Marine Bacteria Colonization Rates on Microplastics in the North Pacific Subtropical Gyre

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Abstract:

Plastic pollution is a growing concern in the microecology of the oceans. Studying bacteria colonization rates on plastic provides one way of understanding of how toxic debris can move through the food chain through ingestion. This process of toxins moving through the food chain is called biomagnification and can eventually reach humans. To evaluate bacterial colonization rates, seawater was collected in coastal waters of Hawaii and near the Pacific garbage patch (GPGP). Seawater was intermixed with 5 different kinds of clean plastics then timed to determine how long it took bacteria to colonize the plastic surfaces. Bacteria on the plastic were counted under an epifluorescence microscope then divided by the time of colonization to determine the rate. Alongside the colonization rate, surface microplastics were collected with a manta net; then sized and classified with a dissection microscope. Seawater was collected from a Niskin bottle attached to a CTD rosette to calculate bacterial abundance with the use of a Guava flow cytometer. The findings of the research displayed little to no correlation between surface bacterial abundance and plastic density, with an R^2 value of 0.1072. Bacteria were found to colonize plastics at 48 and 96 hours in the waters near the Pacific garbage patch with a rate of 7.4E+04 cells/mm. The colonization rates and plastic abundance support evidence of plastics being integrated into the ocean ecology.

Plain language:

Bacteria reside on the smaller scale of ocean life; however, they can be greatly affected by human pollution. Plastic that is littered into the ocean intermixes with the marine life. Bacteria can grow on these plastic surfaces allowing predators to ingest the bacteria and plastic together. The rate of adhesion to the plastic can show us how long bacteria take to intermix with the plastic. To test for this, seawater samples were collected in the surface water near Hawaii and mid-way across the Pacific Ocean in the Pacific garbage patch. Samples were then mixed with plastics and timed for how long the bacteria took to colonize. Bacterial colonization wasn't present until 48 to 96 hours. To further support the research,

surface bacteria levels and plastic abundance were collected and counted. The data showed a slight negative relationship between surface bacteria and plastic abundance. This study shows evidence of human plastic pollution on the small scale of the ocean environment.

Introduction:

Plastic litters our oceans today, affecting marine life in detrimental ways. With many studies conducted on analyzing the relationship between large marine life, such as whales and fish, vs pollution, this study focuses on the small scale of organisms living in the ocean, such as bacteria. Bacteria play a vital role in Earth's environment, through the production of oxygen in the carbon cycle they provide for the health of the land and oceanic life (Lami, 2007). With evidence from past studies, it is shown that marine bacteria colonize plastics and can stay attached and reproduce. In 2019 the Natural History Research Center of Shanghai conducted a study on what kind of plastics marine bacteria colonize. From the study it was found that marine bacteria can easily attach to any plastic surface in large quantities (Batzke, 2019). In this study, clean plastic was mixed with naturally occurring bacteria in the ocean to see if they would colonize. What it did not analyze was the rate of colonization on the plastics. The study additionally analyzed different surfaces of plastic, for example, polyethylene and polypropylene, to see if one had a higher colonization rate. Results displayed polyethylene having higher colonization than polypropylene, which was less colonized (Batzke, 2019). I looked at the rate of colonization of the bacteria on clean polycarbonate filters to determine the amount of time plastic takes to integrate into the oceanic ecosystem. The length of time it takes for the bacteria to colonize can teach us how long the plastic must be in the ocean for it to be affected or consumed by marine organisms.

Filter-feeding organisms will consume the bacteria that have colonized the plastics, as the colonized bacteria hide the plastic. In a study conducted on oysters, it was found that E.Coli coated plastics were more attractive to the filter-feeding oyster and therefore consumed (Fabra, 2021). Plastic consumption from these filter-feeding organisms reaches humans, through a process called biomagnification, which causes these plastics to channel up the food chain as smaller organisms are consumed. A study issued in the journal *Trends in Ecology and Evolution* discovered that filter-feeding organisms near the Gulf of California had over 100 pieces of plastics in their stomachs (Germanov, 2018). Although this may seem relatively high to many individuals, this is actually a very low quantity compared to the other sampling area, Pelagos sanctuary, in which organisms had around 1,000 pieces of plastic in their systems(Germanov, 2018). This can easily be seen in fisheries off the coast of populated cities where pollution is more dramatic. As plastic is pushed up the food chain from the consumption of larger organisms it reaches our fisheries and grocery stores causing humans to be dramatically linked to the effects of marine-life plastic contamination.

Economically, fisheries are most dramatically affected by the plastic pollution, as consumers are less inclined to purchase contaminated seafood (Thushari, 2020). Adding on to this, plastic contaminated seafood creates a multitude of health issues across the population. Evidence from the National Health and Nutrition Examination Study showed a strong correlation between type two diabetes development and high BPA levels from plastic intake (Melzer, 2003). This is just one health concern brought upon by high BPA levels. As plastic toxicity is an active area of research, scientists are working to gain more knowledge about plastic pollution. As stated above, these studies conducted on plastic pollution have shown that higher polluted regions correlate to the quantity of plastic consumed by marine organisms

(Germanov, 2018). Due to this high-density plastic regions, it becomes more difficult for marine organisms to thrive in clean water, hurting the health of the ecosystem.

With knowledge of this, I collected samples in a high plastic density region that crosses through the GPGP (Fig 1), in the Eastern Pacific Ocean and compared the bacteria colonization rate to further understand the harm plastics can cause in the ocean environment.

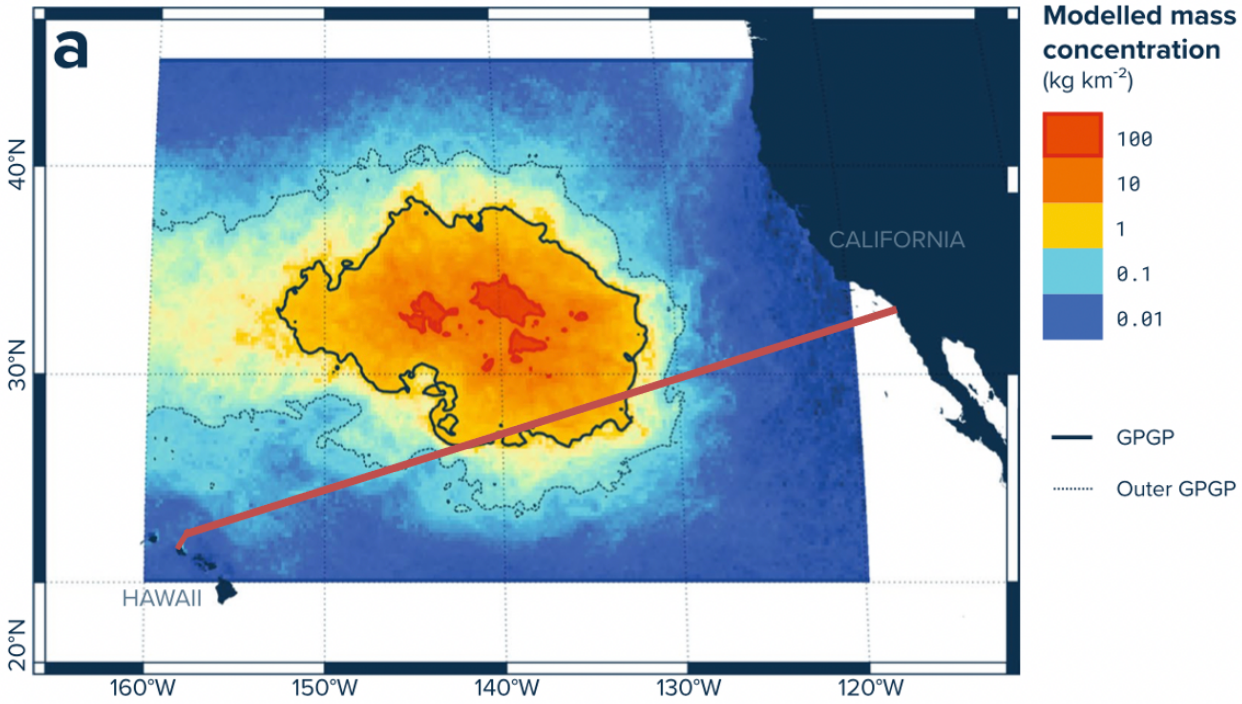

Figure 1 transect from Honolulu, Hawaii to San Diego, California overlayed on graph of plastic density in the great Pacific garbage patch (Lebreton)

This specific section of the Pacific Ocean also is represented by the North Pacific subtropical Gyre. The great pacific garbage patch is formed by the sea surface currents produced through the gyre, resulting in a high region of plastic (Lebreton, 2018). Sea surface winds push along in a pattern that rotates around the middle of the Pacific Ocean, creating a vortex like movement in the middle. As human plastic pollution continues the vortex creates

the potential of a larger GPGP. This continuation of plastic pollution further enhances the negative effects on the environment and microbiome of the ocean. This being said, I hypothesize that bacteria colonization rate will be faster in the pacific garbage patch compared to Hawaiian waters and surface bacterial abundance will display an inverse relationship with plastic abundance. I am hypothesizing this as there is an increase of plastic surfaces for bacterial colonization in the GPGP.

Method and Materials:

Research was conducted on the R/V Thomas G. Thompson from December 18th to the 30th of 2021. Samples were collected from 5 m with a CTD rosette from Niskin bottles at station 7 at 26.329 N, 147.5012 W and station 13 at 29.95275 N, 134.7158 W (Figure 2).

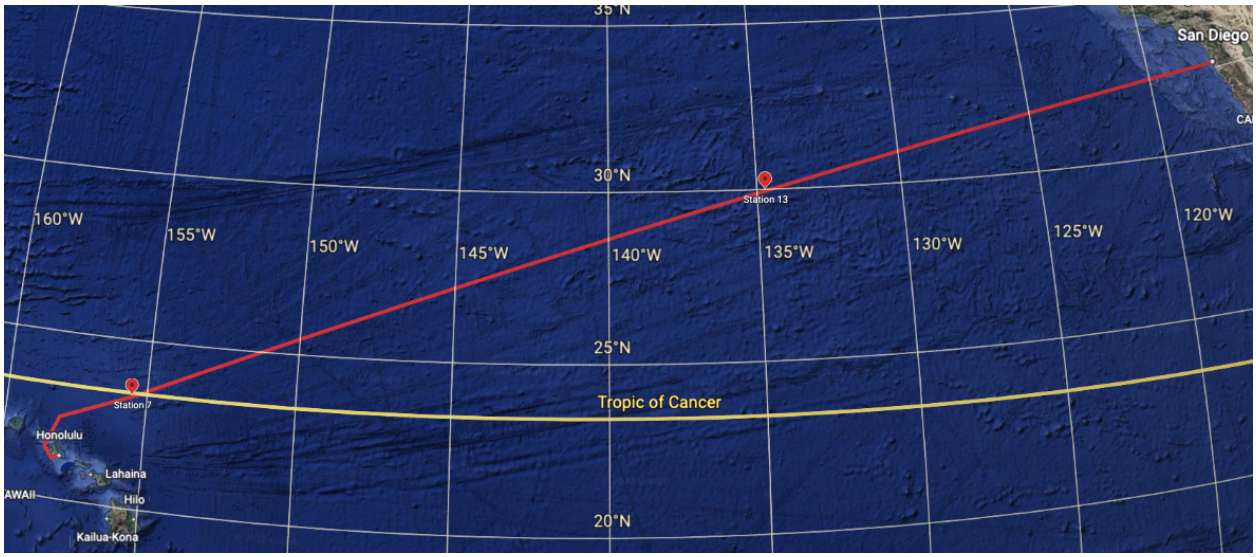

Figure 2 transect from Honolulu, Hawaii to San Diego, California. Station 7 and station 13 marked with pins for where samples were collected for colonization rate.

At each station eighteen 120 mL jars were filled with seawater as depicted in Fig 3.

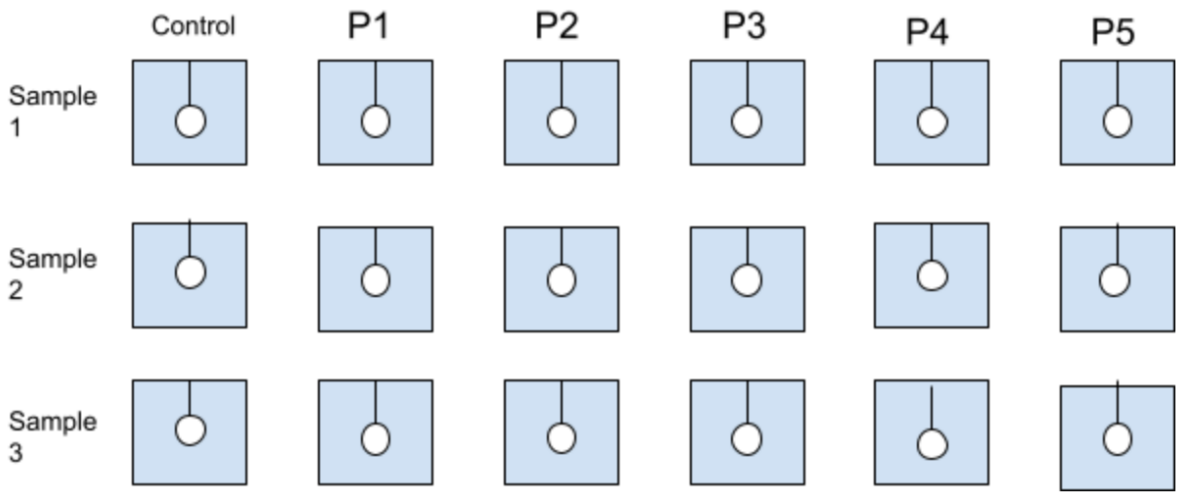

Figure 3 Scientific setup: 3 jars of each plastic type were prepared, with one set as a control, control jars with about 100 mL of seawater and 2 fishing lines hung from the top, 3 “nitrous cellulose filters” jars, 2 filters are hung from two fishing lines in 100 mL of seawater , 3 “Polycarbonate filters” jars, 2 filters are hung from two fishing lines in 100 mL of seawater, 3 Polycarbonate filters jars, 2 filters are hung from two fishing lines in 100 mL of seawater, 3 “glass filters” jars, two filters are hung from two fishing lines in 100 mL of seawater, 3 Black “Polypropylene filters” jars, 2 filters are hung from two fishing lines in 100 mL of seawater.

Jars were placed in a 60 C walk-in cold room roughly the same temperature as the seawater for 24 hours. After 24 hours the jars were removed from the cold room and 450 μm was subsampled out of each jar and placed into a corresponding cryotube and mixed with 50 μm of 10% formalin. Tubes were placed into a -80-degree freezer until analysis in Seattle with the cytometer following the Guava protocol (Vecchione, 2021). Time 0 samples were taken from surface CTD casts at each station and fixed with the same fixing procedure stated above, samples were then analyzed with the guava protocol in Seattle (Vecchione, 2021). All jars, except for the 3 jars with black polycarbonate, were placed back into the chilled room. One black polycarbonate filter was removed with forceps from the 3 jars and placed on Hoefer box wells on top of .2-micron polycarbonate backing filters wetted down with DI water. 30 mL of seawater is filtered with a .2-micron filter into 3 15 mL tubes, (10 mL in each) with 2 μm of formalin pipetted into each tube and gently shaken to mix. Each tube was poured through a well

on top of the filters, then pumped through with the Hoefer box until all water is sucked out of the filters. Slides were labeled correspondingly to the jars and filters and 5 μm of DAPI was pipetted onto the slide. A cover slip was placed on top of the DAPI, and the black filter was carefully removed from the Hoefer box. The cover slip was slid off and the black filter was placed face up on top of the DAPI spot, then covered with a cover slip and pressed down with a kim-wipe to remove excess DAPI. DAPI is then stored in the fridge and covered with tinfoil. Slides were then placed into a slide box and put into a freezer until analysis with an epifluorescence microscope.

Microscopic examination:

To analyze the bacteria on the slides, the ocean 220 protocol from Bob Morris, (Morris 2012) was used with an epifluorescence microscope in Seattle. A drop of immersion oil was placed on top of the coverslip of the slide analyzing and placed under a 100x objective on an epifluorescence microscope. Using computer software, bacteria colonies were counted in different areas of the slide and averaged through excel for the bacteria abundance. This process was repeated for each slide until complete.

Plastic abundance:

A 333 μm mesh manta net was towed behind the vessel for 20 minutes. After collection, plastic was picked out and placed onto pre weighed filters for each station and stored in a fridge until analysis. Filters were then weighed to find the total mass of plastic collected at the specific stations. Using a dissecting microscope plastics were counted, sized, and sorted and results put into an excel file. Plastic abundance was found by dividing the number of plastics by the volume of water filtered.

Results:

The bacteria colonization rate at 24 hours in the waters near Hawaii, station 7, was 0 cells/minute. In the Pacific garbage patch, at station 13, bacteria colonized the

plastic in the highest density around 48 and 96 hours. Samples incubated for 48 hours at station 13 had a colonization rate of 27.1 cells/ minute while the 96 hours of incubation had a rate of 12.0 cells/ minute. The seawater that was subsampled from the station 13 jars for bacterial abundance increased 3.15E+05 cells/mL in the control and 4.56 cells/mL (Figure 4) in the jars containing the 48-hour incubation of polycarbonate.

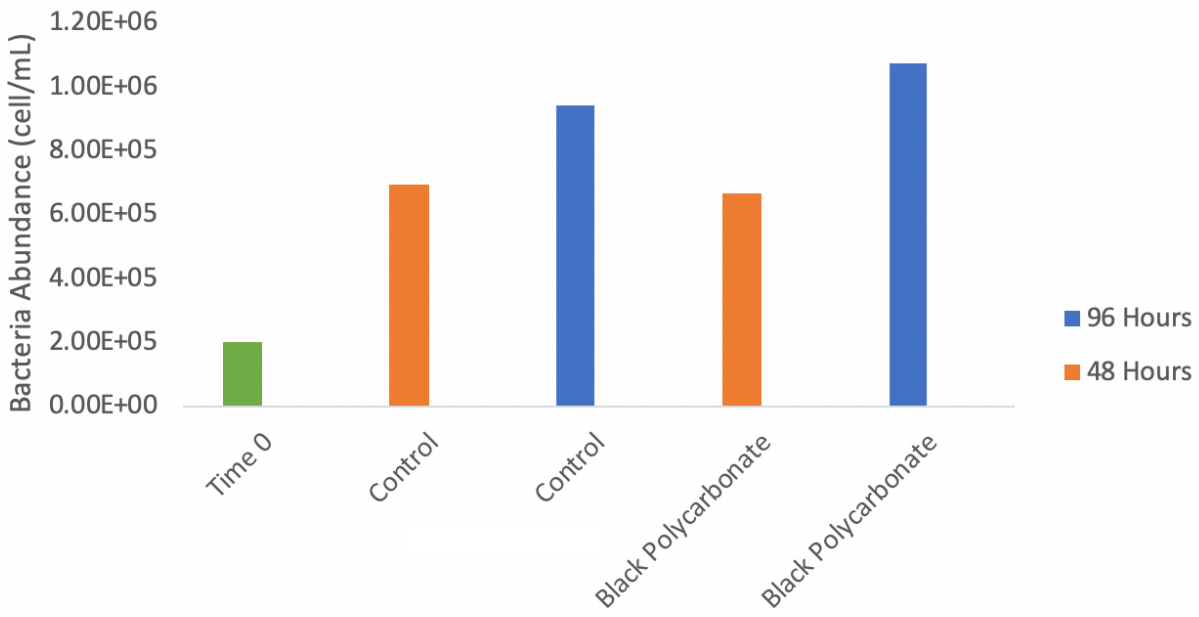

Figure 4 Subsampled seawater for bacterial abundance in jars containing polycarbonate plastic and no plastic at station 13 (control). Time 0 represents bacterial abundance taken directly from CTD cast.

Surface bacteria abundance in the waters around Hawaii remained an average of 4.80E+05 cells/mL until reaching station 10. The surface waters from station 10 to station 20 had an average bacterial abundance of 4.37E+05 cells/mL station 15 had a low of 2.68E+05 cells/mL at station while station 18 had a high of 5.05E+05 cells/mL. In the coastal waters of California, the average bacterial abundance was 7.21E+05 cells/mL.(figure 4)(Vechionne, 2021)

Seawater mixed with different kinds of plastic remained consistent around 5.0E+05 cells/ mL with a standard deviation of 0.4. Nitrous cellulose incubated from time point 24 hours to 48 hours had an increase of 3.85E+05 cells/mL representing the largest difference. Polypropylene had a increase of 2.32E+05 cells/mL from 24 hours to 48 hours (Figure 5)

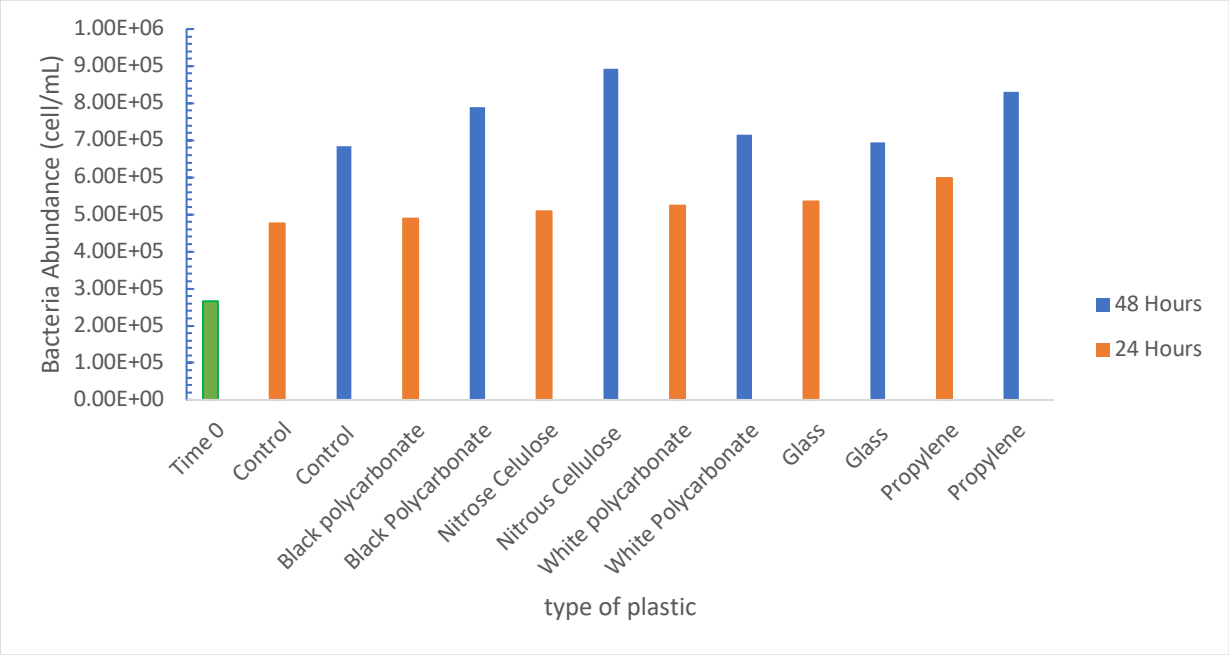

Figure 5 Subsampled bacterial abundance in jars containing different kinds of plastic at 24 and 48 hours of incubation at station 7

Plastic Abundance:

Plastic abundance in Hawaiian waters were an average of 0.08491 plastic per a L while California coastal water had an abundance of 0.02315 plastic per L. The plastic abundance was an average of 0.1902 plastic per L in the pacific garbage patch, station 10 through 18. The

highest plastic abundance was at station 11 with an abundance of 0.7723 per a L. (Fig 6)

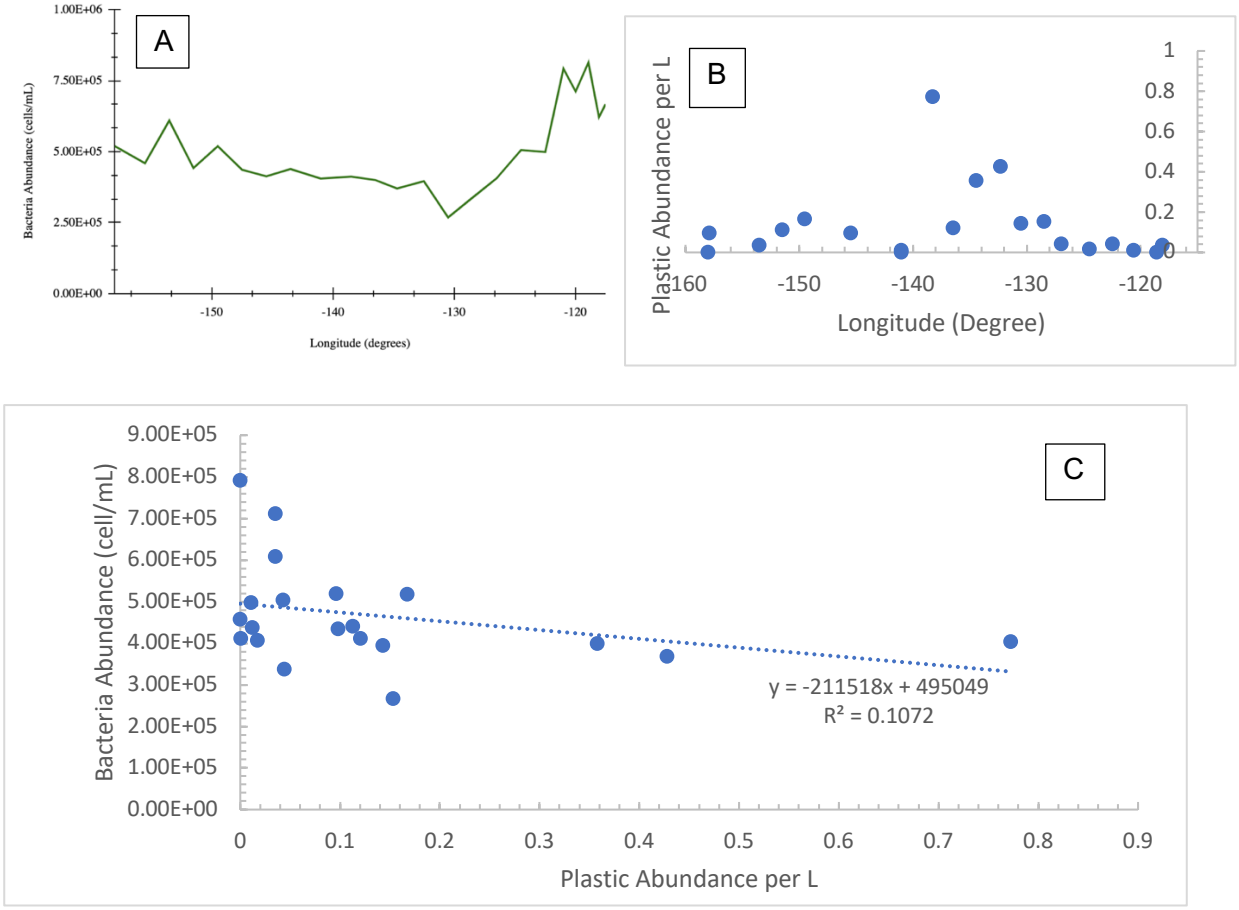

Figure 6 Panel A: Surface bacterial abundance at different longitudes. Panel B: Plastic abundance at different longitudes. Panel C: Plastic abundance compared to bacterial abundance with r squared value and slope.

Discussion:

The prime research I initiated was looking at bacteria colonization rate onto plastics. The first experimental design was set up in the coastal Hawaiian waters. This experiment shown little to no bacteria adhesion at 24 and 48 hours. Levels of bacteria in the jars, what was sub sampled, remained relatively stable with a bit of an increase day after day as growth occurs. This was a continued trend found in the same sample sections with other types of plastic (Thomas, 2021). The second sample collection was near the pacific garbage patch, in

which samples sat with plastic for 48 and 96 hours. At 48 hours more bacteria colonized the plastic in the GPGP compared to at station 7. In addition, there was a decrease in the bacterial abundance, what was sub-sampled, when there was an increase in bacteria on the plastic. This shows some evidence of bacteria moving from the seawater to colonize the plastic. At 96 hours the bacteria that adhered to the plastic almost seemed random. With some having high density and other having little to no adhesion. I conducted three trials with a total of 6 polycarbonate filters to view colonization, only 4 slides showed significant colonization. The plastic that was incubated for 24 hours had no colonization. In a research paper on incubation periods, it was found that an incubation below 24 hours does not show significant bacteria counts (Veloo, 2014). This finding aligns greatly with the data I found in my 24-hour incubation trial.

Plastic abundance was also monitored and collected throughout the track line. In the tows there was an average of 14 pieces of plastic collected at each station in the middle of the Pacific Ocean, what is called the GPGP. Significant amounts of plastic collected were mostly clear and around 1 μm in size, leading us to believe that the surface manta net predominantly collected microplastics. Leaving and before the GPGP there was a smaller abundance of microplastics collected. This comes as suspected as this region is outside of the high density of plastic within the gyre.

The bacteria profiles throughout the entire cruise demonstrate a small to no negative correlation. Surface microplastics and bacterial abundance showed a slight relationship between one another (Fig 6). As for bacterial abundance, there was a continued trend as we passed through the garbage patch. Surface bacterial abundance reached a low within the gyre but climbed up to a higher count as we got closer to California and Hawaii. There are many

hypotheses for this, with my main one being the abundance of nutrients. One thing to keep in mind is this region is a gyre so that could be a reason due to the lack of bacteria. It was found that in a gyre in the south of the Pacific ocean there was a lower abundance of common bacteria in the surface layer of the ocean (Reintjes, 2019). I hypothezise that this would be like the gyre I was looking at as the gyres have similar water movement that cause tehse gyres. With knowledge of that it would make sense why we see this decrease in bacteria towards the center of the gyre.

Finally, looking at the data found among the incubations of different kinds of plastic we can see a relationship from the control and time 0. The control and time zero remain lower than the other plastic incubations at both stations, with a much higher and consistent number of bacteria as incubation time increases. The colonization rate increases closer to the garbage patch with no colonization occurring on any of the plastics at 24 or 48 hours near the water of Hawaii.

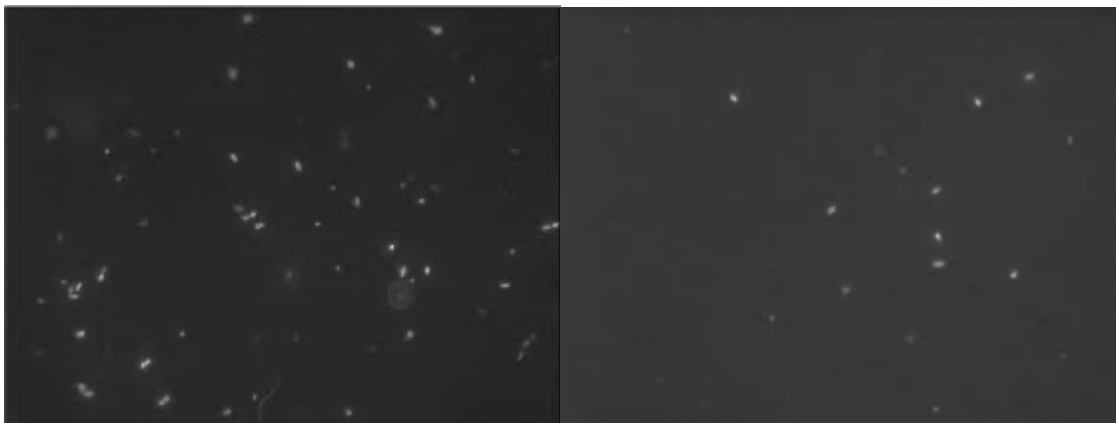

Figure 7 Images captured underneath epifluorescence microscope for plastic incubated at station 13. Right is at 48 hours left is at 96 hours. White dots represent bacteria colonies.

The garbage patch had far more colonization in some areas with not only bacteria but other particles that could be just larger organisms or even dust. Some things to note with this

research is that the filters were in seawater that had more than just bacteria inside of it, so other marine life could have the potential of colonizing the surfaces or consuming the bacteria.

Some setbacks during the experimental process were the need for black polycarbonate filters rather than white polycarbonate filters. The stain used in the lab stained the bacteria white making it difficult to see the bacteria on the white plastic. Since polycarbonate filters are flimsy the experimental set up for the slides did not set-up as well as hoped. The filters in the water folded onto of one another, instead of free floating. In the future this experiment could be set up way user friendly. However, there was just a focus on the surface of the ocean, large amounts of microplastics exist further below the surface to the deep-mid layer of the ocean. A research study based in Australia looked at sediment samples for evidence of microplastics in the ocean floor, the data founded an estimated 14 million tons of microplastics on the ocean floor (Pennino, 2020). This research stands as evidence of the sinking plastic particles and how the microplastics affect every aspect of the ocean environment. With such a high density of plastic residing on our ocean floors, there is no doubt it has hurt the ecology in great ways. A further way to enhance this research is to look at the microplastic and colonization deeper in the ocean, where particles may have sunk. Thinking through these design flaws would help greatly if this experiment was to be repeated with a longer time period.

Conclusion

By looking at the data collected through bacteria abundance and the manta net tows with an R^2 of 0.1072 there is not enough evidence to state a correlation between bacteria and plastic. However, it was found that water near Hawaii and California remained lower in plastic abundance compared to the Pacific garbage patch in the gyre. Colonization rate at station 7 was found to be 0 at 24 hours of incubation. In the samples near the pacific garbage patch, station 13, there was significantly more colonization at 48 and 96 hours. The rate at 96 hours of polycarbonate incubation was 12.0 cells/ minute. The data found supports a part of my hypothesis surrounding colonization rate. I originally thought colonization would be higher in the pacific garbage patch, which was accurate, however the rate was much smaller than predicated. I predicted a rate around 25 cells/ minute. If this was to be researched again having a longer incubation period and different kinds of plastics would be desired. From the research conducted it was clear to see that incubation of plastic must surpass the 24-hour mark to find clear evidence of bacteria colonization. The research I conducted can be used to further our understanding of the interactions between marine life and plastic waste. With this understanding there can be more advocation for greater steps to eliminating plastic pollution in our oceans.

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