trendlines in the size variation of *Prochlorococcus* as the nitrate to phosphate ratio increases, which may indicate two ecotypes reacting differently to changes in nutrient concentrations.

## Conclusion

*Prochlorococcus* accounts for much of the primary production in the NPSG, whereas in the more coastal, nutrient-rich conditions, larger phytoplankton are more abundant, including *Synechococcus* and picoeukaryotes. In this study, *Prochlorococcus* abundance decreased outside of the NPSG, whereas *Synechococcus* and picoeukaryote abundances increased. *Prochlorococcus* abundance negatively correlated with nitrate and nitrite concentrations. All phytoplankton diameters varied on a diel cycle, while the average diameters of *Prochlorococcus* and *Synechococcus* increased and the picoeukaryote diameter decreased outside the NPSG.

In the future, phytoplankton abundance and diameter could be compared seasonally across these same transects. Phytoplankton biomass could be compared to nutrient concentrations in addition to abundance and diameter. Instruments could also measure larger size classes of phytoplankton to supplement the data from SeaFlow to understand the interactions between these different groups. Additionally, for insight into how other regions of the gyre vary, transects measuring picophytoplankton and nutrients west and south from Honolulu could be compared to the Gradients and TN398 cruises. Understanding what controls the spatial distribution of these phytoplankton populations can help predict how they may change in the future due to climate change.

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