Drivers of phytoplankton community heterogeneity in the Eastern Tropical North Pacific

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Abstract

We must understand the drivers of primary producer community composition in the open ocean in order to model accurately carbon and nutrient cycling for our planet. I collected samples along a transect in the Eastern Tropical North Pacific, one of the three oxygen deficient zones (ODZ) on the planet. Here, the shoaling of anoxic waters creates a unique secondary chlorophyll maximum, previously recorded to be dominated by picocyanobacteria. I sampled four depths at each station along the transect and performed size-fractionation of chlorophyll to characterize phytoplankton community composition as well as collected surface net tows to identify phytoplankton. As the ODZ shoaled, the proportion of picoplankton decreased at depth. Station 10, a station geographically located where the ODZ shoals into the euphotic zone, lacked a secondary chlorophyll maximum because of a slight oxygen intrusion, possibly caused by an eddy. This slight difference eliminated an entire deep ocean community. Surface phytoplankton community composition was most strongly influenced by the environmental variables of the depth at which the oxygen concentration fell below 20 µM, sea surface temperature, salinity, phosphate, >10µm chlorophyll concentration, and nitrate. Therefore, the shoaling of the oxygen minimum zone, variations in sea surface temperature and salinity, and patchiness of nutrients played major roles in community structure. Understanding the environmental factors that influence community composition is essential when modeling carbon and nutrient cycling, because different genera can perform different ecological roles, which affect rates and cycling.

Introduction

Primary productivity in the ocean contributes to roughly half of the global net primary productivity. In the most nutrient-poor (oligotrophic) waters of the open ocean, carbon fixation is low, but because its sheer expansiveness, the oligotrophic ocean alone accounts for 23% of the ocean productivity (Field et al. 1998). Patchiness in phytoplankton communities has long been observed in the open ocean (Martin et al. 2003; Bainbridge 1957). There are multiple drivers of heterogeneity, including: localized delivery of nutrients; spatial variability in phytoplankton growth or grazing pressures; and varying behaviors of plankton, such as swarming and vertical migration (Martin et al. 2003). In a time of climate change and global ocean change, it is important to monitor the compositions of our open oceans, including how photosynthetic organisms vary across space and depth. Knowing what factors drive heterogeneity and how they influence community structure in the open ocean as well as the extent of the influence of from coastal systems is paramount in order to create accurate ocean models and estimates pertaining to carbon and nutrient cycling.

Both phytoplankton size and community composition have been shown to influence the biological carbon pump (Price et al. 1994), the upper trophic levels (Duffy and Stachowicz 2006), and the sequestration of carbon and other nutrients (Arrigo 1999, Capone 1997, Capone et al. 2005, Karl et al. 1997, Mackey et al. 2007, Tortell 1999). Carbon export can be more related to net primary productivity than to phytoplankton size (Richardson and Jackson 2007); a community with larger phytoplankton does not necessarily sequester more carbon. Models created using projections of sea surface temperature suggest that open ocean cyanobacterial (<2 µm) communities will experience complex changes in the coming years (Flombaum et al. 2013), which likely will have impacts on the open ocean ecosystem and global ocean biogeochemical cycles. In order to understand how our global oceans are changing, it is important to investigate what other influences besides sea surface temperature drive community changes, and how these influences might affect all size fractions, not just the smallest.

Depth influences the irradiance that reaches a particular location in the water column (Zohary et al. 2009). In addition, unique conditions present only at certain depths can also influence phytoplankton community structure. The Eastern Tropical North Pacific is one of only three locations on the planet where deep water oxygen concentrations reach zero. This is the definition of an oxygen deficient zone (ODZ). Waters with low (<20 µM), but not zero oxygen concentrations are considered oxygen minimum zones (OMZ). The shoaling of the ODZ above the 1% blue irradiance depth likely raises the nitrite maximum, creating favorable conditions for a local secondary chlorophyll maximum (Cepeda-Morales et al. 2009). In the Eastern Tropical North Pacific, the secondary chlorophyll maximum is stronger and more frequent in the south compared to the north (Cepeda-Morales et al. 2009).

In this study, I investigated how the distribution of microplankton (>10 µm), nanoplankton <10 µm and >2 µm), and picoplankton (< 2µm) changed across the stations sampled and with depth. I hypothesized that the phytoplankton communities closest to the coastline would be more similar to each other than to the rest of the stations. This type of similarity would highlight the extent of coastal influences on ocean phytoplankton communities. The eutrophic state of coastal waters, due to upwelling and other coastal enrichment processes such as weathering and nutrient enrichment from runoff, is more favorable for the larger phytoplankton (Chisholm 1992, Utiz et al. 2006), possibly because larger cells can be limited by nutrient diffusion rates at very low nutrient concentrations (Chisholm 1992). If these stations were influenced by coastal processes, then I expected that larger phytoplankton would make up a greater proportion of the communities at these stations and that these larger phytoplankton would be present in higher concentrations.

Due to the ODZ shoaling into the euphotic zone in the southern part of the area sampled, I expected every southern station after this shoaling to have a deep community with a higher concentration and proportion of chlorophyll for the picoplankton size fraction (evidence of a secondary *Prochlorococcus*-dominated maximum) than any of the northern stations where the ODZ does not shoal. Low-light *Prochlorococcus* strains have adapted to live in waters with 0.1 to 2% of surface irradiance in the ETNP (Goericke 2000). Therefore, I expected deeper depths with lower irradiances to host larger proportions of picocyanobacteria than shallower depths.

I also investigated if there were any spatial patterns of community composition of surface microplankton (>10 µm) among the stations sampled. Which environmental factors had the strongest influences on changes in microplankton community composition? I expected the surface phytoplankton communities to cluster together in community similarity based on proximity to one another. I expected similar phytoplankton taxa and proportions at stations closest to the coast if coastal influences reached that far into the open ocean.

Methods

Sample collection

I sampled 12 stations in an oceanic survey in the Eastern Tropical North Pacific off the coast of Mexico (Figure 1) from December 24 to December 28, 2016. Station 15 was sampled on January 1, 2017. Aboard the *RV Sikuliaq*, the science party conducted casts with a CTD (SBE 9) equipped with a rosette of Niskin bottles and SeaBird instruments (oxygen: SBE 43-1, fluorescence: Seapoint) at each station. I collected water samples in the euphotic zone for size-fractionation of chlorophyll in order to characterize phytoplankton community composition. I sampled

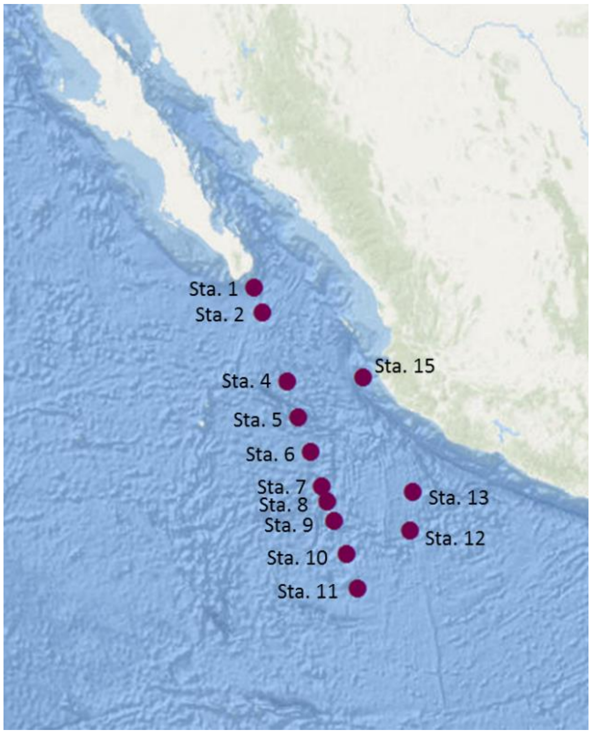

Figure 1. Stations where size-fractionated chlorophyll samples and community surface composition samples were taken. Stations 1-13 are open ocean stations and station 15 is a coastal station sampled for comparison. Station numbers indicate the direction sampled.

the surface, the depth of the first subsurface chlorophyll maximum, the depth of the second chlorophyll maximum at the upper bound of the oxygen minimum zone (present when the OMZ shoaled into the euphotic zone), and a depth between the first and second chlorophyll maxima. For northern stations that did not have a second chlorophyll maximum, I sampled at a depth of 120 m (a decision based on data gathered by Goericke et al. (2000) and Cepeda-Morales et al. (2009)). Station 11 had a secondary chlorophyll maximum, but it was sampled at 120m. On board the *R/V Sikuliaq*, I collected two 1-L samples of water from each sampling depth. I sequentially filtered each liter through three filters of progressively smaller pore size: 10 µm (<10 µm fraction), 2 µm (2-10 µm fraction), and GFF (0.7-2 µm fraction). Filters were flash-frozen in liquid nitrogen, then stored in a -80°C freezer until analysis.

In addition, I collected hand-drawn net tow (mesh size: 10 µm, tow time: 20-40 minutes, speed: variable with no flow meter) samples to investigate the larger phytoplankton community at the ocean's surface. Net tow samples captured morphological diversity. Since the volume of water filtered through the net tow was not recorded, these samples can only provide proportional abundance, not taxa concentrations. Net tow samples were preserved with 5% buffered formalin.

Sample processing

Size-fractioned chlorophyll

At the University of Washington, chlorophyll samples were extracted in 10 mL of 90% acetone for 24 hours in a -20°C freezer. Each sample tube was agitated with a vortex mixer and then spun in a centrifuge for five minutes. Each sample extract was then carefully poured into a disposable glass culture tube and read on a Turner Designs TD-700 fluorometer using the direct concentration method (Turner Designs 2016). Visual transects of the chlorophyll data were created using a linear interpolation method.

Net tow samples

Plankton counts

Net tow samples were allowed to settle, then water was decanted from the top until there was a remaining settled volume of 25mL. This volume was not agitated before removing the aliquots. 50 µL aliquots were taken from each sample and viewed under a light microscope. Each slide was sub-sampled in horizontal transects and then scanned for any large, rare organisms that might have been missed by the sub-sampling. Subsequent aliquots were taken until the number of individual cells counted reached ~200 or more. Organisms were identified to genus when possible and were otherwise assigned a broader category such as pennate diatom or dinoflagellate. Particularly distinct unknown phytoplankton were photographed for later identification and given a unique identifying number. If these same plankton were spotted later in a sample from another station, they were given the same identifier.

Because of the nature of the methods performed, organisms were not always viewed from the angle required to obtain a specific identification. Round phytoplankton with either visible characteristics of dinoflagellates (flagella, presence of a girdle or groove) or seemingly without a regular structure (also a characteristic of dinoflagellates) were consistently identified as generic dinoflagellates. Since these counts were meant to explore differences in community structure, and not the absolute counts of specific organisms distributed across stations, the methods are sufficient for this query. Finally, the number of each taxon counted in a sample was converted into a proportion (number taxon counted/total cells counted for that station) for ordination visualization.

<u>NMS (Non-metric Multidimensional Scaling) phytoplankton ordination</u>

I used the software PC-ORD Version 5 (Grandin 2006) to perform a multivariate analysis of the surface phytoplankton communities. The results of a preliminary NMS ordination indicated a strong, disproportionate influence from large counts of cells that formed chains at a few of the stations. These cells were often spotted in clumps in the samples. In these cases, the chaining cells were most of the only, if not the only visible cells in that clump. There could have been many other organisms, such as dinoflagellates, but only the chaining cells were able to be counted. This could have skewed the counts away from the “true” proportion. In addition, clumps like these could have been scarce in the sample and simply were present in the randomly-sampled aliquot by chance. In this case, these chaining cells would have skewed the community composition.

In order to view the community composition without the skewing effects of these disproportionately numerous chaining phytoplankton, I removed the counts of the clumping taxa from the total post hoc, and adjusted the taxa proportions accordingly. I then performed an NMS statistical analysis and visualized these proportions as a NMS plot. I included a secondary matrix with environmental variables (described below). This visualization enabled me to explore which environmental factors had the strongest influences on differences in microplankton community composition.

In my counts, I identified a total of 89 different taxa. In order to reduce the number of variables and to reduce the effect of sampling bias, I excluded the taxa identified at only one station (n=33 taxa included in NMS). The coastal station was not included in the analysis (See results for details).

Environmental matrix for community ordination

For the environmental matrix to compare to the morphological microplankton community composition, I used depth at which the oxygen concentration fell below 20 µM (m), mixed layer depth (m), sea surface temperature (°C), sea surface salinity (PSU), surface chlorophyll concentration (µg/L) surface chlorophyll concentration of the >10 µm size fraction (µg/L), phosphate (µmol/L), silicate (µmol/L), nitrate(µmol/L), and ammonium (µmol/L). These parameters are reported in table 1 and table 2. When oxygen is less than 20 µM, alternative electron acceptors begin to replace oxygen. This is a common threshold for an oxygen minimum zone (Wright et al. 2012). This depth, mixed layer depth (calculated from viewing plots of density over depth), sea surface temperature and salinity were obtained from CTD instrument data. The surface chlorophyll concentrations were determined from the summation of the size-fractioned chlorophyll samples. Nutrient data was obtained by chemical analysis of surface seawater after the cruise.

Results

Environmental parameters

Along the transect, the ODZ shoaled from north to south (Figure 2, Table 1). Stations 6-13, with the exception of station 10, had a deep secondary chlorophyll maximum due to the shoaling of the ODZ (Figure 3). The primary chlorophyll max around 60m was clear. The secondary chlorophyll maximum was not as distinct, but still present. Stations 7-9 and 11 had the clearest signal at this scale. The deepest depth sampled at station 10 clearly had a lower concentration at the lowest depth than its neighboring stations. Station 15, the coastal station sampled for comparison, also had a secondary chlorophyll maximum. At station 10, there was an oxygen intrusion at the depth where a maximum would have been expected (Figure 4). Higher nitrite values seemed to follow the shoaling of the ODZ slightly, although nitrite appeared above 200 meters before the ODZ shoaled into the euphotic zone. There were two locations above 100 meters with unusually high nitrite values (Figure 5). There was not a single clear pattern in the nitrite data. However, there was a decrease in nitrite values at station 10 around the expected depth of the non-existent secondary chlorophyll maximum.

Along the transect sampled (stations 1-11), the mixed layer depth, total surface chlorophyll and chlorophyll in the 10 µm size fraction were highly variable. The sea surface temperature increased slightly from north to south. There was a very distinct north/south divide for sea surface salinity between station 6 and station 7 (Table 1). Phosphate had a generally decreasing trend from north to south. Silicate also had a decreasing trend with outliers at station 6 and 11. Nitrate and ammonium were variable with large outliers at station 8 and station 10, respectively (Table 2). These findings reflect the patchiness of the open ocean.

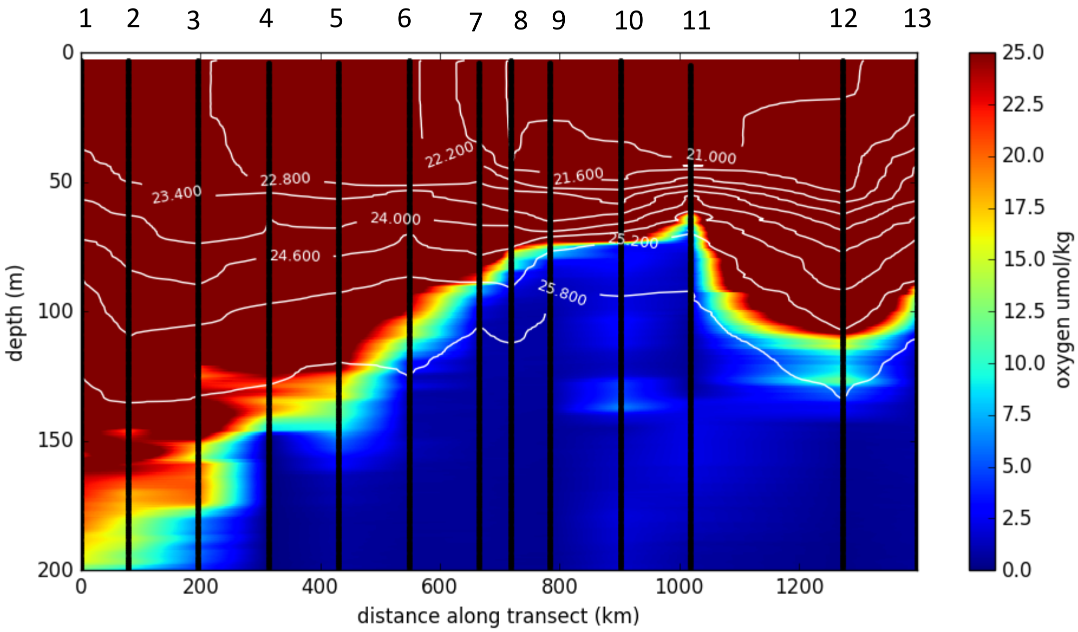

Figure 2. Integrated oxygen concentrations along the transect. The white lines are density contours. The black lines indicate sampling locations. Station numbers are depicted at the top.

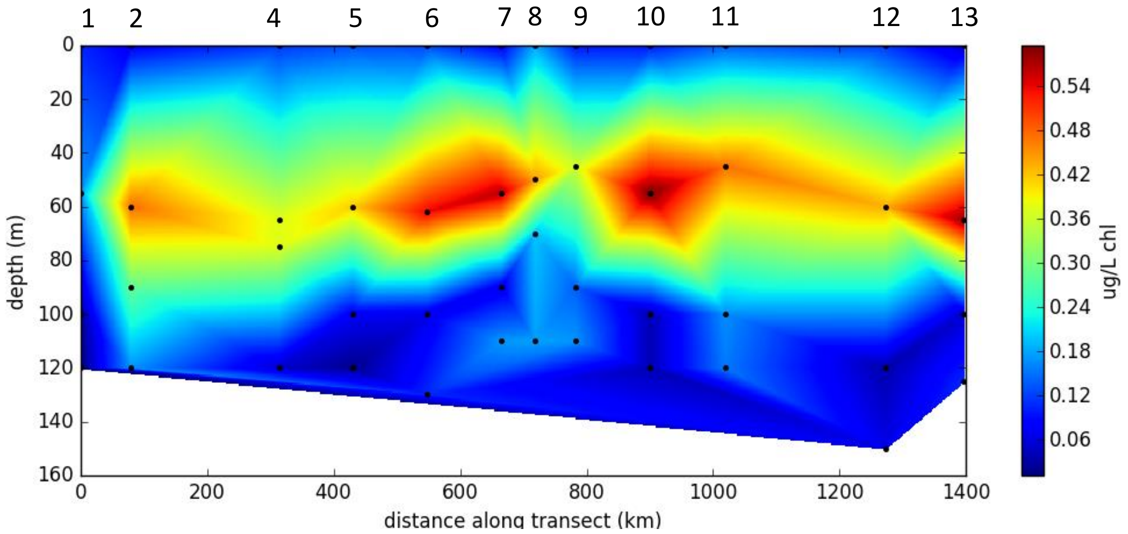

Figure 3. Total chlorophyll concentration (µg/L) integrated across the open ocean stations sampled. The dots indicate sampling location. Total concentration was calculated by summing the size-fractioned concentrations.

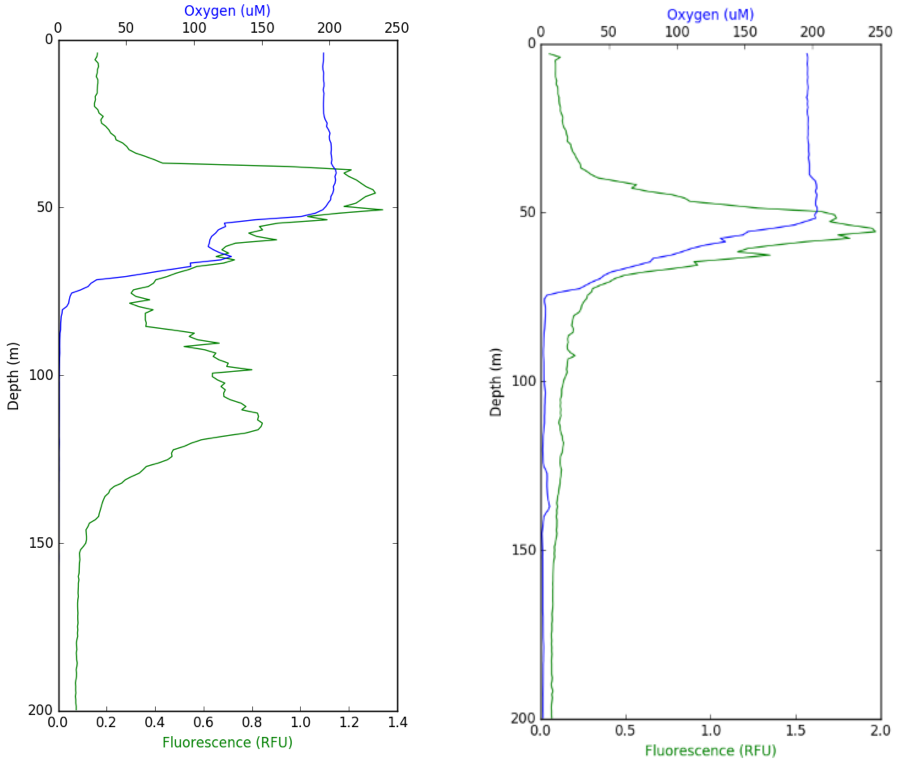

Figure 4. Plots of fluorescence and oxygen for stations 9 (left) as an example of a station with a deep, secondary chlorophyll maximum and 10 (right) which lacked the secondary chlorophyll maximum. Between 125m and 150m, there was a slight oxygen intrusion at station 10.

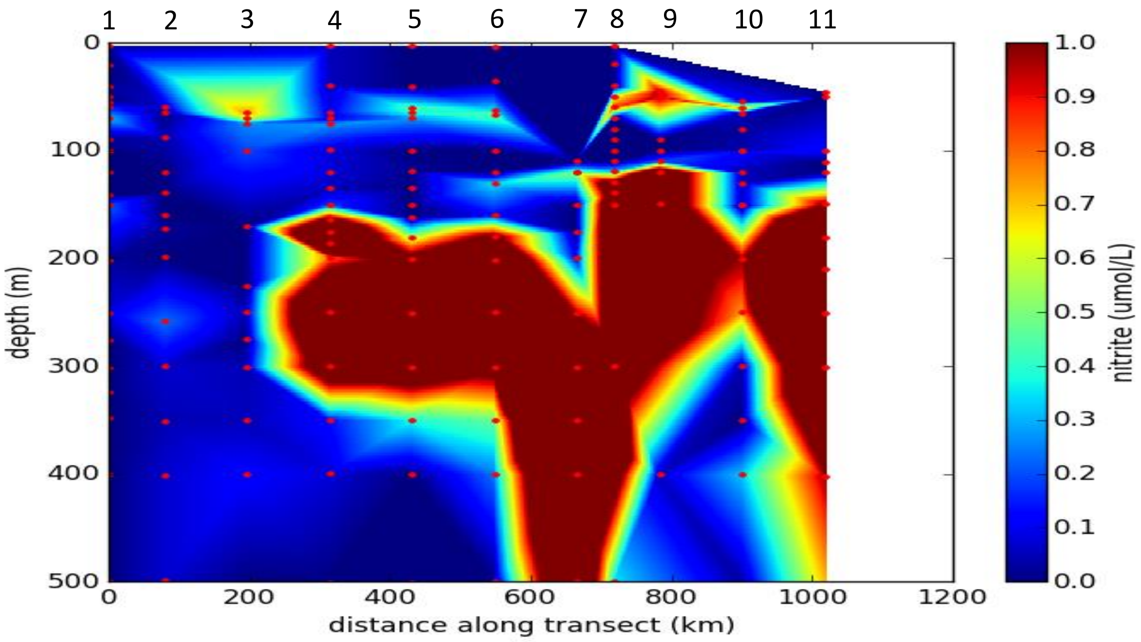

Figure 5. Integrated nitrite measurements along the transect. Station numbers are depicted at the top. The red dots indicate where samples were taken. Nitrite samples were taken and processed by Nicole Travis, a Stanford graduate student.

Table 1. The depth at which oxygen concentrations fell below 20 µM, mixed layer depth, sea surface temperature, total chlorophyll concentrations for the surface waters (determined by summing the surface chlorophyll size fractions), >10 µm surface phytoplankton size fraction, and sea surface salinity for each station sampled. There was no data for the 10 µm size fraction at station 12.

Station	O_2 <20µM (m)	MLD (m)	SST (°C)	Chloro (µg/L)	>10um (µg/L)	SAL (psu)
Sta. 1	182.764	21.861	23.759	0.105	0.008	34.429
Sta. 2	167.878	14.906	25.282	0.078	0.008	34.603
Sta. 4	133.138	36.771	26.310	0.116	0.009	34.563
Sta. 5	125.199	42.735	27.124	0.130	0.010	34.631
Sta. 6	103.350	46.713	27.349	0.127	0.011	34.701
Sta. 7	85.471	23.856	28.142	0.079	0.006	33.499
Sta. 8	76.530	40.753	29.145	0.180	0.019	33.600
Sta. 9	74.544	35.785	29.527	0.096	0.005	33.583
Sta. 10	73.554	31.811	29.492	0.098	0.006	33.590
Sta. 11	61.631	39.764	28.487	0.138	0.010	33.215
Sta. 12	110.318	51.688	29.517	0.116	ND	33.563
Sta. 13	90.440	16.898	28.466	0.052	0.006	33.989
Sta. 15	48.921	10.624	26.017	0.204	0.081	34.670

Table 2. Surface phosphate, silicate, nitrate and ammonium concentrations for each station. *The values from station 15 were from a depth of 10m.

Station	PO_4 (µmol/L)	Silicate (µmol/L)	Nitrate (µmol/L)	NH_4(µmol/L)
Sta. 1	0.392	2.667	0.000	0.001
Sta. 2	0.408	2.982	0.000	0.000
Sta. 4	0.338	2.305	0.220	0.023
Sta. 5	0.403	2.216	0.270	0.011
Sta. 6	0.434	3.188	0.231	0.203
Sta. 7	0.218	1.412	0.259	0.054
Sta. 8	0.318	1.333	0.538	0.005
Sta. 9	0.188	1.585	0.000	0.000
Sta. 10	0.158	1.078	0.029	0.688
Sta. 11	0.249	2.947	0.221	0.026
Sta. 12	0.200	3.209	0.078	0.000
Sta. 13	0.214	1.979	0.050	0.002
Sta. 15	0.450*	3.480*	0.510*	0.000*

Phytoplankton community size fractions

For the open ocean stations (Figure 6), most of the communities sampled were dominated by the picoplankton size fraction (<2 µm). Total concentrations of phytoplankton at the surface ranged from 0.05-0.18 µg/L. For the primary chlorophyll maximum (generally located at about 60 m), total chlorophyll concentrations were 0.18-0.60 µg/L. At the open ocean stations south of station 5, the secondary chlorophyll maximum ranged from 0.07-0.18 µg/L. Station 15, the coastal station sampled for comparison, had much higher chlorophyll concentrations: the surface and first chlorophyll maximum concentrations were 0.20 µg/L and 2.26 µg/L respectively, and the chlorophyll maximum at this station was much shallower (25 m). Unlike the open ocean stations, the microplankton size fraction (>10 µm) made up the majority of the community at station 15. Here, there was a slight secondary chlorophyll maximum (0.11 µg/L) at a depth of 80 m.

After station 6, the northernmost station sampled with a secondary chlorophyll maximum, the proportion of the larger size fractions (microplankton and nanoplankton) increased at the lowest sampled depth. Across the transect, the proportions of the microplankton and nanoplankton size classes generally increased with depth. The picoplankton size fraction was generally the highest in waters closest to the surface. From station 6 onwards (where the oxygen minimum zone shoaled into the euphotic zone), the picoplankton size fraction decreased dramatically (Figure 7).

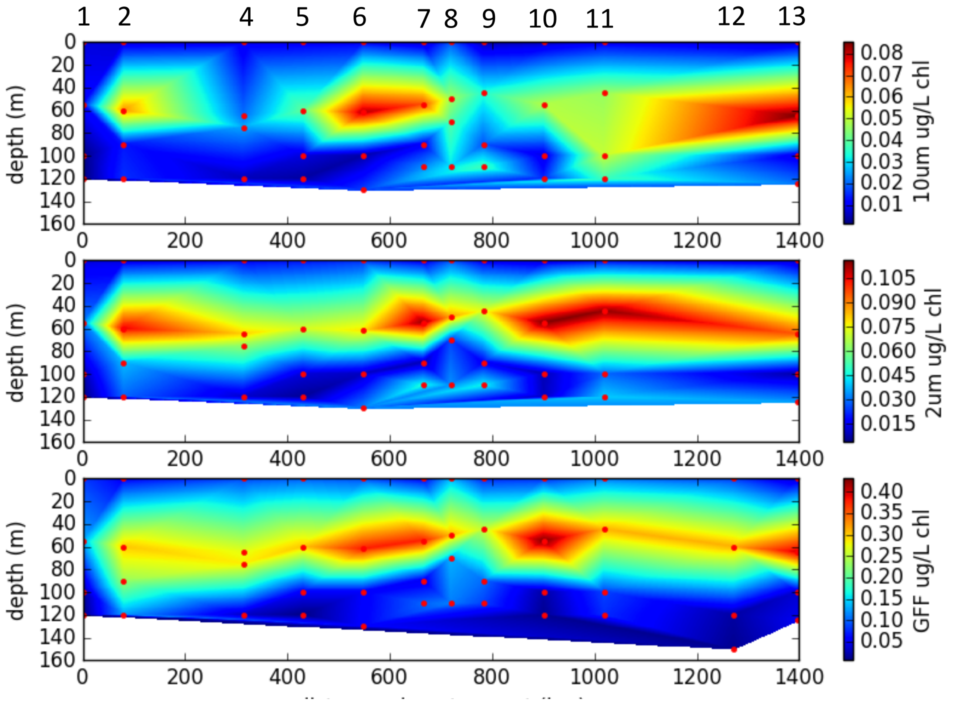

Figure 6. Integrated chlorophyll concentration (µg/L) of microplankton (>10 µm), nanoplankton (<10 µm and >2 µm), and picoplankton (<2 µm) across the open ocean stations sampled. The dots indicate sampling location. Note the different scales on the color bar axes.

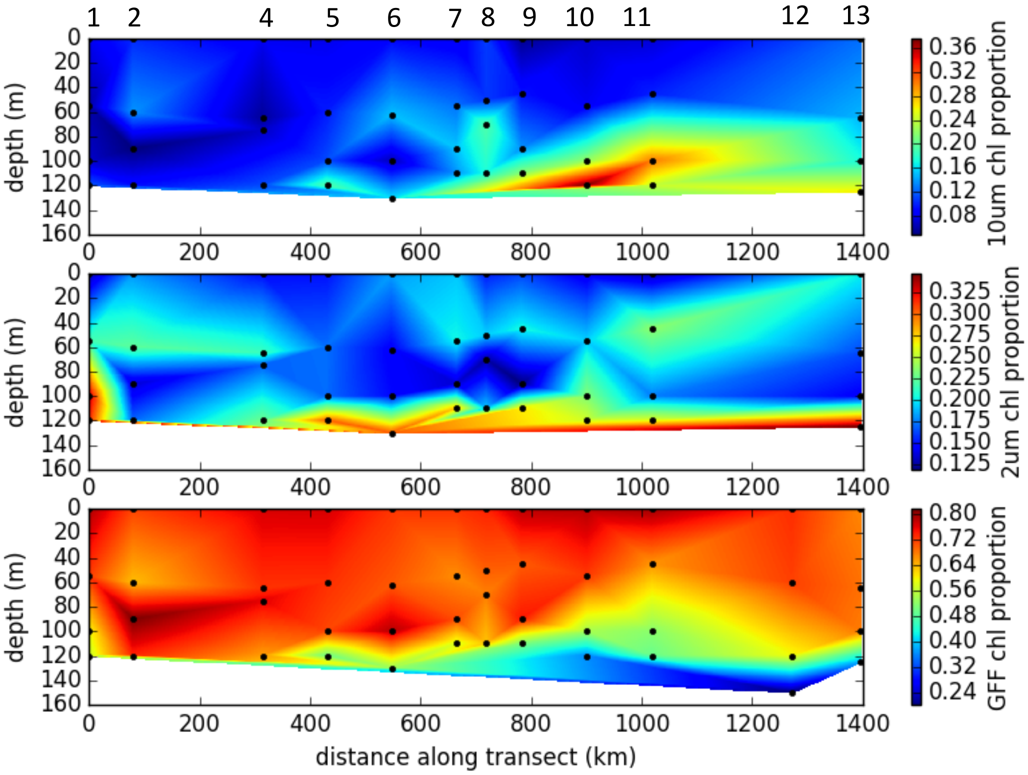

Figure 7. Integrated proportion of microplankton (>10 µm), nanoplankton (<10 µm and >2 µm), and picoplankton (<2 µm) across the open ocean stations sampled. The dots indicate sampling location. Note the different scales on the color bar axes.

Patterns in surface microplankton community composition

The composition of >10 µm surface phytoplankton community did not appear to be organized according to north/south location (Figure 8). Station 15 was not included in the ordination because including it resulted in negative R^2 values for two of the axes (axis 1: R^2=0.806, axis 2: R^2=-0.166, axis 3: R^2=-0.178; ordination only included taxa found at two or more stations). Such a high R^2 value for only one axis indicated that the composition of station 15 strongly skewed the ordination along that axis, which would have made it more difficult to focus on variation among the open ocean stations. For the open ocean station ordination, the axes 2 and 3 explained most of the community arrangement (axis 1: R^2=0.062, axis 2: R^2=0.516, axis 3: R^2=0.270).

The environmental variables of the depth at which the oxygen concentration fell below 20 µM, sea surface temperature, salinity, phosphate, chlorophyll concentration (especially the >10 µm chlorophyll concentration) and nitrate had the highest correlations to the community arrangement for an axis (Table 3). The taxa that had the highest correlation with axis 2 of the NMS ordination were: unknown #1.8 (r=-0.864), *Chaetoceros* (r=-0.853), *Proboscia* (r=-0.852), dinoflagellates (r=0.800), *Oxytoxum* (r=-0.787), *Amphisolenia* (r=-0.787), *Bacteriastrum* (r=-0.758), unknown #8.3 (r=-0.751), *Rhizosolenia* (r=-0.691), *Hemiaulis* (r=-0.576), and *Eucampia* (r=-0.573). The taxa that had the highest correlation with axis 3 of the NMS ordination were: *Cylindrotheca* (r=-0.942), unknown #9.1p (r=-0.927), and unknown #9.1 (r=-0.710). "Dinoflagellates" was a very generic category (See plankton counts in methods for details). Unknown #8.3 was likely a dinoflagellate. Unknown #1.8 was a cell about 125 µm in diameter that appeared to have 6 spines. These two were each identified at only three stations, with only one or two counts per over 200 cells. Unknown #9.1p was a tapered pennate diatom about 75µm in length. Unknown #9.1 was a pennate diatom 65-115µm in length that had rounded ends. These two unknowns occurred at the same 8 stations.

Table 3. Results from the correlation of the phytoplankton community ordination (stations 1-13) and the environmental matrix (See table 1). R values are only reported for axes 2 and 3 because they had the highest r-squared values of all three axes generated by the NMS program (see results above). Environmental variables from top to bottom: depth at which the oxygen concentration fell below 20µM (m), mixed layer depth (m), sea surface temperature (°C), total surface chlorophyll (µg/L), surface chlorophyll in the >10 um size fraction (µg/L), salinity (psu), phosphate (µmol/L), silicate (µmol/L), nitrate (µmol/L), ammonium (µmol/L).

Environmental Parameter	Axis 2	Axis 3
	r	r
<20µM O_2	-0.709	0.250
MLD	0.300	-0.287
SST	0.616	-0.162
Total chloro	0.187	-0.686
Chloro >10µm	0.028	-0.821
Salinity	-0.612	0.017
Phosphate	-0.577	-0.235
Silicate	-0.472	0.380
Nitrate	0.288	-0.787
Ammonium	0.211	-0.127

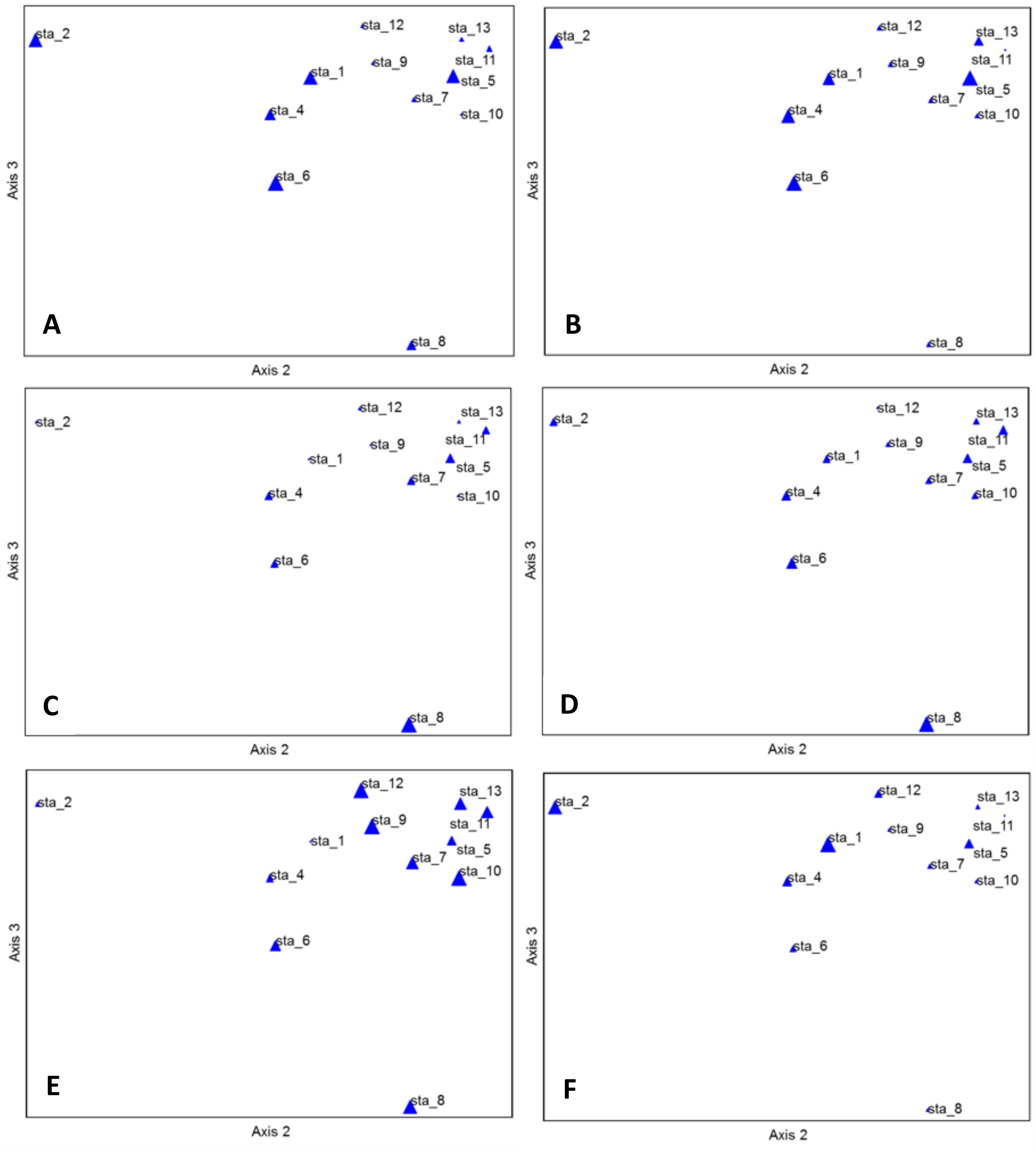

Figure 8. NMS (Non-metric multidimensional scaling ordination) plots of surface microplankton community composition. Ordinations were created without counts of chaining cells from "clumping" taxa. Distance along the axes is a proxy for community difference. Closer stations were more related to each other than they were to stations placed further away in the NMS space. Size of the symbols indicates the directionality of environmental influences on community composition. Phosphate (A), salinity (B), nitrate (C), surface chlorophyll in the 10 um size fraction (D), sea surface temperature (E) and depth at which the oxygen concentration fell below 20 µM (F). Remember that there was no >10 µm chlorophyll data for station 12.

Discussion

There was a notable change in community size composition with depth and across the transect. In my results, the <2 µm size fraction decreased at the secondary chlorophyll maximum once the ODZ shoaled into the euphotic zone. Conversely, the larger size fractions increased at these locations, notably the >2 µm size fraction (Figure 7). However, Goericke et al. (2000) reported that at times the deep chlorophyll maximum was *Prochlorococcus*-dominated, if not a "virtual monoalgal culture". Looking at just the size fractions for the southern stations sampled in 2016, I sampled far from a completely monoalgal culture. Goericke et al. (2000) found evidence of monoalgal cultures by detecting a large contribution of Chl a_2 to the total chlorophyll a. To confirm what types of phytoplankton are living at depth, it would be ideal to be able to sequence the genomes of these populations.

The phytoplankton community composition from the net tow samples showed no clear patterns based on location (Figure 8). There was no north/south division as hypothesized. Stations closer to land did not have similar community structure, which suggests that coastal influences do not reach to this extent offshore, and therefore are not drivers of open ocean community composition. The larger (>10 µm) phytoplankton community composition variation that I observed mainly was due to large scale variations in sea surface salinity, temperature, nitrate, phosphate, and the depth at which the oxygen concentration fell below 20 µM (Table 3). This was evidence that the patchiness of nutrients like phosphate and nitrate, and subtle differences in surface salinity and temperature as well as the depth of the OMZ played a major role in community structure. Data shows that the ODZ is increasing in vertical extent, both shoaling and deepening (Bakker 2017). Therefore, shallower upper bounds of the ODZ in the future could cause changes in open ocean community assemblages.

The lack of a spatial pattern in the phytoplankton community ordination has implications for open ocean modelling. Because only one sample was taken at each station, it was not possible to distinguish station-level differences from sample-level differences. If I had been able to gather surface samples at slightly different locations at each station, then I would have been able to assess within station diversity. Then, I would have been able to determine community variation across the seascape. Since there was no way of knowing how much of the variation between station communities was variation among samples, the scattered community visualization partly could have been due to a degree of within station diversity. In addition, there could have been other important niche parameters that were not able to be tested (e.g. competition, grazing pressure, other trace elements) that did not vary from north to south. If sample variation plays a significant role in community composition, ocean models would have to find a way to generate this diversity in order to better model carbon and nutrient cycling. If non-biological variables other than sea surface salinity and the nutrients tested (Table 1 and Table 2) have an influence on community structure, then modelers would have to have access to that data at an appropriate resolution. If biological interactions (i.e. grazing pressure and competition) have a significant influence on community structure, then it will be much more difficult to model phytoplankton community composition and therefore carbon and nutrient cycling accurately.

The presence of *Cylindrotheca*, *Hemiaulis*, *Proboscia*, *Oxytoxum*, *Amphisolenia*, *Rhizosolenia*, *Chaetoceros*, *Eucampia*, *Bacteriastrum*, unknown #9.1p, unknown #9.1, unknown #8.3, and unknown #1.8 had the most significant influences on community structure. Determining the identity of the unknown pennate diatoms observed in this study would greatly help to explore the ecological role of these organisms.

In a previous study conducted in the oligotrophic northern Adriatic, researchers found that *Cylindrotheca closterium* forms aggregates. The appearance of these clumps was associated with higher salinity (Najdek et al. 2005). *Cylindrotheca* was observed at stations 1-6, station 8, and station 15. Stations 1-6 and station 15 had the highest salinity values of the transect (Table 1). Local or landscape level changes of salinity likely drove the presence and abundance of *Cylindrotheca* across this transect.

The chaining diatom *Hemiaulis* hosts nitrogen-fixing cyanobacterial symbionts (Villareal 1991). This ability gives this diatom a competitive edge in extremely nutrient poor patches and enables it to act as a facilitative species for the other members of the phytoplankton community. *Hemiaulis* was present at two of the three stations that had undetectable amounts of nitrate (stations 1 and 2, Table 2). However, it also was counted at stations with some of the highest nitrate in the transect (stations 4, 5, 6, 12, 15). However, no *Hemiaulis* were counted at station 8, which had very high concentrations of nitrate relative to the other open ocean stations. This suggests that these cells might not be good competitors in relatively high nutrient patches in the open ocean.

Rhizosolenia and *Proboscia* are two very similar diatoms (*Rhizosolenia* and *Proboscia* are sometimes synonyms). *Rhizosolenia* may be favored in locations with low phosphorus (Carlsson and Graneli 1999). This finding is not consistent with the findings of this study. These genera were found at stations 2, 4, 6 and 15. *Rhizosolenia* form mats at the surface of the ocean. As an adaptation to the oligotrophic open ocean, these diatom mats perform extensive vertical migrations (~100+ m) to obtain nutrients. When a mat returns to the surface, nitrogen taken up by the cells leaks into surface waters and benefits the surrounding community (Singler and Villareal 2005).

On this cruise, I observed the shoaling of oxygen-deficient waters from north to south, also reported by Goericke et al. (2000) and Cepeda-Morales et al. (2009). The appearance of high nitrite (Figure 5) where the ODZ shoaled into the euphotic zone was consistent with previous studies. The top of the ODZ is the depth of the maximum rate of nitrite reduction (Buchwald et al. 2015). Here, nitrite can be both oxidized and reduced, so it can be used by several coupled and competing reactions in low oxygen water. Beman et al. (2013) reported that in the Eastern Tropical North Pacific, nitrite oxidation rates were the highest where the dissolved oxygen concentrations were <5µM. This was also where nitrite accumulated and nitrate reductase genes were expressed.

In terms of ocean modelling, it is necessary to understand what conditions will cause dramatic changes, like the absence of a secondary chlorophyll maximum, in order to properly represent oxygen production, and carbon and nutrient cycling. At station 10 (15.002°N 106.568°W), there was no secondary chlorophyll maximum, unlike the stations on either side of this location (Figure 3). Here, at a depth of about 130 m, there was a slight oxygen intrusion. From 100 m to 150 m oxygen values were low, but not zero. In comparison, at neighboring station 9, the oxygen values reached zero at about 80 m (Figure 4). An eddy could have created a local disturbance at station 10 on the day it was sampled, thereby infusing the water column between 100 m and 150 m with oxygen. Comparing CTD data from station 9 and station 10 emphasizes how ephemeral the open ocean can be. With just the slightest difference, a whole sub-surface oceanic community can be rendered virtually non-existent. During the WOCE cruise during December 2007-January 2008, an intrusion of relatively high oxygenated water around 80 m was reported at about 14.5°N 105°W (WOCE Pacific Ocean Atlas). This similar phenomena happening at a similar location at a similar time of year suggests that oxygen intrusions like this could be seasonal and reoccurring.

Community composition is important for ecosystem functioning. Genera-specific classifications of phytoplankton matter when modelling carbon and nutrient cycling because different genera can perform different ecological roles. Morphological diversity helps us classify and understand functional diversity, which is essential for accurate ocean modelling. In this way, microscopic work like this can be more valuable than a strictly phylogenetic approach. Determining if a genome is different enough from other genomes to require a separate classification can be somewhat arbitrary. Functional classifications are more relevant to ecosystem functioning and to chemical and biological cycling. By exploring the ecology of individual diatom genera, we can better understand seemingly random compositions of open ocean phytoplankton communities. With more research into the ecological niches of specific phytoplankton, we will be closer to predicting community composition and therefore carbon and nutrient budgets from environmental factors.

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