



Bacterial Biomass along the Kuroshio Extension

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NONTECHNICAL SUMMARY

Bacteria are essential for the remineralization of nutrients in the ocean between different energy levels. Therefore, understanding what control bacterial abundance in the ocean is important. This study compares the bottom-up and top-down controls of bacteria along the Kuroshio Extension in order to further understand the processes involved. The Kuroshio Extension is the largest sink for anthropogenic CO₂, which creates an interesting environment for bacterial populations. The data indicated an importance of grazers and viruses controlling bacterial abundance more so than nutrients.

ABSTRACT

Bacteria are essential for the remineralization of nutrients in the ocean between different energy levels. The amount of CO₂ fixed by the food chain is dependent on new nitrate in the euphotic zone. Bacterial population aid in creating dissolved inorganic nitrogen, which is a food source for phytoplankton. The amount of carbon in sediment depends on the bacterial loop, which recycles dissolved and particulate organic carbon. Therefore, understanding what control bacterial abundance in the ocean is key to further understanding the roles of the microbial loop and it's involvement in the carbon cycle. The Kuroshio Extension is the largest sink for anthropogenic CO₂ with approximately 25 mol of anthropogenic CO₂ per square meter. While, the North Pacific region has an average of 15 mol of anthropogenic CO₂ per square meter. Bacteria abundance along the Kuroshio extension play a vital role in the carbon cycle. Therefore, this study compares the effects of inorganic nutrient [nitrate, nitrite, and phosphorus], viral lysis and grazing [heterotrophic nanoflagellates (HNF)] as well as physical and chemical within and without the Kuroshio Extension on the abundances of bacteria in order to further understand the processes involved. The data indicates grazers and viruses may be the controlling factor for bacterial abundance, which was seen in a deviation from normal distributions.

Bacteria aid in the regeneration of nutrients between higher and lower trophic levels. Bacteria are generally $5 \times 10^5 \text{ mL}^{-1}$ in the euphotic zone of the oceans. In some cases, material flux through bacteria in marine plankton food webs can exceed primary production (Ducklow and Carlson 1992). Therefore, bacteria are an important link between detritus, dissolved organic matter, and higher trophic levels because they remineralize nutrients (Azam et al. 1983; Lee et al. 2001; Lalli and Parsons. 1997; Sherr and Sherr 1984). They are responsible for converting dissolved organic

matter (DOC) to its inorganic constituents (Azam et al. 1983).

Since bacteria play an important role in the microbial loop and carbon cycle, the lack of bacteria in a region have substantial effects. The loss of bacterial biomass is mainly caused by grazing and viral lysis, which have different consequences in carbon fluxes and elemental cycles (Fuhrman and Noble 1995; Weinbauer and Peduzzi 1995). If the loss is due to viruses, carbon is burned in a dissolved organic carbon-bacteria-DOC loop (Bratbak et al. 1992). If protists

primarily graze bacteria, the carbon will be transferred to a higher trophic level (Tuomi and Kuuppo 1990). Primary grazers of bacteria are heterotrophic nanoflagellates (HNF) (Lee et al. 2001; Lalli and Parsons. 1997; Sherr and Sherr 1984). Because of the importance of bacteria in the carbon-bacteria- DOC loop and the transferring of nutrients to different trophic levels, understanding the controlling factors of bacteria will increase the understanding of the biological processes in the carbon cycle.

A region with large amounts of carbon creates an unique environment for studying the controlling factors of bacteria. The Oyashio current from the Arctic Ocean flows southward by the Bering Sea collides with the Kuroshio Current originating from Taiwan flowing into the North Pacific Current transporting enormous amounts of heat northward (Tally 1997) altering the region's sea surface temperature and height (Fig. 1). Together they form the North Pacific Current.

The Kuroshio Extension is the greatest sink of anthropogenic CO₂ with approximately 25 mol of anthropogenic CO₂ per square meter. While, the North Pacific region has an average of 15 mol of anthropogenic CO₂ per square meter. (Hideyuki et al., 2011; Sabine et al. 2002). Therefore, the Kuroshio Extension is a key influence on the carbon cycle, which has

properties that involve physical and biological processes.

Biological processes are important to the carbon cycle and the balance of CO₂ in the atmosphere. Specifically, biological processes including bacteria are essential for the cycle. The amount of CO₂ fixed by the food chain is dependent on new nitrate in the euphotic zone. Bacteria aid in creating dissolved inorganic nitrogen, which is a food source for phytoplankton. Additionally, the amount of carbon in sediment depends on the bacterial loop, which recycles dissolved and particulate organic carbon (Lalli and Parsons, 2010). Because bacteria play an important role in the carbon cycle and the Kuroshio Extension is a key influence on the North Pacific's carbon cycle, a study was created for understanding controlling factors abundance of bacteria along the Kuroshio Extension

This study compares the effects of inorganic nutrient [nitrate, nitrite, and phosphorus], viral lysis and grazing [heterotrophic nanoflagellates (HNF)] as well as physical and chemical within and without the Kuroshio Extension on the abundances of bacteria on the R/V *Melville* from February 24 to March 17, 2013 in order to understand the relative effects of the controls of bacterial populations (Fig.1).

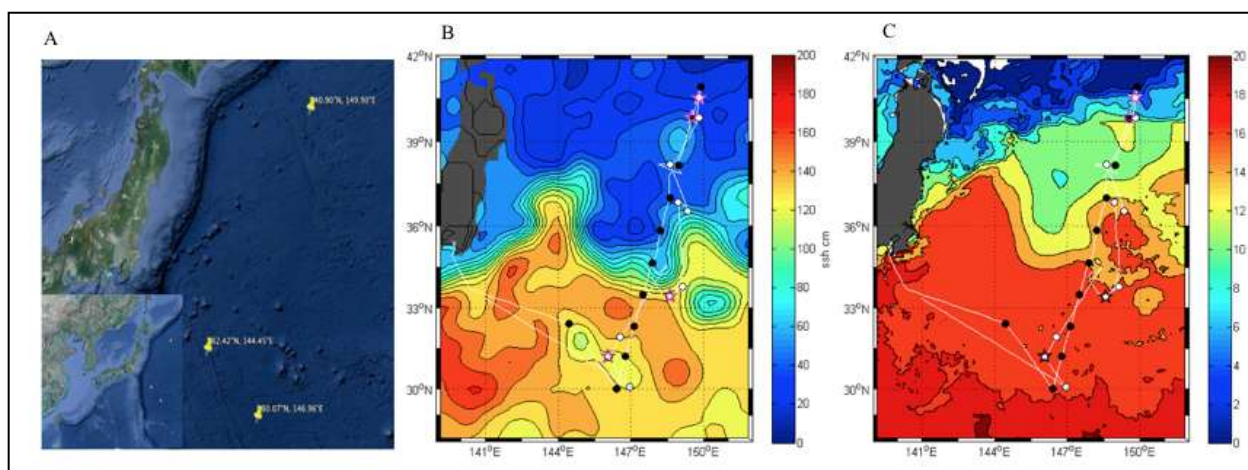


Figure 1. (A) Map of the beginning, middle and end of survey site off the coast of Japan. (B) Average sea surface height (SSH) from March 1-7, 2013, accessed from Archiving, Validation and Interpretation of Satellite Oceanographic (AVISO) with a $\frac{1}{4}^{\circ}$ by $\frac{1}{4}^{\circ}$ resolution. (C) Sea surface temperature (SST) on March 1, 2013, provided by NASA PO.DAAC. Sampling stations are indicated by black (leg 1) and white (leg 2) dots. Starred stations indicate samples that were analyzed. Starred stations from left to right are 22, 20, 14 and 11.

METHODS

Sample collection

From February 24 to March 17, 2013, I collected water samples along the cruise track (32.42°N, 144.45°E) to (40.90°N, 149.90°E) to (30.07°N, 146.96°E) on the R/V *Melville* (Fig. 1). However, due to problems with sample preservation only two water samples from the southern region were available for analysis. Therefore, net tow surface water samples from the northern portion of the track were analyzed for comparison. The southern samples were collected by CTD and fixed with 1% glutaraldehyde (final conc.). The northern samples were collected by a handheld 20 μm mesh net with a 0.25 cm diameter and fixed with 10% formaldehyde. The net tows were spooled out to 10 meters, which corresponded to the shallow chlorophyll maximum, and run through the water three times before the sample was collected onboard. Water samples for nutrient data were taken from the CTD except for station 22; it was collected from an uncontaminated seawater line (5 m depth). Nitrate, nitrite and phosphate were sampled and processed using SEAL Analytical segmented continuous-flow AutoAnalyzer 3 (AA3) by Dan Schuller, Scripps Institution of Oceanography.

Microscopy

Water samples for bacterial and HNF counts were filtered onto 0.22 μm black polycarbonate filters and stained with 4'-diamidino-2-phenylindole (DAPI) (0.1 $\mu\text{m l}^{-1}$ final conc.) for 20 minutes. Samples for viral counts were filtered on 0.02 μm and stained with 5x solution of SYBR for 20 minutes. Three filters per sample were made for bacterial and viral-like cell counts, whereas two filters per sample were made for HNF counts due to the limited water available for analysis. Counts for virus-like particles, bacteria, and HNF were conducted by epifluorescence microscopy and abundance was calculated by averaging 20 fields of view per

filter. Error bars were calculated from the standard deviation of the mean abundance of virus-like particles, bacteria, and HNF.

RESULTS

Bacterial, Virus-like particles, HNF counts

Bacterial abundance range from 7.87×10^5 mean/mL to 9.00×10^5 mean/mL, with no north-south trend. The maximum was measured at station 20 with a value of 1.77×10^6 mean/mL (Table 1, Fig. 2).

Virus-like particles were found to be more abundant in the north with an average of $9.541.90 \times 10^6$ mean/mL than in the south with an average value of 1.90×10^6 mean/mL (Table 1, Fig. 2).

HNF were found to be very similar in the north with an average northern value of 6.08×10^4 mean/mL (Table 1, Fig. 2). HNF in the southern stations were an order of magnitude different with an average value of 9.02×10^4 mean/mL. Station 22 had the value of 1.04×10^4 , while station 20 had the value of 1.70×10^5 mean/mL (Table 1, Fig. 2).

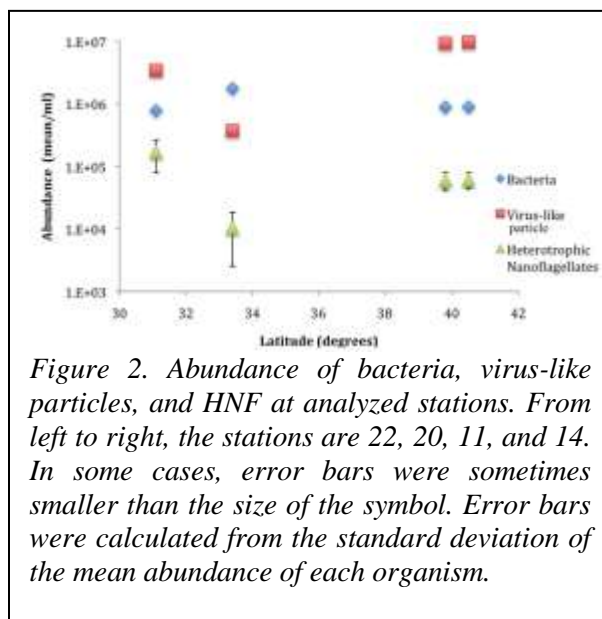


Figure 2. Abundance of bacteria, virus-like particles, and HNF at analyzed stations. From left to right, the stations are 22, 20, 11, and 14. In some cases, error bars were sometimes smaller than the size of the symbol. Error bars were calculated from the standard deviation of the mean abundance of each organism.

Station Num.	Latitude (degree)	Bacteria (mean/mL)	Virus (mean/mL)	HNF (mean/mL)	Bacteria: Virus	Bacteria: HNF	Nitrate ($\mu\text{mol/L}$)	Nitrite ($\mu\text{mol/L}$)	Phosphate ($\mu\text{mol/L}$)	Temperature ($^{\circ}\text{C}$)
22	31.1	7.87E+5	3.42E+6	1.70E+5	.23	4.63	0.53	0.14	0.05	17.80
20	33.4	1.77E+6	3.73E+5	1.04E+4	4.75	170	2.30	0.10	0.18	17.11
11	39.8	8.93E+5	9.36E+6	6.01E+4	0.0954	14.9	9.63	0.13	0.72	10.54
14	40.5	9.00E+5	9.72E+6	6.14E+4	0.0926	14.7	20.42	0.16	1.67	2.77

Table 1. Master sheet of data analyzed from stations 20, 22, 14, and 11 including the abundance of bacteria, virus-like particles, and HNF; ratios between bacteria and virus-like particles and HNF; nutrient values of nitrate, nitrite, and phosphate; the physical property temperature.

The ratio between bacteria and virus-like particles were similar in the northern stations differing by 0.0026. The bacteria: virus-like particle ratio in the southern stations differed. Station 20 showed a difference against the other stations by an order of magnitude in ratios between bacteria per HNF (170) and bacteria per virus (4.75) (Fig. 4).

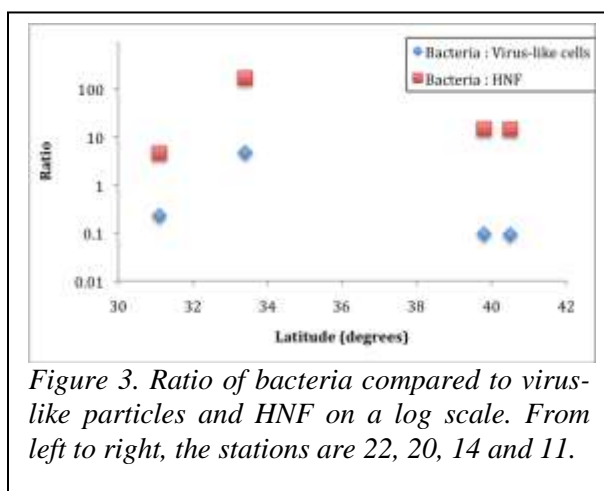


Figure 3. Ratio of bacteria compared to virus-like particles and HNF on a log scale. From left to right, the stations are 22, 20, 14 and 11.

Nutrients

Concentrations of nitrate, nitrite, and phosphate increased with increasing latitude (Fig.

4). Nitrate concentration ranged from 0.53 $\mu\text{mol/L}$ to 20.42 $\mu\text{mol/L}$, with a south-north trend (Fig. 2). Nitrite concentration ranged from 0.14 $\mu\text{mol/L}$ to 0.16 $\mu\text{mol/L}$, with no south-north trend (Fig. 2). Phosphate concentration ranged from 0.15 $\mu\text{mol/L}$ to 1.67 $\mu\text{mol/L}$, with a south-north trend (Fig. 2).

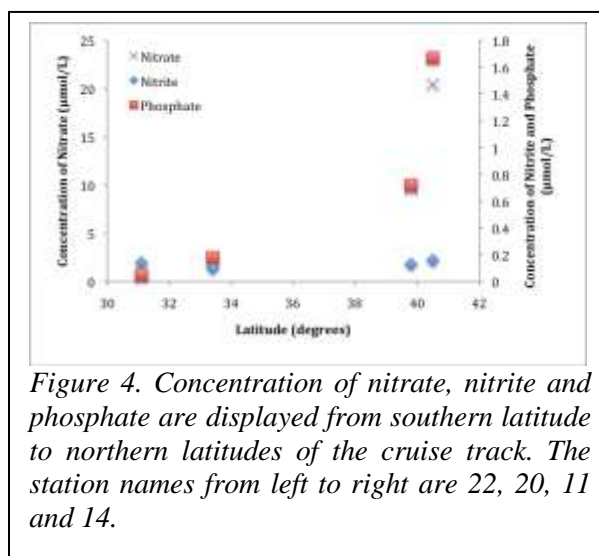


Figure 4. Concentration of nitrate, nitrite and phosphate are displayed from southern latitude to northern latitudes of the cruise track. The station names from left to right are 22, 20, 11 and 14.

SST

The water temperature decreased as the longitude increased (Fig. 5). Temperature ranged

from 17.80°C to 2.77°C, with a north-south trend (Fig. 3).

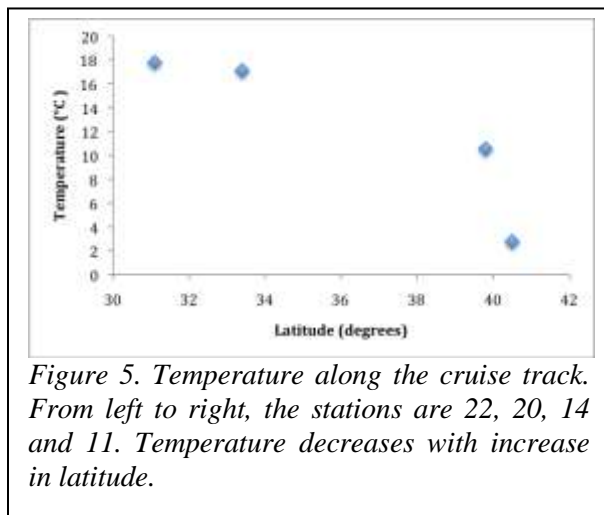


Figure 5. Temperature along the cruise track. From left to right, the stations are 22, 20, 14 and 11. Temperature decreases with increase in latitude.

DISCUSSION

The cruise track along the Kuroshio Extension offered a distinct opportunity to investigate microbial communities over a range of environmental variables. The cruise track surveyed over the southern Kuroshio Current collision with the northern Oyashio Current creating a northern, cold high-nutrient region and a southern, warm low-nutrient region of the Extension.

Microbial distribution and abundance

Bacteria

The increase in bacterial abundance from southern stations to northern stations correlates to what has been seen in previous studies of bacteria. Physical properties such as temperature has often been a major factors correlated with variability of bacterial abundance in coastal ecosystems (Shia and Ducklow, 1994). However, viral lysis and grazing can be a controlling factor based on correlations between abundances at the sample sites. As hypothesized, the bacterial abundance increased with increased latitude possible due to decrease in temperature and increase in nutrients. The abundance of bacteria was slightly higher in concentration than the ocean average of 5×10^5 mean/mL with an average of 1.09×10^6 mean/mL. However, the outlier of these values was found at

station 20 with a value of 1.77×10^6 mean/mL. The other stations had an average of 8.60×10^5 mean/mL. Because the nutrient data shows no distinct changes in abundance, the outlier may be a result of lack of grazers and viral lysis.

Nutrients and bacteria

Bacteria are the chief limiting function of gaseous nitrogen from the atmosphere and depend upon combined nitrogen present in the sea for their growth (Waksman et al., 1933). The data shows that nitrate concentrations are significantly larger than nitrite and phosphate concentrations. However, there is a consistent increase in concentration, which does not explain the outlier at station 20 for bacteria. The nutrient data could indicate that a large concentration of nitrate would cause an increase in bacterial abundance because of the night levels of nitrate in the northern stations and because the food chain is reliant on nitrate as a food source (Waksman et al., 1933).

Virus-like particles and Bacteria

Virus abundance increases rapidly through cell lysis, thus concentrations of viruses proliferate following the peak and decline of the host populations (Thomson et al., 2010). In a study of the Southern Ocean, viral abundance, virus to bacterial ratios and 50-100% loss of bacterial production due to viral infection indicated that viral abundance plays a key role in bacterial abundance (Marchant et al., 2000; Guixa-Boixereu et al., 2002). In this study, an increase in viral abundance further north the station correlated with a decrease in bacterial abundance (Fig. 3). The ratio values of .0954 and 0.0926 show that the bacterial abundance was much lower than the bacterial abundance. A significant, positive correlation was found ($r= 0.99$) between the abundances of viral-like particles and bacteria excluding the outlier of station 20. With station 20 the correlation was negative and less significant ($r= -0.70$). From this data, it can be inferred that viral abundance is suggested to be a cause of bacterial mortality. However, station 20 shows a deviation from the hypothesis; there were more bacteria than viruses relative to all other stations.

HNF and Bacteria

I found a strong, negative correlation between HNF and bacteria with stations 22, 11, and 14 ($r = -0.99$), which is supported by other surveys (Thomson et al. 2010). The strong correlation suggests bacteria were prey for HNF. This is supported by a similar experiment conducted in the Southern Ocean where the negative correlation between bacterial and HNF abundance was considered to be a result of high grazer-induced bacterial mortality (Thomas et al. 2010).

Figure 3 shows a steady ratio bacteria and HNF in stations 11 and 14, which may be caused by close station proximity and latitude. Stations 20 and 22 have radically different ratio values, which may be caused by the location of each station. Station 20 is closer to the mixing of the Oyashio and Kuroshio Current. However, when r was calculated with all stations the value was -0.73 . Station 20 deviated from overall HNF concentration; HNF populations were unexpectedly low, measured at 1.70×10^4 mean/mL instead of the other station average of 9.72×10^4 mean/mL.

Station 20

Station 20 is located nearest the mixture of Oyashio and Kuroshio Current. Its proximity to the combination of low-temperature, high-nutrient water and high temperature and low-nutrient water would be justification for its uncharacteristic traits. The HNF and viral abundances were radically lower than the bacteria (Fig. 3). The nutrients at this station did not indicate any deviation from hypothesis, which indicates a property other than nutrients, is a causing factor (Fig. 2). Low abundances in predation by grazers and viral lysis are a strong explanation for this result. Temperature and sea surface height are in a state of frequent flux, which could explain the change in HNF and viral abundances. HNF and viral abundances were larger at stations 22, 11, and 14. However, the constant flux in water flow could make the region less inhabitable for HNF and viral-like particles.

This study was designed to detect the controlling factors of bacteria along the Kuroshio Extension in order to understand the relative effects of the controls of bacterial populations. The

findings of this research have implications for research concerning HNF and viral controls of bacteria. Further work in this region could be to normalize data used to compare the northern and southern portions of the cruise track. Usage of an incubation experiment could further explain the effects of viral-like particles, HNF and nutrient have on the bacterial abundance. Studying the possible effects a monthly flux by currents on HNF and viral abundances will help in understanding the interactions between the environment and resource availability have on these microorganisms furthering the knowledge of the microbial loop. Additional research directions within this field would be finding the correlation between dissolved organic carbon and matter to bacteria along this track, which could further describe the trophic pathway.

CONCLUSION

Understanding the abundances of HNF, viral-like particles as well as nutrients is important in understanding the controlling factors of bacterial populations. Bacterial populations control carbon and nitrogen processes in the ocean. A deviation from normal distributions of abundances in the ocean affects the understanding of the biological process. The data presented in this study indicates that HNF and viral-like particles play an important role in bacterial abundance based on the outlier at station 20 and the lack of outlier in the nutrient data. Results have implications for studying HNF and viral-like particles on bacterial populations in a fluctuating environment to further understand the biological process.

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