Marine Bacterial Growth Rates in the Presence of Microplastics

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3/11/2022

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ABSTRACT

This study investigated the effects of microplastics (MPs) on the growth of marine bacteria in the open ocean to try to obtain a general picture on whether or not bacteria can be harmed by or benefit from the presence of MPs in their environment. Samples of were collected in the vicinity of the Great Pacific Garbage Patch (GPGP), just outside the Hawaiian islands, and just outside the California coast. The bacteria were grown in the presence of Teflon, polycarbonate, and polypropylene filters as well as a control sample with no filter. Generally, plastics enhanced the growth of bacteria compared to the control samples and the highest hourly growth rate was experienced by a sample with polypropylene, being 1.59*10^4 bacteria per hour. One control and one Teflon sample, each at a different station, experienced a loss of bacteria. There are still many unanswered questions about the relationship between MPs and bacterial growth, namely which species benefit from MP accompaniment and which species are potentially beneficial to the ocean.

PLAIN LANGUAGE SUMMARY

With the enormous amount of plastic in the ocean worldwide, this study was conducted to find out if these materials could be put to use in growing bacteria. Instead of plastic simply being pollution contaminating seawater, can it be used to grow organisms that can make marine life and the environment healthier? This question was answered by collecting water off the coast of Hawaii, in the middle of the Pacific, and near the coast of California and growing the bacteria with several types of plastic filters. Then, the samples were put into a machine that counted all of the bacteria in them to determine which plastic filter helped enhance bacterial growth the most. The primary goal of the study was to determine if plastic can help marine organisms survive in

their environment rather than be something for them to choke on, especially since there is very little information about the effects of plastic on microscopic organisms.

INTRODUCTION

Pollution from man-made trash is among the most notorious and seemingly unfixable threats facing the ocean. Of all the pollutants that lurk in the ocean, oceanographers and conservationists are becoming more concerned about microplastics (MPs) flaking off of bigger plastics (Law and Thompson, 2014). They might be miniscule and incapable of entangling marine life (Andrady 2011), but their small sizes actually make them more deadly to marine life since a wide range of organisms will mistake them for food, ingest them, incur tissue damage, and bioaccumulate organic pollutants (Curren and Leong, 2019). This has been observed in organisms as big as pelagic fish and birds, as small as shellfish and lugworms, and as microscopic as zooplankton (Andrady 2011; Curren and Leong, 2019). Any one of these organisms could eat a microplastic thinking that they are getting a snack when they are actually getting a poison. Another key reason for MPs making such an environmental impact is their huge quantity. There is an estimated five trillion pieces of plastic in the ocean altogether (Brandon et al., 2016). On top of that, an estimated 4.8-12.7 million metric tons of plastic are added to that number every year and the majority of these pieces are MPs (Brandon et al., 2016). MPs are also found everywhere in oceanic environments, from sediment to polluted water to pristine water (Curren and Leong, 2019). As much as the scientific and environmentalist communities would love a solution to cleaning up every last microplastic in the global ocean, such an answer would take a colossal amount of time and scrutiny to collect every last one.

Other microscopic inhabitants of marine habitats with an intense negative reputation are marine bacteria. Several bacterial genera, including *Vibrio* and *Arcobacter*, are confirmed to have multiple members that are pathogens and can be transmitted to humans with life-threatening results (Curren and Leong, 2019). However, marine bacteria have also been regarded

as a key player in the nitrogen cycle (Jetten 2008). These microbes are confirmed to do the vast majority of fixing and denitrifying dinitrogen gas in the entire cycle so that it may be turned into a form of nitrogen that is useful for other organisms in and out of the cycle (Jetten 2008). A new tool called Recirculation Aquaculture Systems (RAS) has even been invented to use these nitrifying bacteria in biofilters to reduce ammonia toxicity in aquaculture farms, which is crucial to keeping the fish healthy (Ruiz et al., 2020). The overall image of marine bacteria as a whole is complicated and multi-faceted; some species are viewed negatively for their tendencies to spread disease while others are viewed positively for their abilities to create chemical compounds all organisms need to survive. On the other hand, the image of microplastics in the ocean is overwhelmingly negative; they are only pollutants that harm aquatic life and must be dealt with.

The intent of this study was to investigate if there is more nuance to be seen in the effects of microplastics on marine ecosystems, specifically how they affect the growth of marine bacteria. The field of studying MPs' effects on bacterial growth has already turned up some interesting results. When investigating how MPs and their additives affect the growth of diazotrophs, Fernández-Juárez et al. (2021) discovered that there was no definitive trend in the ways in which the plastics affected bacterial growth; some plastics and additives deterred growth, others enhanced it, and others did both simultaneously. MPs have also been found to shield bacteria from harmful UV radiation and can act as templates for the formation of bacterial biofilms (Shen et al., 2021). While investigating the effects of MPs on the methods of wastewater treatment/disinfection, Shen et al. (2021) speculated that wastewater bacterial pathogens, such as *Escherichia coli*, could use MPs as a means of creating biofilms and bypassing wastewater treatment processes. However, the growth of nitrogen-fixing cyanobacteria has also shown to be greater in the presence of MPs (Fernández-Juárez et al.

2021). Further still, MPs found along tropical coastal environments have shown growth of a mammoth mix of bacteria. Curren and Leong (2019) found over 400 bacterial taxa and estimated over 400 species present on MPs at each of their study sites in Singapore. These species range from the virulent *Pseudomonas*, proven to cause serious infections in hospitalized and immunocompromised people (Palleroni 2015), to the hydrocarbon-degrading *P. alcaligenes*, which has been used at oil spills to decompose toxic chemicals (Curren and Leong, 2019). MPs have a complicated relationship with the growth of marine bacteria.

The goal of this experiment was to further investigate how MPs affect the growth of marine bacteria, specifically in the Great Pacific Garbage Patch (GPGP). Did bacteria experience enhanced growth in the presence of MPs? Did they experience stunted growth?

METHODS

Seawater was collected at three different stations along the route of the R/V Thompson's cruise TN398 from December 18th-30th 2021. The sampling locations were station 3 around the Hawaiian Islands, station 11 near the GPGP (near the center of the cruise track), and station 19 closer to the US mainland (Figure 1). These locations were chosen for sampling to compare the growth of bacteria in the GPGP and in two different locations outside of it. At each location, a CTD Niskin bottle was utilized for collecting the water. Four different treatments of marine bacteria were prepared, one being a control with just seawater and the other three being seawater with a different type of plastic filter for the bacteria to grow in the presence of. The three types of filters were Teflon (often used in non-stick cookware, nail polish, waterproof clothes, paint, and a general non-stick spray; https://www.nes-ips.com/what-are-the-uses-of-teflon/), polycarbonate (often used in bullet-resistant windows, storefront window displays, and greenhouse glass; https://www.acmeplastics.com/content/popular-uses-polycarbonate-plastic/), and polypropylene

(often used in plastic furniture, bottles of cleaning products, and medical syringes; https://adrecoplastics.co.uk/polypropylene-uses/).

Each type of sample had three replicates prepared in 20 mL scintillation vials, making for 12 total vials per station and 36 vials for the entire study. Each vial was incubated in a walk-in fridge with a temperature kept near 6°C for approximately 24 hours. Afterward, the replicates were prepared for analysis and storage based on a protocol written by Bob Morris for the UW OCEAN 220 course. 450 μL of seawater from each replicate were placed in a 2 mL cryotube along with 50 μL of filtered formalin. The cryotubes were then frozen at -80°C in a Ziplock bag for the remainder of the cruise. The samples were then transported back to the University of Washington's School of Oceanography for analysis. 100 μL of each sample was dispensed into a single well of a 96-well plate and stained with 3μL of SYBR Green genetic stain using a Rainin P10 multichannel pipette. After 30 minutes of incubation in the dark, the stained samples then had their bacteria counted with the Guava Technologies Flow Cytometer in Bob Morris' lab at a flow rate of 0.59 μL/s.

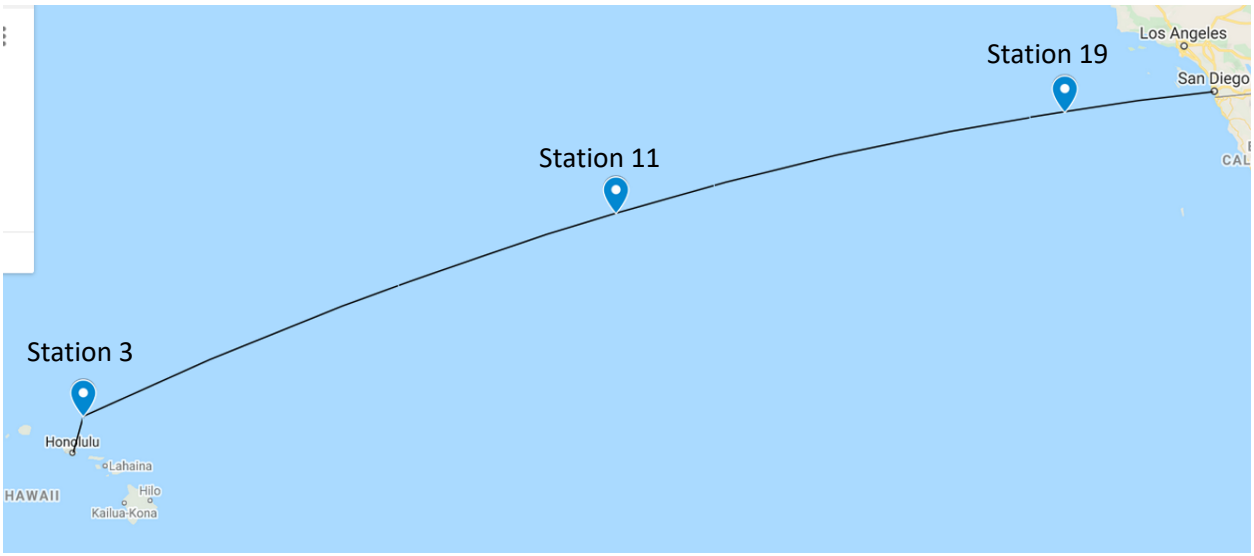

Figure 1: A map of the cruise track. The locations of where water and bacteria were sampled from are marked by the blue markers along the cruise route. From left to right, they are stations 3 (22.5°N 157.51°W), 11(29.01°N 138.5°W), and 19 (32.11°N 122.5°W).

RESULTS

Bacterial abundance after 24 hours was compared to the abundance of bacteria at time 0 (the instance immediately after the seawater was sampled from the CTD) to determine which samples experienced bacterial growth. At station 3, the control sample experienced the biggest bacterial growth with an abundance of 5.12*10^5 cells per mL, and the Teflon sample was the only one to lose bacteria over 24 hours (Figure 2).

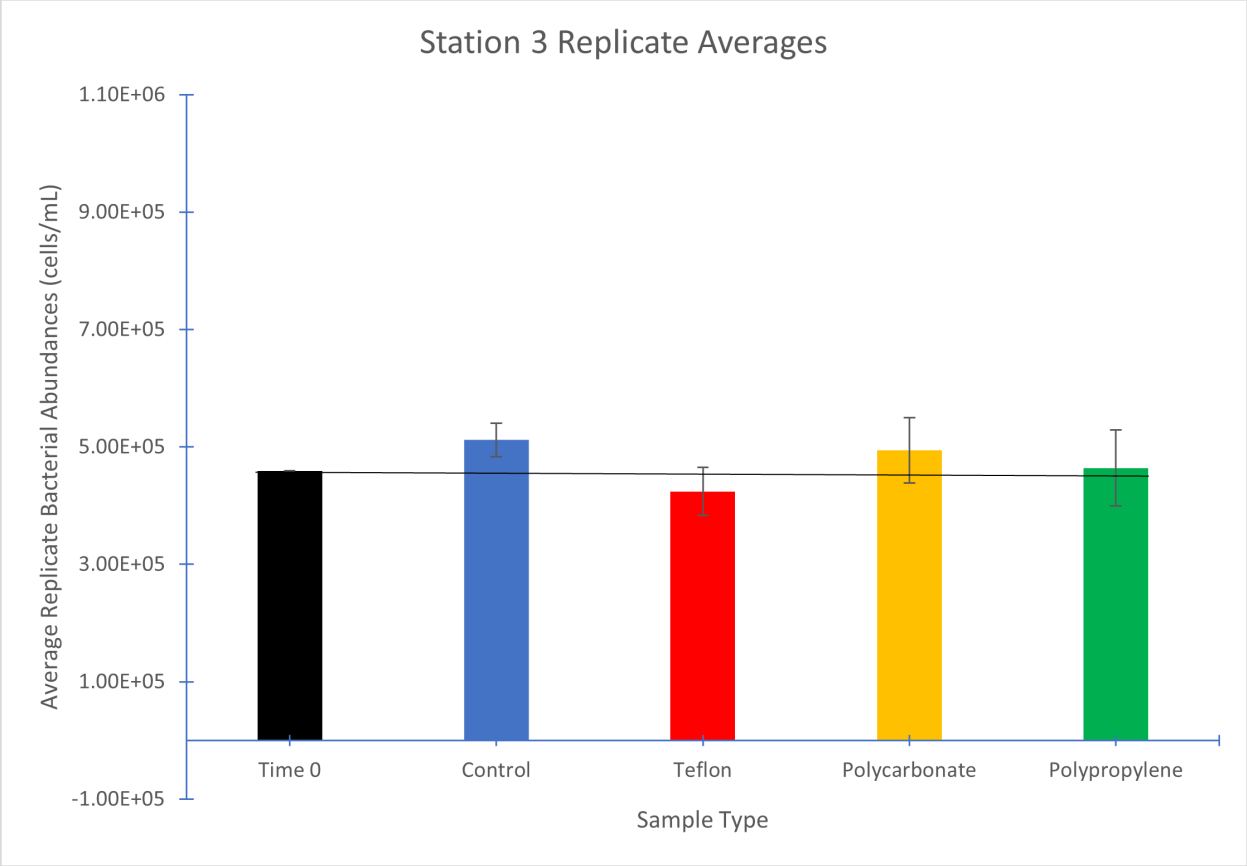

Figure 2: The average growths of each sample at Station 3. The black horizontal line extending from Time 0's bar is to help with comparison from time 0 to 24 hours of incubation. The control, polycarbonate, and polypropylene samples all experienced growth while the Teflon sample lost bacteria. The highest growth was observed in the control sample.

At station 11, the polypropylene sample experienced the highest growth with an abundance of 6.32*10^5 cells per mL, and the control sample was the only one to lose bacteria over 24 hours (Figure 3).

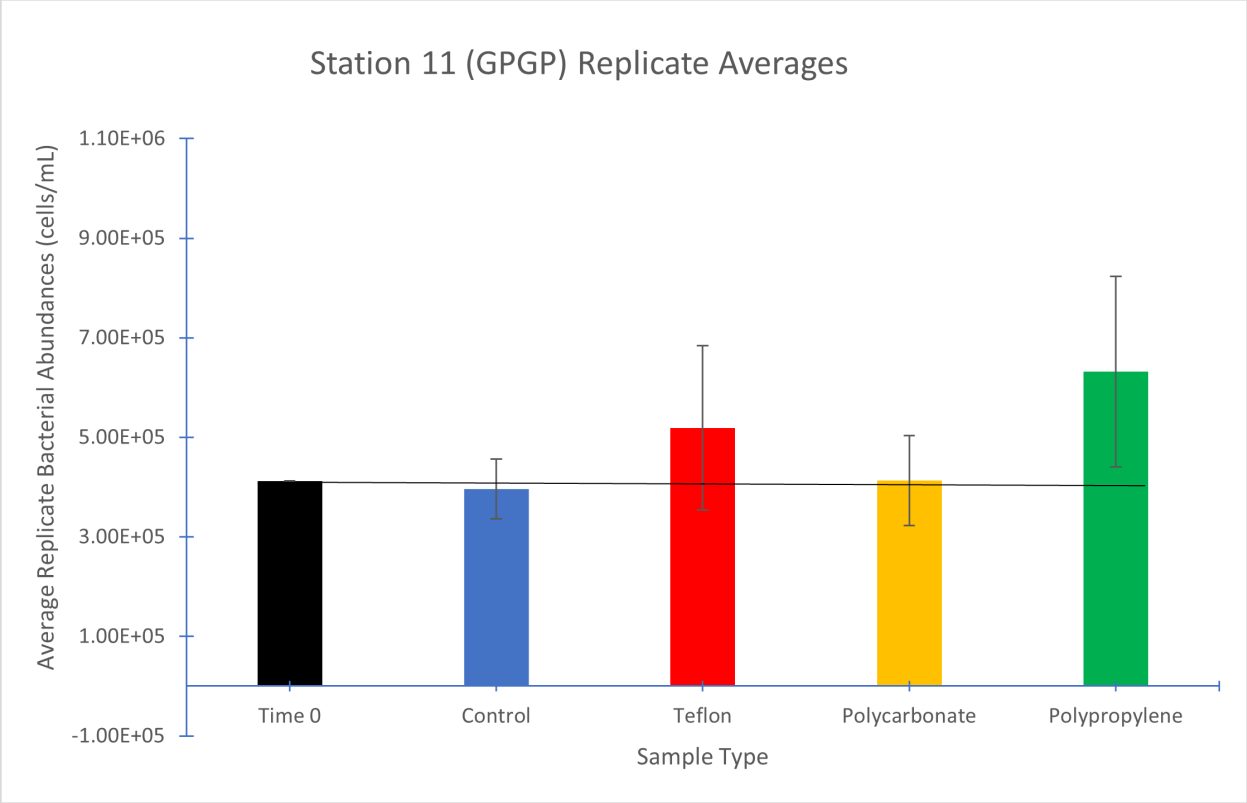

Figure 3: The average growths of each sample at Station 11 and within the real GPGP. The black horizontal line extending from Time 0's bar is to help with comparison from time 0 to 24 hours of incubation. The Teflon, polycarbonate, and polypropylene samples all experienced growth while the control sample lost bacteria. The highest growth was observed in the polypropylene sample.

Station 19 was where every sample type had their highest bacterial abundances. Once again, polypropylene had the highest growth with 8.8*10^5 cells per mL. None of the samples lost bacteria at this station (Figure 4).

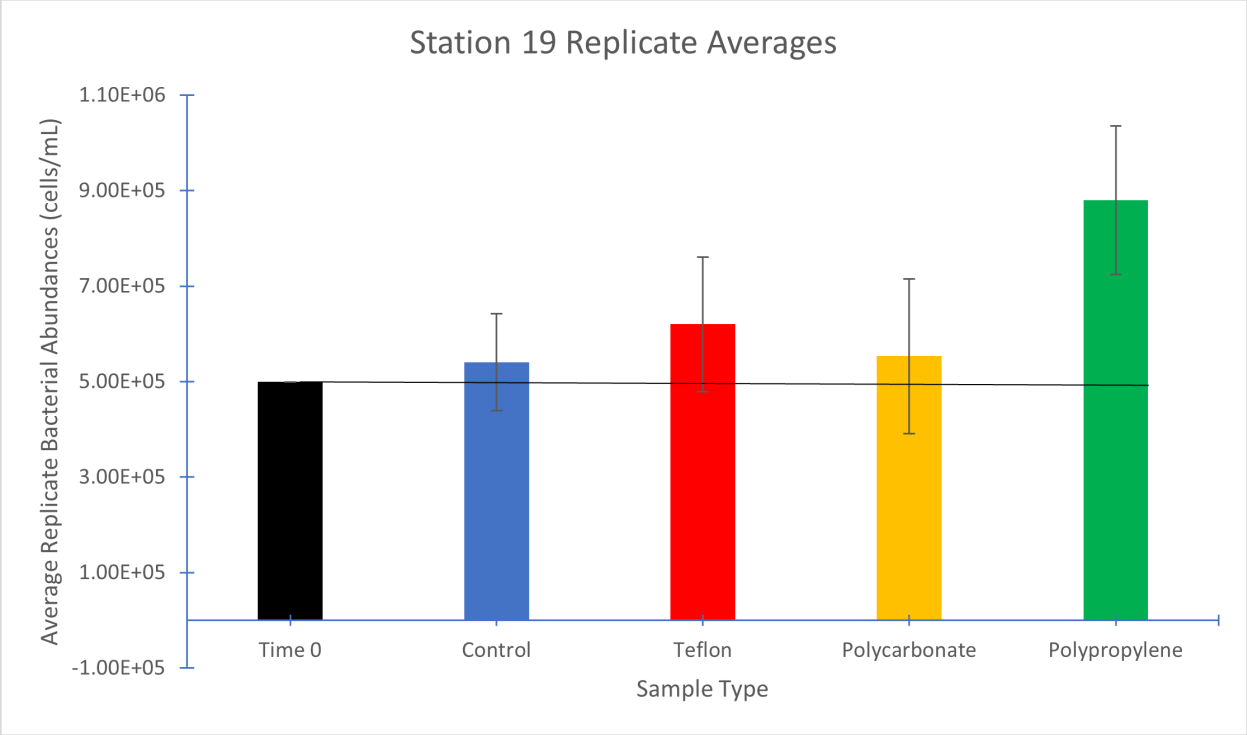

Figure 4: The average growths of each sample at Station 19. The black horizontal line extending from Time 0's bar is to help with comparison from time 0 to 24 hours of incubation. Every sample experienced growth and the highest growth was observed in the polypropylene sample.

In terms of hourly growth rates, every plastic sample experienced a positive rate except for Teflon at Station 3 (Figure 2), which was also the highest average loss of bacteria experienced among all of the samples in the study. The control samples also usually experienced positive growth with the exception of Station 11. Every plastic sample type experienced their highest growth rate at Station 19 and the polypropylene sample here had the highest growth rate overall of 1.59*10^4 bacteria per hour. The control sample experienced its highest growth rate at Station 3 with 2.21*10^3 bacteria per hour. Figure 5 shows the average bacterial growth rates per hour of each sample from each station.

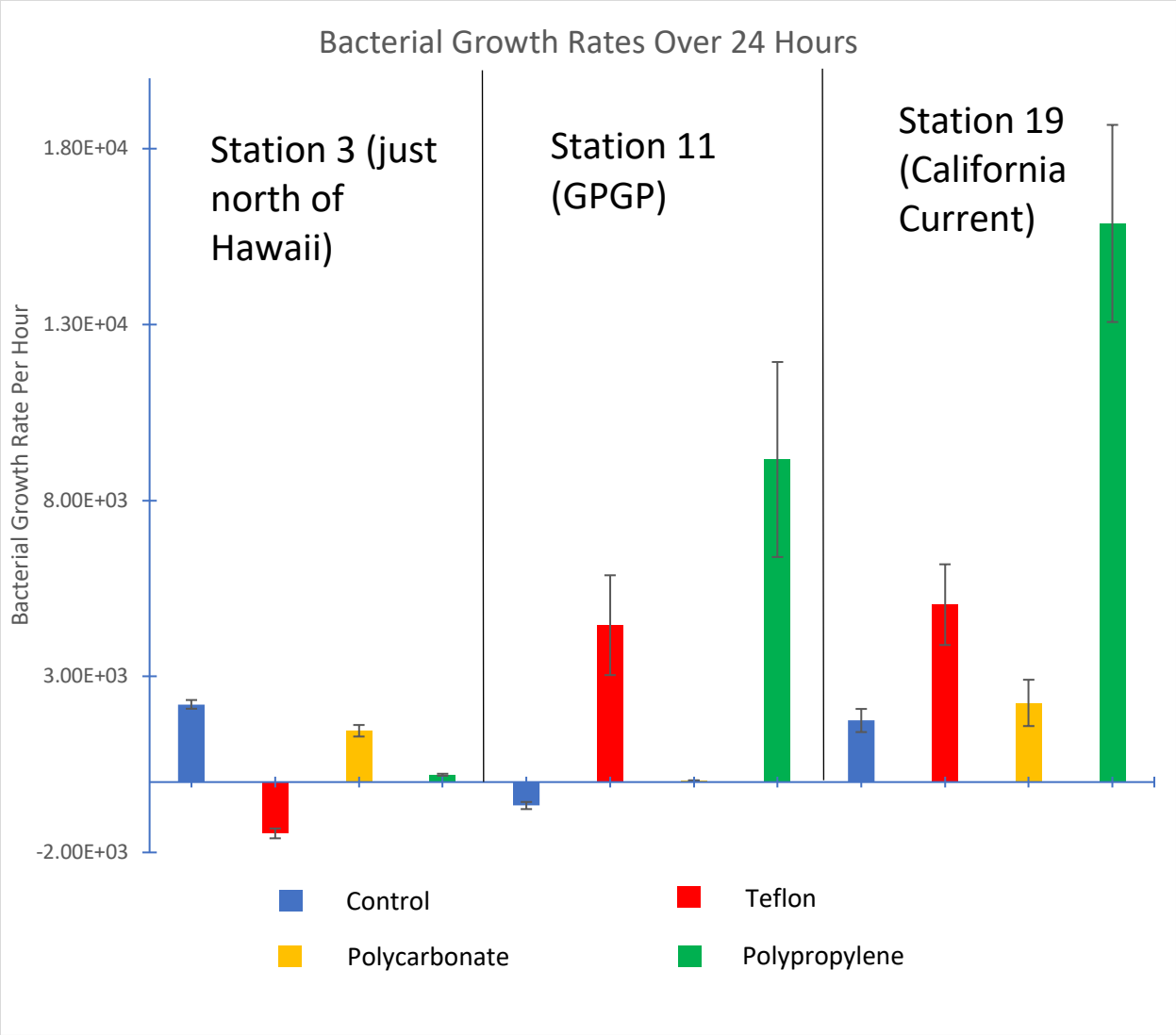

Figure 5: The hourly growth rates of all 12 samples separated by station number and sample type. The greatest overall loss of bacteria was experienced by the Teflon sample at Station 3 and the highest growth of bacteria was experienced by the polypropylene sample at Station 19.

DISCUSSION

The plastic that overall had the most positive effect on bacterial growth was polypropylene, having the highest bacterial abundances and hourly growth rates at both Stations 11 and 19 (Figure 5). A study conducted by Bellón et al. (2001) found that bacterial biofilms did grow on surgical prosthetics made from polypropylene due to the filament's structure, which corroborates the material's samples having a great effect on bacterial growth in this study.

Polycarbonate did not have a particularly strong effect on bacterial growth, positive or negative. This plastic's history of growing bacteria is not well-known, but studies that have been conducted showed different results. Polycarbonate filters have proved useful in growing bacterial biofilms to determine if certain species can grow in anaerobic conditions (HØjberg et al., 1997) and polycarbonate has also been engineered into unique polymers to kill infectious bacteria (Nimmagadda et al., 2017). Teflon was the only plastic to cause a reduction in bacterial abundance (Figure 5). If the bacteria experienced a higher growth rate in the Teflon samples, it likely was not because they grew on the filter itself. Teflon is a hydrophobic substance and its use on ceramic surfaces deters bacterial adhesion (Zore et al., 2020). Yet, the control sample also experienced a reduction in bacterial abundance within the GPGP (Figure 5). One possible explanation for these losses is that predators of the bacteria were in the sample and ingested some of them. With the exception of Station 3, all three types of plastic consistently yielded a higher average growth rate of bacteria than the control sample.

Station 3 was the only station to experience a loss of bacteria in a plastic solution and Station 11, the station located in the GPGP, was the only one to experience a loss of bacteria in the control solution. According to a study conducted by Bryant et al. (2016), several bacterial taxa inhabiting the GPGP are adapted to living on the surfaces of plastic particles and have many taxonomic differences from the free-living picoplankton in the same region. If these bacteria were placed in a sample where there were no plastic surfaces to stick to, then they would have a harder time enduring with their atypical survival methods. Station 19 showed increases in bacterial abundance in every sample and the highest growth rates for all three plastic samples. This station was close to the coast of California, and Alli Miller's research showed that surface

bacterial abundance was higher in this region compared to those of Hawaii and the GPGP, so that could be an explanation for why this station showed higher numbers of bacteria.

CONCLUSION

This experiment was conducted to see if certain types of plastic could augment the growth rate of marine bacteria and if these types of bacteria could be used for beneficial purposes. This could lead to potential uses for a pollutant that has been largely seen as hazardous and seemingly unremovable. While the species of bacteria that were cultivated from the samples could not be determined, there were definitive changes in bacterial growth in plain seawater versus seawater containing plastics. The results of this study indicate that MP's enhance bacterial growth overall and that polypropylene is the most conducive to it. However, the Teflon samples were observed to cause both positive and negative growth rates in the bacteria and polycarbonate's effects ranged from positive to neutral. These observations indicate there is no universal answer to how MPs affect marine bacterial growth, which matches the previously mentioned study conducted by Fernández-Juárez et al. (2021) about diazotroph growth in the presence of MP's. The control sample of Station 11 losing bacteria was unexpected. Some possible explanations for this occurrence are bacterial predators in the sample or bacteria within being unable to survive without a plastic surface to colonize (Bryant et al., 2016).

Future experiments should be designed with more stations and sample types to allow for more replicates of more different types of plastic, like polystyrene or polyvinyl chloride (PVC). Another suggestion would be to take a droplet from the samples after incubation and before placement in the cryotubes to place underneath a microscope to observe or take a picture of and possibly identify which species of bacteria were growing in them. Bacteria could also be counted and identified by examining the plastic filter itself underneath a microscope to see if any bacteria

grow on its surface. Knowing which species of bacteria are growing with these plastics could answer the question of whether they are benign or dangerous and if the use of MPs or plastic filters to grow bacteria would be wise and beneficial for the health of other marine organisms or humans.

ACKNOWLEDGEMENTS

Special thanks go to all of the crew members of the R/V Thompson who facilitated the voyage this experiment was conducted on and kept the operations running smoothly. Thanks go out to Kathy Newell for her assistance in the procedure for sample collection and preparation and Rick Keil for his similar assistance in project planning and graph formatting. Finally, I would like to thank Dylan Vecchione for providing the information on the flow cytometer operating procedure, Alli Miller for her sharing her research in surface bacterial abundance, and both of them for their assistance in operating the flow cytometer and collecting the bacterial abundance data for this study.

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