Changes in bacterial concentrations with depth, temperature, and chlorophyll fluorescence in the Equatorial Pacific Ocean

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Abstract

It is important to consider the abundance of marine bacteria, as they play important roles in biogeochemical processes that are significant in the carbon cycle. Marine bacteria can give us insight about factors such as the amount of nutrients in the water, dissolved organic matter (DOM) production by phytoplankton, and photosynthesis occurring in the water. High bacterial abundance indicates that there are high levels of nutrients and organic matter, and examining bacterial abundances at different depths can reveal the state of the ocean such as nutrient levels and bioactivity. My study was carried out by collecting water samples from the Equatorial Pacific Ocean and processing the samples through a flow cytometer to get heterotrophic bacterial concentrations measured in cells/mL. Here we show that bacterial abundance is the highest in the top 200 meters of the ocean and decreases with depth. I found that bacterial abundance mirrors the presence of phytoplankton as heterotrophic bacteria absorb nutrients from dead phytoplankton. My results show that temperature and chlorophyll fluorescence share the same trend as bacteria, of increasing at the surface and decreasing at depth, which seems to relate to the availability of sunlight with depth in the ocean. In addition, bacterial abundance is higher south of the equator compared to north of the equator. This could be due to sunlight and the angle of the sun's rays on the ocean surface being more direct on the water, resulting in increased sunlight available for photosynthesis in phytoplankton and thus more food for bacteria.

Plain Language Summary

Heterotrophic bacteria are bacteria that use dissolved organic matter for food, which is released by phytoplankton. Therefore, phytoplankton are a main food source for heterotrophic bacteria. Autotrophic bacteria are bacteria that are able to produce their own food through sunlight and photosynthesis. In this experiment, I focused on heterotrophic bacteria and how their abundance is affected by temperature, chlorophyll fluorescence, and depth. Chlorophyll fluorescence is able to be used to tell the abundance of phytoplankton, because phytoplankton are also autotrophs and need chlorophyll in order to carry out photosynthesis. Therefore, the levels of chlorophyll fluorescence also represent phytoplankton abundance in the ocean. This experiment was carried out by collecting water samples from the Equatorial Pacific Ocean and processing the samples through an instrument to obtain heterotrophic bacterial concentrations measured in cells/mL. I found that bacterial abundance is the highest in the top 200 meters of the ocean, where temperatures are the warmest and chlorophyll fluorescence is the highest. It is important to study marine bacteria because they are responsible for incorporating and redistributing organic matter and inorganic nutrients throughout the ocean and regulating the marine food chain.

Introduction

Marine bacteria play critical roles in the global nutrient cycle as they incorporate and redistribute organic matter and inorganic nutrients throughout the oceans (Tang et al., 2012). They are involved in the transformation of dissolved organic carbon (DOC) in the ocean, which is correlated between bacteria and primary production (Jiménez-Mercado et al., 2007**)**. Primary production occurs where light is the most available for autotrophs to carry out photosynthesis. Sunlight is required for photosynthesis to occur, and the warmth from the sun causes water temperatures to rise, which results in increased respiration rates and bacterial growth rates (Jiménez-Mercado et al., 2007). Respiration of marine bacteria is linearly related to their growth rate, and the slope of the line appears to depend on other nutrients, principally nitrogen for protein synthesis (Fenchel & Blackburn, 1979).

Half of the ocean's primary productivity is channeled by heterotrophic bacteria to the microbial loop (Djaoudi et al., 2020). The role of the microbial loop is important as it rapidly recycles nutrients above the thermocline caused by bacteria consuming sinking particles and reintroducing the energy back into the food web when they get eaten by larger creatures (Wangersky 1977). This pathway drives a wide range of biogeochemical processes, such as assimilating and transforming dissolved organic matter (DOM) in marine ecosystems, that are necessary for the carbon cycle (Bunse and Pinhassi, 2017). The Sheldon (1972) particle-size model describes how organisms tend to use particles one order of magnitude smaller than themselves. Phytoplankton releases DOM, which is a significant process that provides energy and nutrients to bacteria and is returned to the main food chain via a microbial loop of bacteria-flagellates-microzooplankton (Azam et al., 2017). Bacterial production and growth rates appear to be limited by DOM in the equatorial pacific, which results in bacterial production following primary production over large spatial and temporal scales (Kirchman & Rich 1997**).**

Bacteria feed on organic matter, such as dead phytoplankton, and are also responsible for the uptake of phosphate and ammonium. In the ocean, bacteria consume NH_4^+ for their growth, which reduces the availability of NH_4^+ in the water for phytoplankton to use. Therefore, phytoplankton ultimately turns to NO_3^- as a substitute nutrient source as NH_4^+ availability decreases. This indicates that when bacteria grazing is not recycling enough NH_4^+ back into the system, phytoplankton use more NO_3^- in response to the lack of NH_4^+. (Kirchman 1994). Giebel et al. (2021) found that prokaryotic cells, which include bacteria, follow patterns of Chl *a*, particulate organic carbon (POC), and particulate organic nitrogen (PON) in the ocean, resulting in enhanced levels of Chl *a*, POC, and PON in the upper 100 meters of the ocean at the equatorial upwelling zone, where temperatures are also warmer (Giebel et al., 2021**)**.

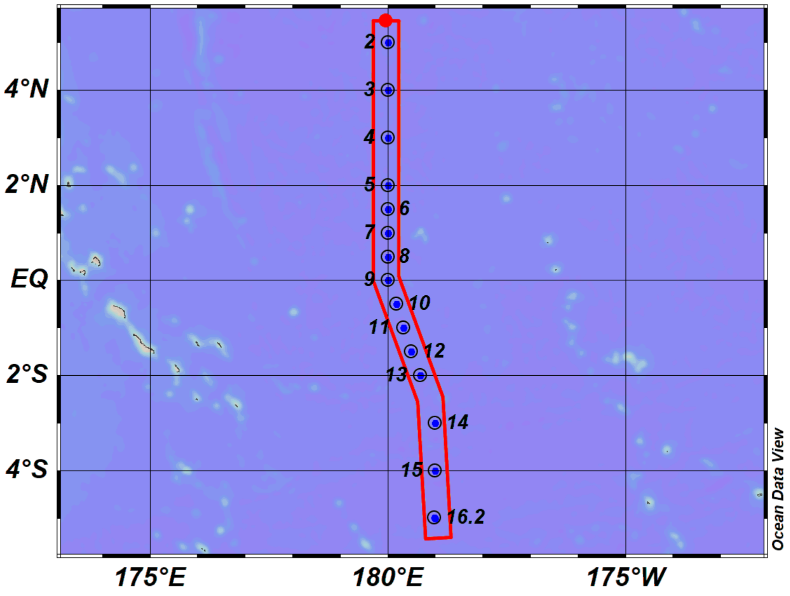
Since marine bacteria play such important roles in the ocean, I think it is essential to observe where bacterial concentrations are most abundant, because it could give us insight of marine bacteria's ability to recycle organic matter and factors that can help determine the health of ocean environments such as primary production and nutrient concentrations. The question I have is how bacterial concentrations will change with depth, and what environmental factors regulate the abundance of heterotrophic bacteria in the Equatorial Pacific Ocean. I hypothesize that there will be high bacterial concentrations in the surface mixed layer and decrease as depth passes the mixed layer into the intermediate layer, about 500-1000 meters below sea level. Fast growing microbes are abundant in the upper column, whereas slower growing microbes are relatively more abundant at depth (Nguyen et al., 2022**)**. I assume that there will be higher concentrations of bacteria where there is an abundance of phytoplankton due to dead phytoplankton and released DOM being one of the main intakes by bacteria. Following the same logic, I predict there to be less bacterial abundance as depth increases past the mixed layer because phytoplankton concentrations decrease as there is less light available and phytoplankton are unable to carry out photosynthesis. Deep waters are colder, which indicates a surplus of nutrients that are available for bacteria as there are no phytoplankton utilizing it, as well as particulate organic matter sinking through the oceanic water column into the deep ocean (Bergauer et al., 2017). Jing et al. (2013) found that species richness and diversity were higher for bacterial communities in the intermediate water layers than for those in surface and deep waters in the Pacific Ocean, but HÖfle & Brettar (1995) stated that bacterial species richness was the highest in the bottom water due to carbon and oxygen rich sites being known to carry more diverse communities than near bottom waters that suffer from oxygen depletion. (Jing et al., 2013; HÖfle & Brettar 1995). Another paper found that the diversity of community structure was uniform horizontally between different sites than vertically, which supports my hypothesis of bacterial abundances changing with depth (Koskinen et al., 2010**).** With these papers, I infer that with high species richness and diversity, there will also be high bacterial counts, and by collecting my own data I can test if there are more bacteria in the surface, intermediate, or deep waters.

Fluctuations of environmental factors could affect the bacterial abundance in surface waters compared to the deep waters in the Equatorial Pacific because of westerly wind events such as El Niño. These winds cause changes such as an increase in sea surface height (SSH), deepening of the mixed layer, and an increase in sea surface temperature (SST) (Vecchi & Harrison 2000**)**. During El Niño, winds decrease, which causes upwelling to weaken, and cold, nutrient rich waters from the deep waters are unable to rise to the surface. With a lack of nutrients in the surface water, there are fewer phytoplankton, which affect organisms that feed on phytoplankton, such as bacteria.

Methods

I carried out my experiment aboard the R/V Thomas G. Thompson from February 23 to March 23, 2023, on a transect from Honolulu, Hawaii to Suva, Fiji. Depths were chosen based on rapid changes in chlorophyll fluorescence, oxygen, and salinity – properties that may affect bacterial abundance – using the conductivity, temperature, and density (CTD) instrument as it was being lowered into the ocean. I chose 10 different depths, ranging from 5 meters to 1000 meters below sea level. Most samples were taken from the surface waters between 5 meters and 200 meters where chlorophyll fluorescence levels were the highest. I took these samples from 5ºN -5ºS in the Equatorial Pacific Ocean (Fig 2). From the CTD, I filled a 15 mL falcon tube with water from the different depths and then transferred 1uL of each sample into a cryotube and then flash froze them in the -80º freezer. The samples were transported from Fiji to Seattle in a dry shipper filled with liquid nitrogen. In the lab in Seattle, the cryotubes were organized by station and depth. I filled duplicate 96 well plates with 150 uL of sample and added 3 uL of SYBR green into one of the duplicate plates. This SYBR was used to stain the DNA of the bacteria in the samples so that the Guava easyCyte flow cytometer would be able to count them. Once the plates were run into the flow cytometer, it gave me a cell count in cells/mL of the heterotrophic and autotrophic bacteria combined in the samples. Heterotrophic

Figure 1. Thesis expedition map with markers representing sampling locations from 5ºN -5ºS in the Equatorial Pacific Ocean.



bacteria concentrations were obtained by subtracting bacterial abundance counted by the flow cytometer without SYBR green from bacterial abundance counted by the flow cytometer stained with SYBR green. Staining the samples before running them through the flow cytometer gives a count of all of the bacteria in the sample, whereas the non-stained samples give a count of autotrophs and phytoplankton. By finding the difference between the two, counts of heterotrophic bacteria were able to be obtained. With this data, I made figures using OceanDataView, and was able to layer different variables measured from the CTD and compare them to bacterial abundance to see how they were affected. Then, in order to better understand the correlation between chlorophyll fluorescence and bacterial abundance, I used the multiple linear regression (MLR) tool in Excel and combined chlorophyll fluorescence, light transmission, and temperature. I used these three variables to assess whether they could be used to predict heterotrophic bacterial abundance.

Results

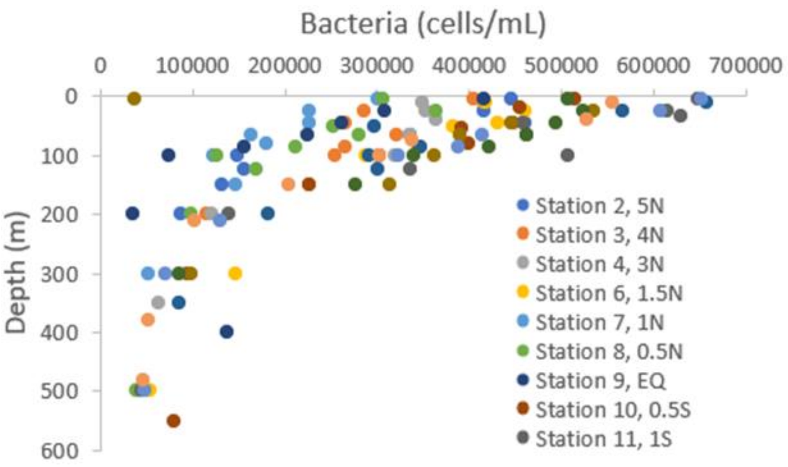


Figure 2. Heterotrophic bacterial abundance measured in cells/mL from the Equatorial Pacific Ocean on the x-axis and depth measured in meters on the y-axis. Stations labeled in legend, represented by different colored dots.

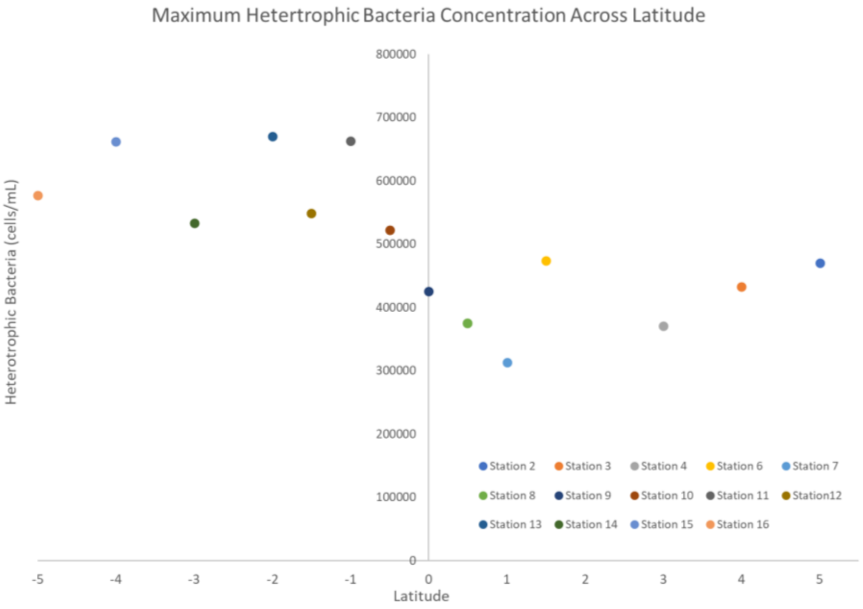


Figure 3. Greatest bacterial concentrations from each station on the y-axis measured in cells/mL against latitude from 5N to 5S in the Equatorial Pacific Ocean on the x-axis. Stations labeled in legend, represented by different colored dots.

Bacteria concentrations are highest from 0 to 200 meters below sea level. As depth increases, bacterial abundance decreases (Fig 2). The bacterial depth profiles were consistent with every station from 5N to 5S, but there was a difference in the maximum bacterial concentrations depending on if the samples were taken north or south of the equator (Fig 3). Heterotrophic bacterial concentrations were higher in samples taken from the stations south of the equator, ranging from concentrations of 500,000 cells/mL to 700,000 cells/mL, whereas concentrations of bacteria taken in the northern hemisphere ranged from 300,000 cells/mL to 500,000 cells/mL (Fig 3). Using the heterotrophic bacterial counts, a heat contour map was created through OceanDataView (ODV) with depth, measured in meters on the y-axis and latitude from 5N to 5S on the x-axis and heterotrophic bacterial concentrations on z-axis represented by the different colors (Fig 4). Although there are extrapolations evident as an impact of ODV contouring, it represents the spatial distribution of heterotrophic bacteria along latitude and depth, providing a visual of how bacterial concentrations are highest at the surface than with depth, and is higher south of the equator than the north, with a minimum amount at the equator. (Fig 4).

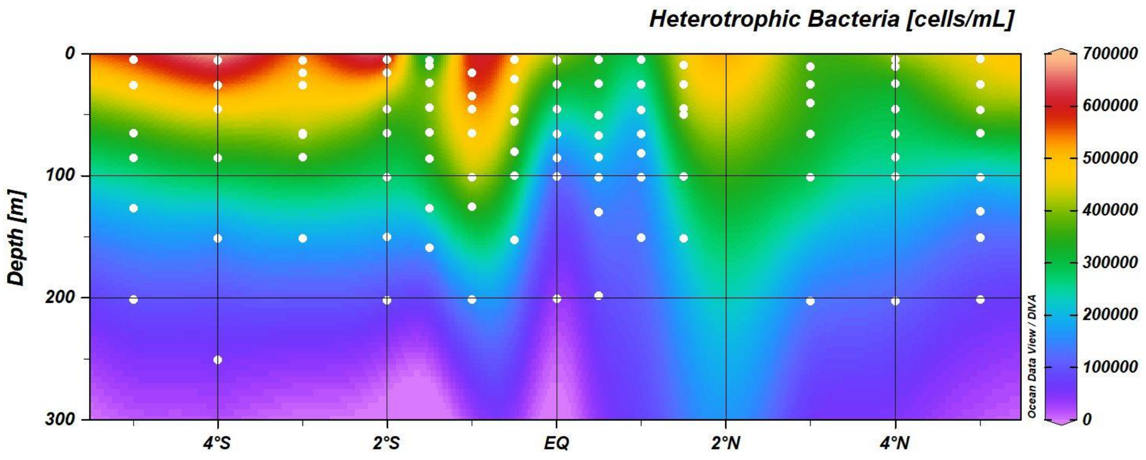


Figure 4. Heat contour map of heterotrophic bacteria (cells/mL) against depth. White dots on the figure represent depths where samples were taken.

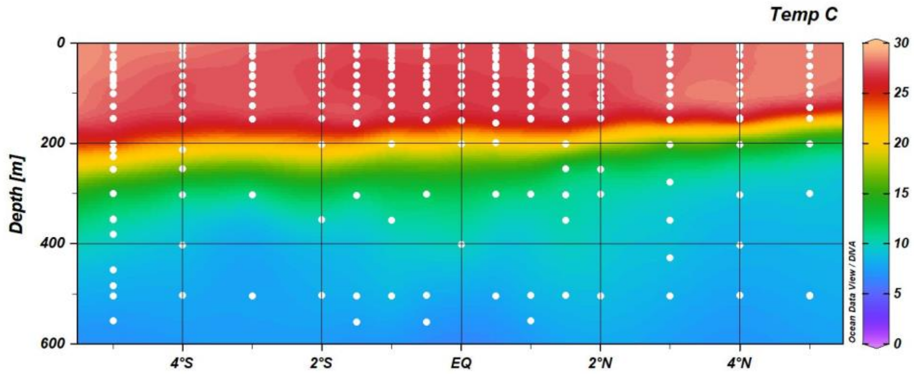


Figure 5. Heat contour map of temperature against depth along latitude. The warmest temperatures are shown in red and changes colors according to the scale on the right with depth and temperature.

In the Equatorial Pacific Ocean, waters were warmest at the surface and became colder with depth, and the thermocline of the ocean changed with latitude. (Fig 5). This was similar to the bacterial abundance of the samples taken because bacterial abundance was highest at the surface, where the temperature of the water was the warmest, and decreased in abundance as temperature decreased as well. Chlorophyll fluorescence was also seen to follow the same trend, as levels were highest at the surface and decreased with depth, along with the concentration of bacteria (Fig 6). The chlorophyll fluorescence ranged from 1.5 to 1 RFU when bacteria concentrations were the highest and ranged from 0 to 0.25 RFU when bacteria concentrations began to decrease (Fig 6). Bacteria concentrations, temperature, and chlorophyll concentrations all displayed similar trends of maxing at the surface and decreasing with depth.

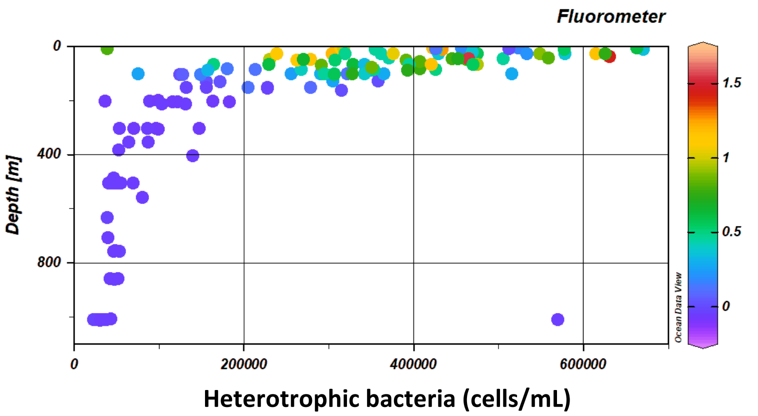


Figure 6. Bacterial depth profile, with colors of the dots representing chlorophyll fluorescence data at each sample. Red dots represent the highest chlorophyll fluorescence and purple dots represent the lowest chlorophyll fluorescence. Bacterial abundance on the x-axis and depth on the y-axis.

Discussion

Bacterial abundance is highest at the surface primarily due to their correlation with chlorophyll fluorescence. Bacterial abundance is correlated with chlorophyll fluorescence because where there is high fluorescence, there is also high primary production activity, which includes phytoplankton (Fig 7). After dying, phytoplankton becomes a source of food for bacteria, which is why bacteria are usually abundant where there are high levels of phytoplankton. Since primary producers need sunlight in order to carry out photosynthesis, they are found in the warmer surface ocean where sunlight is the strongest. Sunlight cannot penetrate deep into the ocean, and with decreased sunlight is a decrease in temperature and a decrease in primary production. Therefore, bacterial abundance is seen to correlate with the trends of chlorophyll fluorescence with depth.

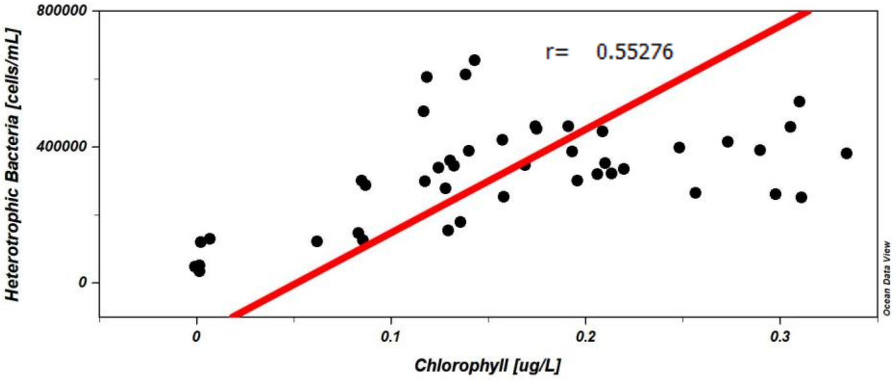


Figure 7. Comparison of heterotrophic bacteria concentrations against calculated chlorophyll concentrations from all stations

However, bacterial concentrations did not appear to correlate with temperature. When the temperature was the highest at around 27 degrees, heterotrophic bacterial concentrations varied from 100,000 cells/mL to 600,000 cells/mL. This significantly large

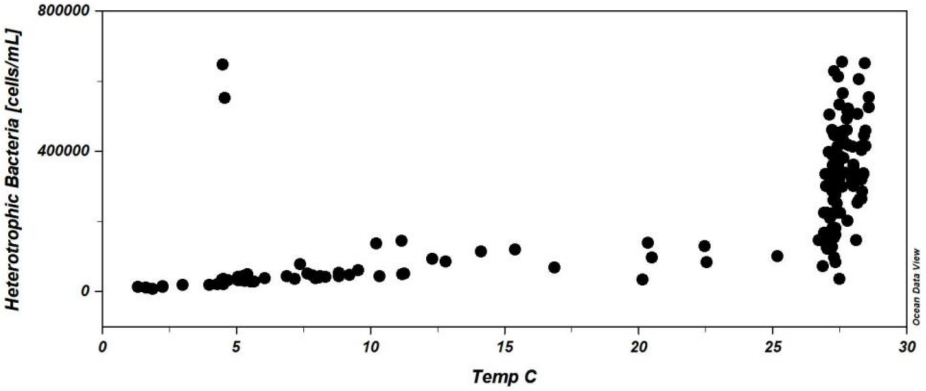


Figure 8. Heterotrophic bacteria compared against temperature from all stations and depths.

variation in data does not demonstrate a correlation between bacterial concentrations and depth (Fig 8).

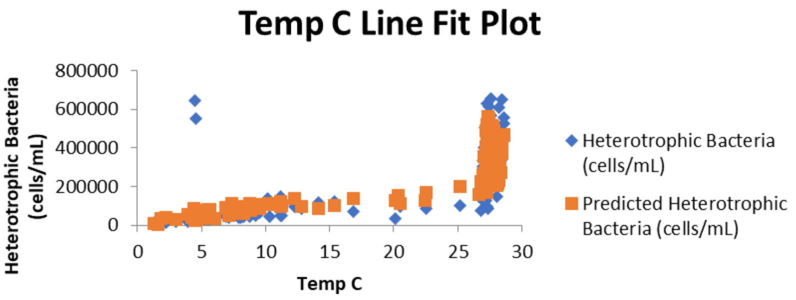


Fig 9. Line fit plot of heterotrophic bacteria measured in cells/mL on the y-axis and temperature in ˚C on the x-axis. Measured heterotrophic bacteria represented by blue diamonds and predicted heterotrophic bacteria using multiple linear regression represented by orange squares.

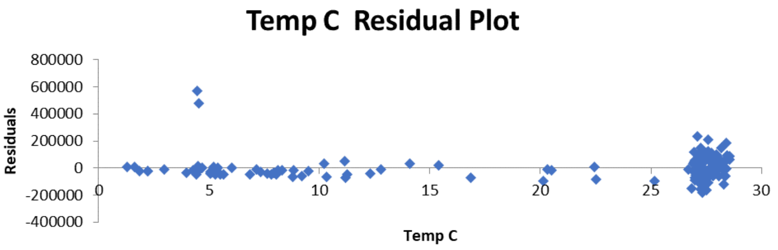


Fig 10. Residual plot of chlorophyll fluorescence, with residual data from line fit plot on the y-axis and chlorophyll fluorescence on the x-axis.

Using the MLR, I made line fit plots for all three variables individually. There were significant overlaps in the measured heterotrophic bacteria abundances and predicted (Fig 9). In addition, I used the line fit plot to create residual plots for the variables. The residual is what is left after subtracting the predicted bacteria from the observed bacteria; the positive values mean that the prediction was too low, and negative values mean the prediction was too high, whereas zero means the prediction was accurate. The residual values of each variable clustered toward 0, so I can conclude that a linear model is appropriate for predicting heterotrophic bacterial concentrations (Fig 10). I had 146 samples of heterotrophic bacterial data and found that the three variables combined accounted for 72% of the variability in bacterial abundance, which is represented by the R squared value. The multiple linear regression fit was 0.85 – represented by the R value. Seeing as temperature, chlorophyll fluorescence, and light transmission could be used to predict bacterial abundance with an accuracy of 72%, I can assume that there is a correlation between heterotrophic bacterial abundance, chlorophyll fluorescence, and depth. When the multiple linear regression test was done without light transmission, the linear regression only accounted for 55% of the variability. Since light transmission accounts for about 20% of the variability in bacterial abundance, it is safe to assume that light transmission is significant when considering heterotrophic bacterial abundance. Light is most available at the surface and decreases with depth, and although there is no direct correlation with depth and bacteria alone, the results of this multiple linear regression test indicates that there is a correlation between bacterial abundance and depth when light is considered. The results that I found were similar to Aniefiok (2015) when researching bacterial abundance-chlorophyll a concentration relationship in Southeastern Nigeria (2015). Aniefiok's overall results concluded that there is a correlation between bacteria cell abundance with chlorophyll a concentration. He took samples from two stations and found a weak positive correlation at 57'N, 18'E with an r value of -0.587. The second station at 45'N and 35'E had a strong positive correlation with an r value of 0.599. This finding was consistent with my results as I found that the correlation between heterotrophic bacteria and chlorophyll concentration had an r value of 0.55 (Fig 7).

Conclusions

My hypothesis was correct in that bacterial abundances were highest at the surface waters and decreased with depth. This is primarily due to the correlation with chlorophyll fluorescence. Bacterial abundance is correlated with chlorophyll fluorescence because where there is high chlorophyll fluorescence, there is also high primary productivity, which includes phytoplankton. Although there is no direct correlation between bacterial abundance and temperature, bacteria do grow faster in warmer temperatures, which occurs in the upper 200 meters of the ocean and decrease with depth. This is because as depth increases, the sunlight able to penetrate into the ocean decreases, which causes water to be colder and denser. As a result, light availability in the ocean decreases, limiting primary production in the deeper ocean. When phytoplankton decreases, primary production decreases, resulting in less food available for bacteria, decreasing their abundance. In addition, although there is also no direct correlation between bacteria abundance and depth, variables of temperature, chlorophyll fluorescence, and light attenuation combined can be used to predict bacterial abundance with an accuracy of 72%. This indicates that depth plays a significant role when considering light availability in the ocean, which contributes to bacterial abundance. However, this also means that there are other important factors to consider as 30% of the variability of bacteria still remains unaccounted for. It is important to understand these factors and how they affect bacteria because bacteria play an important role in nutrient cycles by incorporating and redistributing dissolved organic matter and inorganic nutrients throughout the oceans, which reveals the state of the ocean such as nutrient levels and primary production. If this experiment were to be repeated, I suggest taking duplicates of each sample in order to ensure more useful and reliable data in order to eliminate any ambiguity in the results. In addition, I would like to take nutrient samples to see how they play an effect on bacterial abundance variability.

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