Limiting Nutrient Concentrations and Nutrient Assimilation and Storage Gene Expression across the Transition Zone Chlorophyll Front in the North Pacific Ocean

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Abstract

This study investigates limiting nutrient-relevant cyanobacterial gene expression across the Transition Zone Chlorophyll Front (TZCF) in the north Pacific Ocean. An increase in available nutrients due to upwelling in the sub-arctic ocean gyre is expected to result in an increase in expression in genes associated with nutrient uptake and storage. This expected trend was not observed. Rather, there was a weak, negative correlation in all instances except ferritin expression in *Synechococcus*, which showed a weak positive correlation. The departure from the expected correlation is a likely a result of unexpected anthropogenic inputs of nutrients, or differences in nutrient usage among ecotypes of *Prochlorococcus* and *Synechococcus.*

Plain Language Summary

This project aimed to grow understanding about how cyanobacteria, photosynthetic bacteria, are able to react to changes in environmental conditions. I focused on one region north of the Hawaiian Islands, where two ocean gyres converge. This region is referred to as the Transition Zone Chlorophyll Front (TZCF) and is a region of very high biological productivity. This study specifically looked at the way that cyanobacteria are able to make proteins to allow them to use nutrients that are available for them in the water; more available nutrients would mean that cyanobacteria are producing more proteins in order for them to use the nutrients to grow and reproduce. As the TZCF is approached and crossed, the amount of available nutrients increases, the expectation is that the amount of proteins that cyanobacteria are making to also increase; organisms are using the nutrients available to them. The data shows that this relationship is not generally true, with that trend holding true for one gene in one type of cyanobacteria, while the rest showed the opposite trend.

Introduction

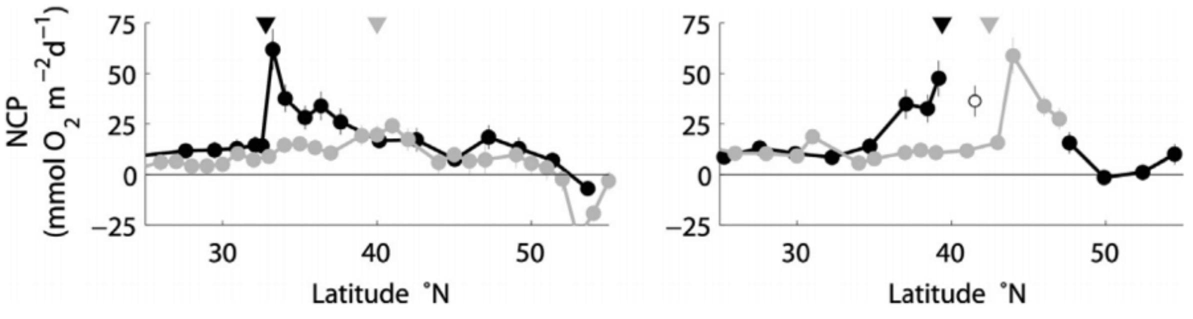
The sub-arctic subtropical transition zone spans the Pacific basin between the Kuroshio Current Extension and the California Current and exists between 35°N and 45°N with seasonal fluctuation (Ayers and Lozier 2010). The transition zone is defined by the region between the sub-arctic gyre, and the subtropical gyre and between two salinity fronts associated with the two gyres respectively. Contained within the sub-arctic subtropical transition zone is the transition zone chlorophyll front (TZCF), a chlorophyll gradient with a position defined by the 0.2 mg C/m^3 isopleth (Polovina et al. 2017). This front marks the boundary between the subarctic frontal zone and subtropical front and is a zone of enhanced phytoplankton net community production, as well as an important region for pelagic fauna. Originally identified through a study of 9 loggerhead sea turtles, 6 of the 9 surveyed turtles spent significant time along a sharp surface chlorophyll gradient that was identifiable using remotely sense sea surface color data (Polovina et al. 2001). In addition, a survey of US albacore tuna catch data found that the highest proportion of catch came from trolling within proximity of this chlorophyll gradient, suggesting its importance to existing fisheries. It seems to be an important region in loggerhead sea turtle migration and feeding and in the location of albacore fisheries (Polovina et al. 2001).

The location of the transition zone chlorophyll front (TZCF) fluctuates between 40-43° N and 31-34° N, migrating about 1000km seasonally between the northern and southern bounds of the transition zone. The mechanisms that drive this seasonal migration are still the subject of study, however, recent research by Ayers and Lozier (2010) found that the location of the TZCF may follow the boundary between the two ocean gyres.

The high productivity to the north of the TZCF is a result of waters supplied by nutrient rich upwelled subarctic gyre. This seasonal migration of the TZCF is a function of wind stress with strong winter westerlies driving the front southward into the subtropical gyre. In the summer, these westerlies are less intense, allowing the TZCF to relax northward (Ayers and Lozier 2010). When modeled, significant correlation is found between increased levels of horizontal Ekman transport due to increased strength of westerlies and the wintertime location of the TZCF and through surveyed years, represented the southern extent of the TZCF well (Ayers and Lozier 2010). This finding, as well as by Bograd et. al. (2004) is consistent with the assumption that the advection of nutrient rich water from the upwelled sub-arctic gyre sustains productivity in the northern subtropical gyre, resulting in a steep chlorophyll gradient (TZCF) (Bograd et al. 2004). The processes that drive the seasonal migration of the TZCF may also drive nutrient supply to this region, which controls productivity.

Net community production is the difference between the total amount of carbon fixed through photosynthesis, and ecosystem respiration. Net community production was observed on multiple cruises in proximity to and across the TZCF. Primary productivity was 1.5-4 times higher at or near the TZCF (Fig. 1) than other locations on the transects that were surveyed (Juranek et al. 2020). The presence of elevated productivity could point to the TZCF's importance in air-ocean gas exchange in the north Pacific sink region (Juranek et al. 2012) as fixed organic carbon is taken to depth rather than respiration as particulate organic carbon. The

Fig. 1. Meridional trends in productivity across 4 transects. Trends for spring cruises in April 2003 (black) and March 2006 (grey) appear left. Trends for two summer cruises, October 2003 (black) and September 2008 (grey). Inverted triangles point to location of TZCF for each cruise (Juranek et. al.,



TZCF delineates a picoplankton community boundary as well, with *Prochlorococcus* dominating abundance to the south, and *Synechococcus* dominating abundance to the north. The cause of this increased productivity is assumed to be correlated to vertical mixing, as well as a weakly stratified top 200 m and a shallow nutricline (Juranek et al. 2012). Vertical mixing results in decreased stratification, or a homogenous mixed layer. This means, along with a shoaling of the nutricline, that nutrients are closer to the surface and more available for photosynthetic organisms, thereby increasing productivity. There are many nutrients involved in increases in productivity. Two among them, and the focus of this project, are nitrate and iron. Nitrate and nitrite (NO_2 and NO_3 respectively) are essential nutrients required for growth and development. Iron is another essential nutrient and is often a limiting nutrient in oligotrophic conditions. A large flux of iron to the oligotrophic ocean is terrestrial dust, which is blow from surrounding continents and settles into the ocean. Like nitrate and nitrite, this terrestrial iron is not immediately bioavailable, requiring intermediate reactions with molecules such as siderophores to become available for use by biota (Morrissey and Bowler 2012). The productivity in the TZCF region is thought to be limited by the import of nutrients from higher latitudes and that it is the presence of these normally limiting nutrients that drives the increase in productivity.

The location and productivity of the TZCF are likely subject to change as a result of anthropogenic climate change due to weakening westerlies (Polovina et al. 2017). This will result in a decrease in wind stress on the surface ocean, decreasing the strength of Ekman transport moving nutrient waters to the south, and cause detrimental effects to fisheries and other biological communities that rely on the TZCF as less nutrient availability and less primary productivity in lower latitudes will compromise foraging grounds for fauna, and lead to less overall ecosystem productivity. Consistent with output from NOAA's Geophysical Fluid

Dynamics Laboratory's Earth System climate model with biogeochemical and phytoplankton components, the TZCF's northern boundary is projected to migrate northward approximately 500-1000km (Polovina et al. 2017). This northward migration is attributed to increases in vertical stratification and weakening and poleward shift of westerlies, which drive mixing and southward migration of the TZCF in the winter months (Polovina et al. 2001).

This project tests the hypothesis that nitrate is the limiting nutrient for increased primary productivity associated with the TZCF and can be correlated with increased expression of gene NarB in cyanobacteria. This results in increased nitrate reductase due to the importance of this gene to nitrate assimilation and the conversion of nitrate to bio-available nitrite. As well, iron limitation is considered by testing whether expression of ferritin, a protein that sequesters and oxidizes iron, can be positively correlated with increased primary productivity associated with the TZCF due to iron being a limiting nutrient for growth. To test this hypothesis, the correlation between nutrient availability across the TZCF, and cyanobacterial gene transcript levels of *Prochlorococcus* and *Synechococcus*, is studied. These primary producers form the base of the marine food web.

Methods

This study relies on historical data as well as interpretation of that historical data, namely, metatranscriptomic and nutrient data from the second of three Gradients Cruises (MGL1704), which sailed in the spring of 2017 between 23.14 degrees north and 42.43 degrees north along -158 degrees west. Samples collected on this cruise measured a number of biological parameters including nitrate, phosphate profiles, iron concentrations along the transects, chlorophyll a, primary productivity and net community productivity. The Gradients 2 cruise is used because the data for prokaryotic gene expression is the most complete. This cruise sailed across the TZCF and took metatranscriptomic data along transect. The metatranscriptomic data from this cruise serve as the primary data set for the study, supported by other chemical and biological data such as nutrient profiles.

The metatranscriptomic data was collected by first taking triplicate sea water samples at 15 meters depth at sunrise (roughly 6 A.M.). Two size fractions were taken on filters at 3-200 μm and 0.2-3 μm. For this study, I will focus on the 0.2-3 μm filter which captures the small sized cyanobacteria. Samples were then flash frozen and transported to the lab for extraction. RNA was then extracted from filters, where ribosomal RNA was largely removed so that it does not interfere with the ability to sequence target mRNA due to rRNA abundance. RNA was then sequenced using the Illumina Platform. DNA short reads were then mapped against cyanobacterial genomes and counts were recorded in transcripts per million (TPM) against single cell genomes from a collection of different ecotypes using a software called "Salmon" (Patro et al. 2017). This data was then compiled into a data set that contains information such as mean TPM at various latitudes through the MGL1704 transect, associated ecotypes, and CyCOG (Cyanobacterial Clusters of Orthologous Groups of proteins) ID for target genes.

Using the CyCOG ID number, specific genes in the data set and their transcript abundance (in TPM) along the transect were obtained. This allowed me to plot the TPM of nutrient-related transcripts as a function of latitude and compare that with nutrient profiles taken on the same cruise. To aid in plotting, Ocean Data View (ODV) was used to construct surface profiles, allowing TPM to be plotted against latitude. The use of ODV also allowed for the construction of scatter of TPM vs. latitude. ODV contains statistical analysis tools that were also utilized in this study. Excel was also used to plot and to perform statistical tests on the data. The data also differentiated between the genera of the sequences, as well as between ecotypes within genera. As well, data normalization for TPM counts was done to offset the effect of abundance on transcription counts. To do this, bacterial abundance data from Gradients 2 was used and was sorted into bins corresponding to station coordinates from the metatranscriptomic data; 0.5 degrees north and south of station coordinates (+/- 0.5 degrees). Bacterial abundance from these bins was averaged, then used to normalize TPM counts. This was done by comparing the maximum average abundance, as well as the TPM count, as well as the average abundance for that bin. The following formula was used to normalize the TPM data.

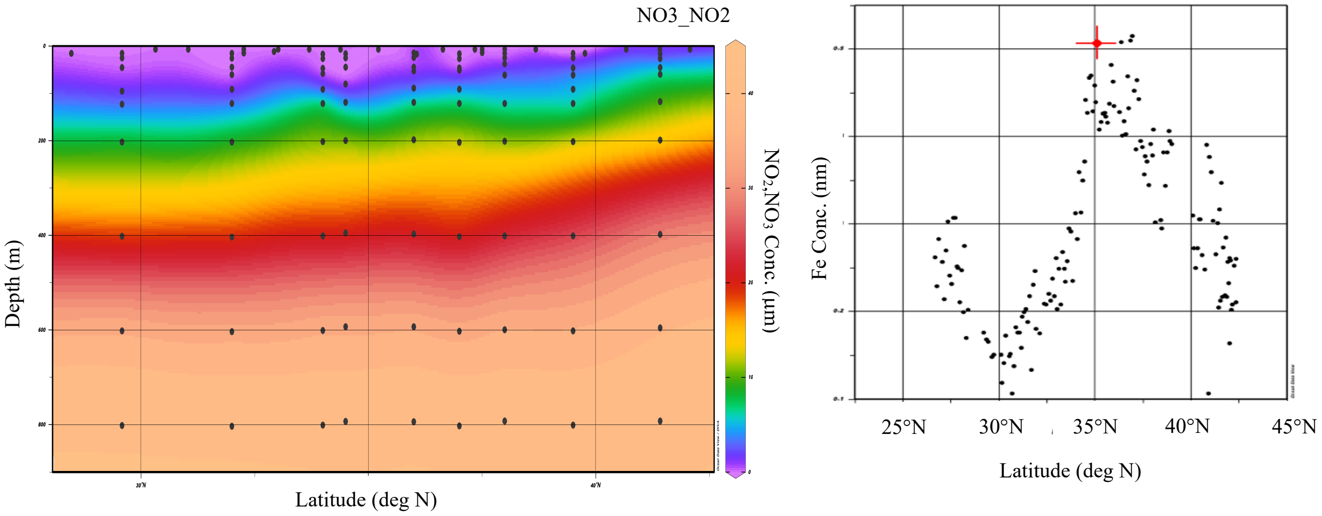
Abundance Normalized TPM = max. average bin abundance*(mean TPM/average abundance)

This data was then plotted vs. latitude in order to assess for patterns in transcription count through the transect.

Results

Nitrate (NO3-) and Nitrite (NO2-) concentrations increase northward and start to shoal at approximately 39.5 degrees north (Fig. 2A), with black dots representing individual

Fig. 2A. (left) NO3 & NO2 profile taken from Gradients 2 cruise showing a shoaling of concentration northward along cruise tract. Fig. 2B. (right) Iron surface concentration plot showing increase in concentration at approximately 35 degN.



measurements. In the south, nitrate and nitrite concentrations, which are measured together because the assay cannot distinguish between the two and nitrite is in small concentrations compared to nitrate, are depleted due to the downwelling nature of the sub-tropical gyre in the northern Pacific Ocean, increasing in concentration as the gyre edge and boundary zone is approached at approximately 35-39 deg N (fig. 2A). Iron, the other nutrient of interest, does not follow as expected a profile, with concentrations increasing northward. Iron initially decreases as distance from Hawaii increases, then concentrations begin to increase northward, until they peak at 0.51nm at approximately 35 degrees north. After this peak, concentrations decrease northward until the end of the cruise tract (fig. 2B). NO3 and NO2 increase in concentration going northward as expected.

The use of metatranscriptomic data allows for an estimate of whether the available nutrients are being used by organism. Metatranscriptomic data was separated by genus (*Prochlorococcus* and *Synechococcus*), and by target gene to understand how each genus reacts to nutrient availability. First, considering NarB, which is a gene that codes for nitrate reductase, a protein involved in the first step of nitrate assimilation and the conversion of nitrate to bio-available nitrite, *Synechococcus* shows an increase in NarB transcript abundance northward, with an increase around 30 degN and at approx. 40 degN (Fig. 3A).The slope of the trend line is negative with an r^2 value of 0.0348. *Prochlorococcus* showed a generally stable transcript abundance, with a slight increase in transcriptions northward (Fig. 3B). The slope of the trend line for *Prochlorococcus* is negative with an r^2 value of 0.0034. The second of the two target genes, ferritin, is a protein involved in intracellular iron storage and uptake.

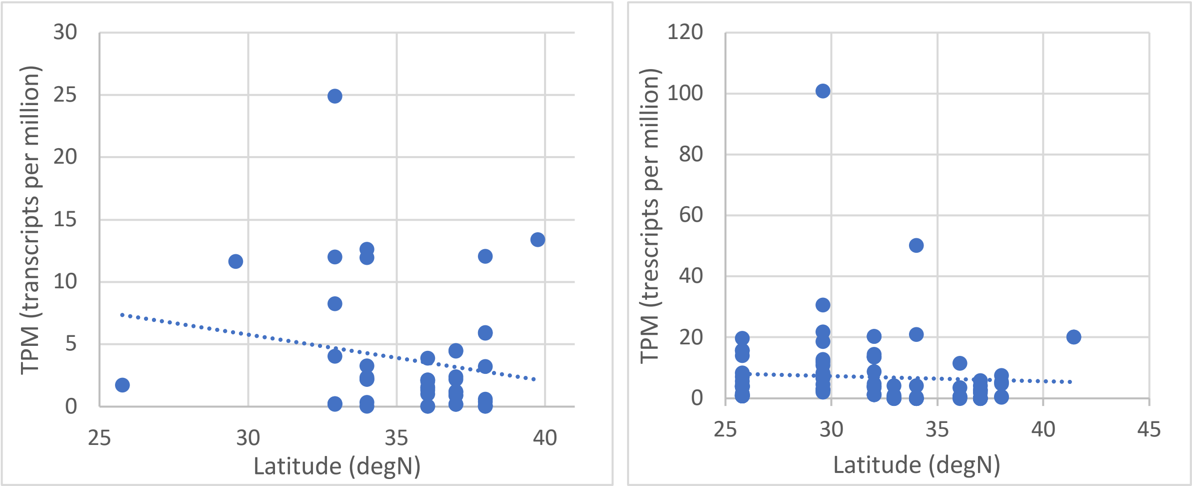


Fig. 3A. Plot of TPM (transcripts per million) of NarB gene in *Synechococcus* as it varies with latitude along the cruise tract. Fig. 3B. Plot of TPM (transcripts per million) of NarB gene in *Prochlorococcus* as it varies with latitude along cruise tract.

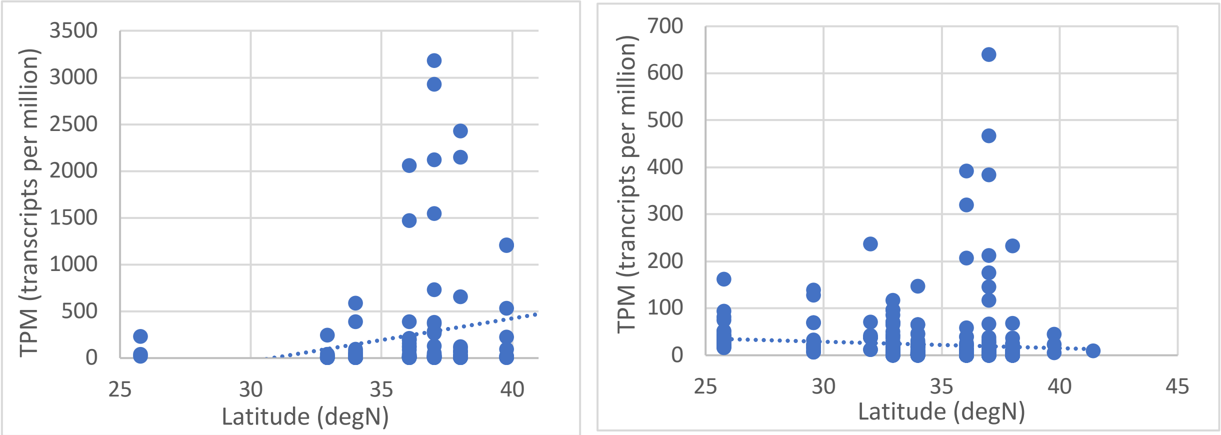


Fig. 4A. Plot of TPM (transcripts per million) of Ferritin gene in *Synechococcus* as it varies with latitude along cruise tract. Fig. 4B. Plot of TPM (transcripts per million) of Ferritin gene in *Prochlorococcus* as it varies with latitude along cruise tract

Synechococcus showed a clear increase in transcript abundance northward, with a small increase in TPM around 35 degN (Fig. 4A). The slope of the trend line for *Synechococcus* is positive with an r^2 value of 0.05. *Prochlorococcus* also showed a slight increase in TPM northward, however had a clear increase around 30 degN (Fig. 4B) and had a negative trendline with an r^2 value of 0.0065. Along with Scatter plots, box plots were constructed to look at distribution of transcription counts south of the TZCF, and north of the TZCF. For NarB in both genera, there was more variation south of the TZCF than there was to the north (Fig 5A). The opposite was true for Ferritin (Fig 5B). In both genera, there was greater variation in TPM counts south of the TZCF.

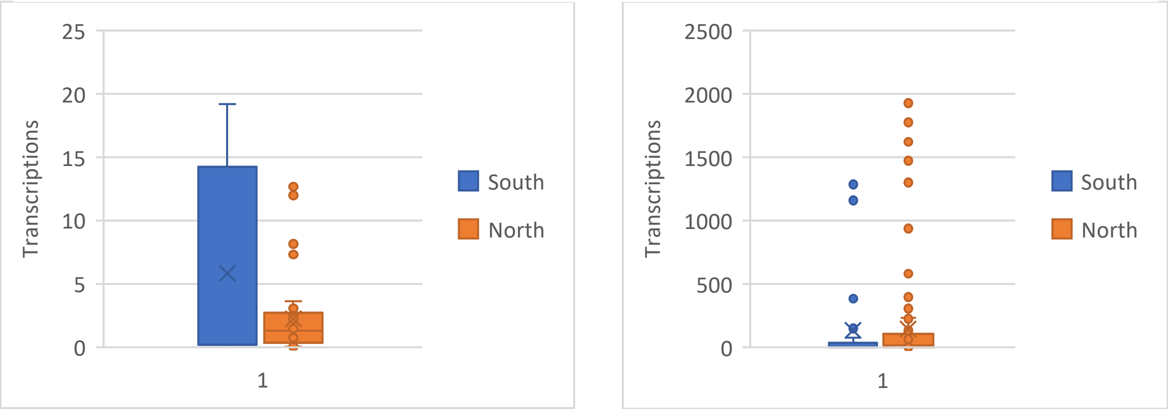


Fig. 5A (left) Distribution of NarB TPM counts in *Synechococcus* south and north of TZCF. Fig. 5B (right) Distribution of Ferritin TPU counts in *Synechococcus* south and north of TZCF.

Discussion

Generally, the trend of increased transcription the further north in the cruise tract was not correlated as expected, although there was much greater variation in TPM northward (Fig. 5A & 5B), which could have been a result of differing ecotypes of *Prochlorococcus* and *Synechococcus*. I expected to see a positive correlation between nutrient availability, denoted by latitude, and TPM count. While there was a positive trendline for the *Synechococcus* Ferritin gene, the correlation was still weak. The rest of the measured genera and gene pairings all had negative trendlines with generally weak correlations. Factors such as anthropological inputs of nutrients, differences in ecotypes, or differences in cyanobacterial abundance could explain the departure from the expected correlations. One potential explanation pertaining to the iron profile, as well as *Prochlorococcus* and *Synechococcus* ferritin transcription is an increased supply of iron to the region of interest via aerosols from industrial activities in Asia. This input contributes between 21-59% of dissolved iron between 35 degN and 40degN (Pinedo-González et al. 2020).

The results of this study do not necessarily confirm my hypothesis, as the expected positive correlation between nutrient availability was only seen in ferrtin in *Synechococcus* expression. In all other instances considered regarding genera and target gene in this project, there was a negative correlation between nutrient availability and expression. This lack of expected correlation in this study means that it is hard to determine whether or not this region experiences a bottom-up control on productivity. Ferrtin in *Synechococcus* would point to bottom-up control due to the positive correlation between nutrient availability and transcript count, however the lack of this trend in the other conditions considered does not support this. Ecotype differences could also have influenced TPM reads, with different ecotypes inhabiting different regions of the research tract and reacting differently to differing environmental conditions and nutrient availability. To test this, I would investigate the distribution of ecotypes along the cruise tract, as well as investigate the nutrient requirements of each ecotype.

The largest concern regarding the results in this study was the amount that data was being extrapolated while using Ocean Data View (ODV). Outliers may have biased the results in one direction, leading ODV to over emphasize their importance and thereby either amplifying a signal an overstating its importance, or the opposite. To fix this, I focused on scatter plots rather than heat maps, to avoid ODV extrapolation. As well, the data did not have complete coverage of the study area, so there were some gaps in the data between stations. This also led to potential over-extrapolation from ODV, which also could have made some trends seem more significant. To mitigate this, the study could be repeated with more frequent data collection, so as to not have large gaps between samples. As well, incorporating more target genes could result in clearer results as there are many nutrients being utilized by cyanobacteria. This would help in identifying which nutrients are likely the ones limiting productivity.

Conclusion

The TZCF is one of many biological gradients that exist in the ocean between gyres and other geographical and oceanographical features. It is characterized by an increase in productivity measured via a 0.2 mg C/m^3 chlorophyll isopleth proxy. It is known that this front marks the boundary between the subarctic frontal zone and subtropical front and is a zone of enhanced phytoplankton net community production, as well as an important region for pelagic fauna, however, ecological control of this increase in productivity was still relatively unknown. I expected to see an increase in transcription of genes that pertain to nutrient uptake due to increasing concentrations northward due to the upwelling nature of the sub-arctic gyre. This would be consistent with the idea of bottom-up control, or that and increase in productivity of producers results in increased production of the ecosystem. The expected trend was somewhat followed, however showed weak correlations that likely resulted from an effect of cyanobacterial abundance, ecotype interactions, or some other control. The increased iron availability at 35-40 degN due to anthropogenic aerosol input was perhaps the most surprising find, as it did correlate to some of the patterns seen in ferritin transcription (Pinedo-González et. al 2020). A follow up study would look at more genes involved in nutrient uptake, assimilation, and metabolism. This would provide a more complete understanding of the biochemical interactions happening in the region. Additionally, more focus could be given to the different ecotypes of each focus genera. Different ecotypes react differently to environmental conditions due to differences in nutrient requirements and how efficiently they use nutrients. This could have explained the trends in expression that were observed. As well, as study that test whether the region is top-down controlled would likely provide interesting results, allowing us to conclude more concretely about ecological control. This could take the form of exclusion experiment, isolating *Prochlorococcus, Synechococcus,* and other pelagic producers in the water column free of consumers.

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