The Effect of Acidification on the Abundance of *Synechococcus spp. WH7803*

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Abstract

The purpose of this study was to evaluate how the ongoing acidification of the ocean would affect the abundance of cyanobacteria. In order to investigate this, the environmental pH of *Synechococcus spp. WH7803*, a strain of cyanobacteria, was manipulated to four different pH levels. After an incubation of 168 hours, the abundance was quantified using a simple plate count method. The results showed a significant positive linear correlation between the environmental pH and the percentage of cyanobacteria. These results indicated that the acidification of the ocean would have a negative effect on this particular strain of cyanobacteria, thus disrupting multiple factors in the ocean, including the aquatic carbon cycle and the trophic web in the open ocean.

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In the face of the growing concern of climate change, researchers are met with the challenge of studying the many different aspects of human-caused effects on the environment. One such aspect is the acidification of the Earth's oceans. In 2019, the Government of Canada (2019) evaluated that the ocean had an average pH of 8.0. However, they projected the ocean's pH would decrease to between 7.5 and 7.7 by the year 2100. Despite these numbers appearing close and inconsequential, the logarithmic pH scale indicates that proportionally, the acidity is expected to triple within the next 80 years. The source of this acidification is primarily the increasing amount of carbon dioxide (CO_2) gas into the Earth's atmosphere.

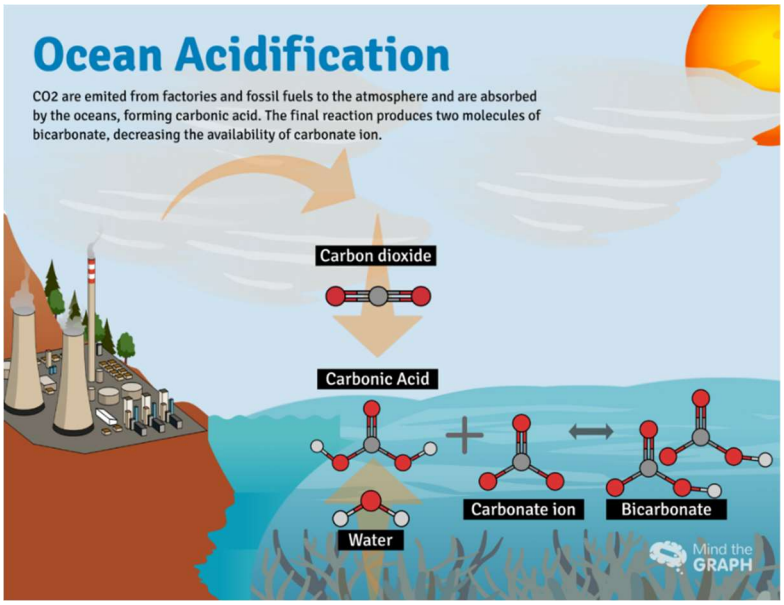

Figure 1. *The chemical reactions present in the ocean due to an excess of CO_2 in the atmosphere. $CO_2(g)$ reacts with H_2O to produce carbonic acid, carbonate and bicarbonatein an equilibrium reaction. Image source: https://www.legacyias.com/wp-content/uploads/2020/10/Ocean-Acidification.png*

As Figure 1 shows, the ocean rests in a state of chemical equilibrium. As atmospheric CO_2 reacts with the seawater, resulting in a carbonic acid (H_2CO_3), a weak acid being formed. Because carbonic acid is weak, the acid very dilute and the change in pH is slow (Smithsonian Ocean, n.d.). However, as more CO_2 is put into the atmosphere through the burning of fossil fuels, the equilibrium reaction is pushed to form H_2CO_3 in the water, resulting in the gradual lowering of the pH of the ocean.

The acidification of the ocean affects countless aspects of ocean life, biotic and abiotic and its ecosystems, benthic and pelagic. For instance, the balance between the blood cells of fish and the seawater will result in health problems in fish, due to the lowering of their blood's pH (Smithsonian, n.d.). In plants, it was found that in some cases, the acidic environment could be favourable for the growth of certain species (Smithsonian, n.d.). Whether acidification affects species favourably or not, the ramifications can be observed in multiple levels of the ocean's trophic web. While every trophic level is important in the ocean environment, as disturbances begin affecting the lower trophic levels, the ramifications become biomagnified to the higher trophic levels.

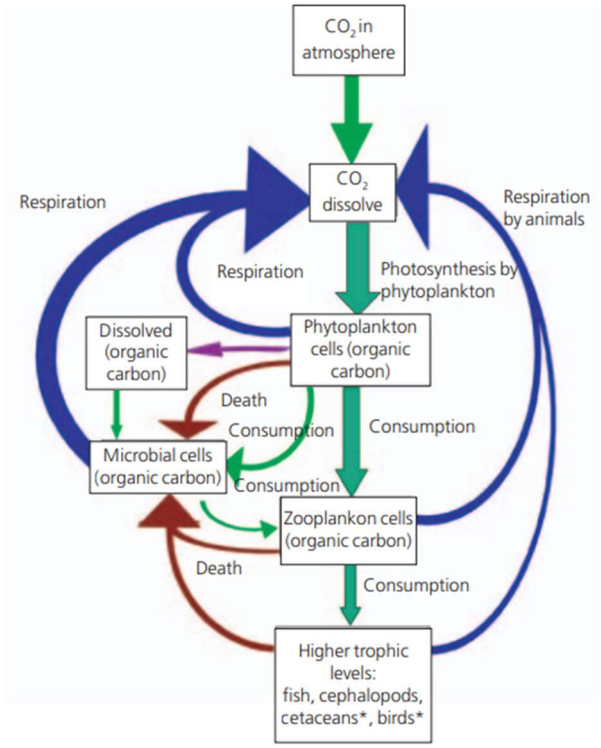

Figure 2. *A representation of the carbon and respiration cycle within ocean life. The consumption of carbon (green), respiration (blue) and decomposition (red) indicate how carbon moves through the cycle. Image source: https://royalsociety.org/-/media/Royal_Society_Content/policy/publications/2005/9634.pdf*

Furthermore, the ocean's producers play a significant role in the ocean's carbon cycle. Their conversion of dissolved CO_2 into organic carbon provides a carbon sink in the ocean (The Royal Society, 2005). However, increased CO_2 levels in the ocean have shown to have little effect on the rate of photosynthesis on the producers (The Royal Society, 2005). As demonstrated in Figure 2, the role the primary producers play is particularly important at sustaining life and cycling nutrients in the ocean. The dissolved carbon is consumed and used for photosynthesis by photosynthetic organisms, and cycled through the consumers through decomposition, ingestion and respiration. Photosynthetic organisms of particular importance in the ocean are

cyanobacteria. These single-celled photosynthetic prokaryotes, sometimes known as blue-green algae, are paramount to life in the ocean. According to Traving et al. (2014), they produce about 64% of the ocean's primary production. Waterbury (2005) explained that by expelling oxygen (O_2) as a waste product of their photosynthesis, cyanobacteria help to provide oxygen, an essential nutrient for the life of many organisms, aquatic and terrestrial. Furthermore, it is believed that cyanobacteria are responsible for most of the oxygen in the atmosphere (Waterbury, 2005).

Given what is known about the importance of cyanobacteria, and the threat of the ocean's acidification, one could question whether or not there could be a link between the two. To test this possible connection, an experiment was created. The cyanobacteria of focus in this experiment were *Synechococcus sp. WH7803* (hereafter referred to as simply *WH7803*). For this study, it was hypothesized that the lowering of the pH would have an effect on the abundance of the *WH7803* in saltwater samples. According to previous results in studies conducted by Rai and Rajashekhar (2016), in addition to Traving et al. (2014), it was predicted that as the pH decreased, so would the abundance of the cyanobacteria. Furthermore, there would be a steep drop-off in abundance of cyanobacteria with pH levels under 7. This experiment aimed to examine the relationship between the *WH7803* with its environment's pH levels.

Methods

Setup

The *Synechococcus spp. WH7803* culture was grown aseptically in a mixture of seawater and SN media. The SN media's recipe was provided by Willey et al. (1987). The culture's regular environmental pH was measured as 7.1. During the experiment, the temperature and salinity were be kept constant, and the amount of light was regulated to 14 hours of simulated day and 10

hours of simulated night with a lamp equipped with a timer (see Figure 3). A spectrophotometer was also used to provide backup data throughout the experiment.

Figure 3. *Final set-up of the incubation station. Left: treated culture test tubes and plates. Right: spectrophotometer.*

Dilution Factors

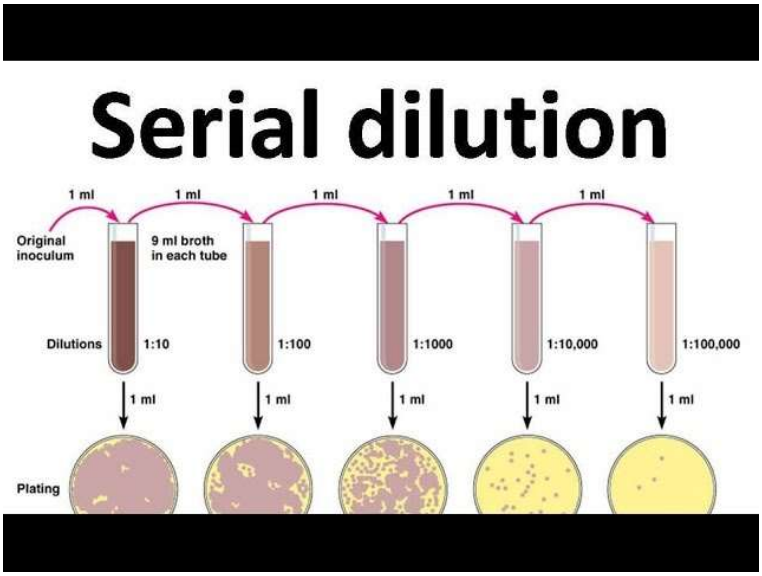

Figure 4. *Serial dilution procedure. Each dilution will thin out the abundance of colonies of bacteria on each plate, enabling clearer bacterial count. Image source: https://www.youtube.com/watch?app=desktop&v=loyeVy1D-3o*

The first step required for this experiment was to determine the optimum sample dilution required for the plating of the *WH7803* cultures. So, a serial dilution was performed, using the procedure shown in Figure 4. The dilution ratios tested were 1:10 and 1:100. Each dilution was plated in a culture media composed of a mixture of sea water and nutrient agar, and left to incubate a few days, until visible bacterial colonies grew. Once this incubation period was completed, the colonies on the plates were counted. Plates with a range of 20 to 200 colonies were considered viable. The dilution factors of 1:1, 1:10 and 1:100 all had viable numbers of colonies, so two replicates were done of each plate, using these dilutions factors.

Treatment of Bacterial Cultures

Once the dilution factors were determined, the next step in the experiment was to test the cyanobacteria's growth in different pH environments. To do this, four flasks of SN media were

treated with filter-sterilized 0.1 M HCl and 0.1 M NaOH, drop by drop, until each flask reached the following pH levels: 6.5, 7.1, 7.5 and 8.1 (Figure 5). Next, 15 mL of each treated SN media was pipetted into three different test tubes. Finally, 5 mL of bacterial culture was pipetted into each test tube. This resulted in four testing solutions, each with three replicates.

Figure 5*. pH papers with the initial pH values of each SN media, before the bacterial cultures were added.*

Plating and Counting

Initial Abundance (T0)

The initial abundance was determined by pipetting 5 mL of the original bacterial culture into 15 mL of untreated SN media. The resulting mixture was diluted to 1:10 and 1:100 with additional untreated SN media. Then, the plates of initial abundance were made by pipetting 250 µL of each dilution onto a plate of nutritional agar mixed with seawater. The plates were left to incubate at room temperature for one week and the colonies on the plates were then counted. Control plates for each of the treated SN media were also plated, in order to rule out the

possibility of contamination of the SN media during the earlier stages. In order to provide comparative data for the results, the dilutions were also run through the spectrophotometer, with a wavelength of 460 nm.

Timepoint Plating (T48 and T168)

Once the treated cultures had incubated for 48 hours, the samples were prepared for plating. For the first timepoint, the dilutions used for plating were 1:1 and 1:10. The dilutions were performed for each of the test tube replicates, and each dilution was plated on the nutrient agar plates, with 250 μL of each dilution pipetted on the plates. The plates were left to incubate at room temperature for one week. The colonies were then counted and recorded. Like the initial abundance samples, each of these samples were run through the spectrophotometer and their absorbance values were recorded.

These methods were repeated after 168 hours (one full week) of the 12 treated cultures' incubation, in order to determine a long-term effect of the acidification on the abundance of the cyanobacteria. The sole difference was the dilution factors used for this timepoint: 1:10 and 1:100 due to the growth of the bacteria during the treated cultures' incubation time. After the plating and the week-long incubation of the T168 specimen of bacteria, the plates were once again counted and recorded.

Statistical Analysis

Once the results were recorded, a correlation analysis was evaluated as being the preferable statistical analysis. The environmental pH was the independent variable and the percentage of the counted colonies of *WH7803* was the dependent variable. Both variables were input as continuous variables. Therefore, using a correlation provided a clearer illustration and analysis of the results. Significance of the data would be determined if $p < 0.05$.

Results

The resulting plates were counted once growth occurred. Because growth occurred in all the plates, the data taken from the spectrophotometer was not needed for the data analysis. As both dilutions of each pH value had a high number of colonies, the plate with the lowest number of colonies was counted. Figure 6 shows an example of the bacterial plates. Both the *Synechococcus spp. WH7803* and the companion bacteria were counted, and the percentage of *WH7803* was calculated and used for the statistical analysis. The data compiled from the T168 plates was used for the statistical analysis. A preliminary visual comparison of the plates revealed a trend of a higher ratio of *WH7803* colonies as the pH increased.

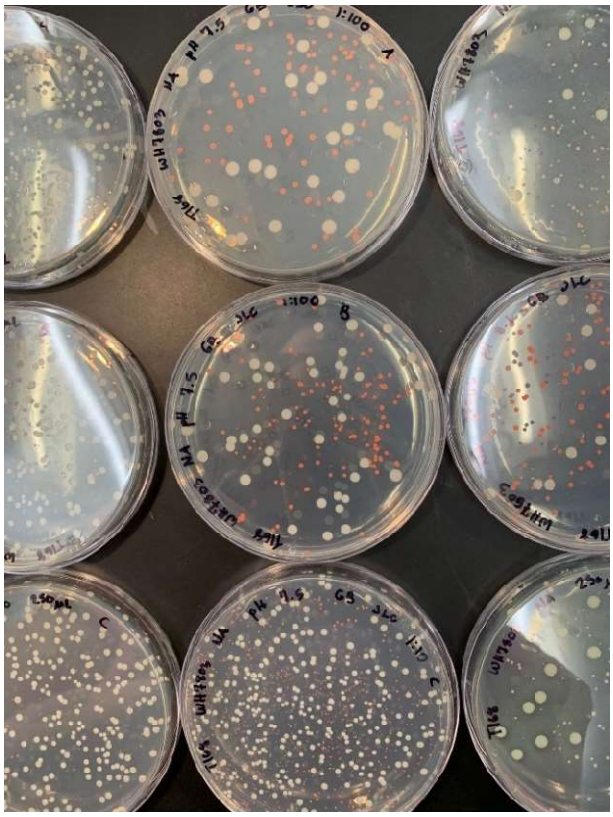

Figure 6. *Bacterial plates after incubation. The culture from these plates were at a pH of 7.5 and a dilution of 1:100. The pink colonies are WH7803 and the white colonies are unknown companion bacteria.*

The graph that was generated from the results (Figure 7) was a scatterplot with the pH value on the x-axis and the percentage of *WH7803* colonies counted on the y-axis. The general trend of the data was a positive linear correlation of the data, as shown on the graph, particularly illustrated by the line of best fit. However, in order to determine the true significance of the data, a statistical analysis was performed on the results as well.

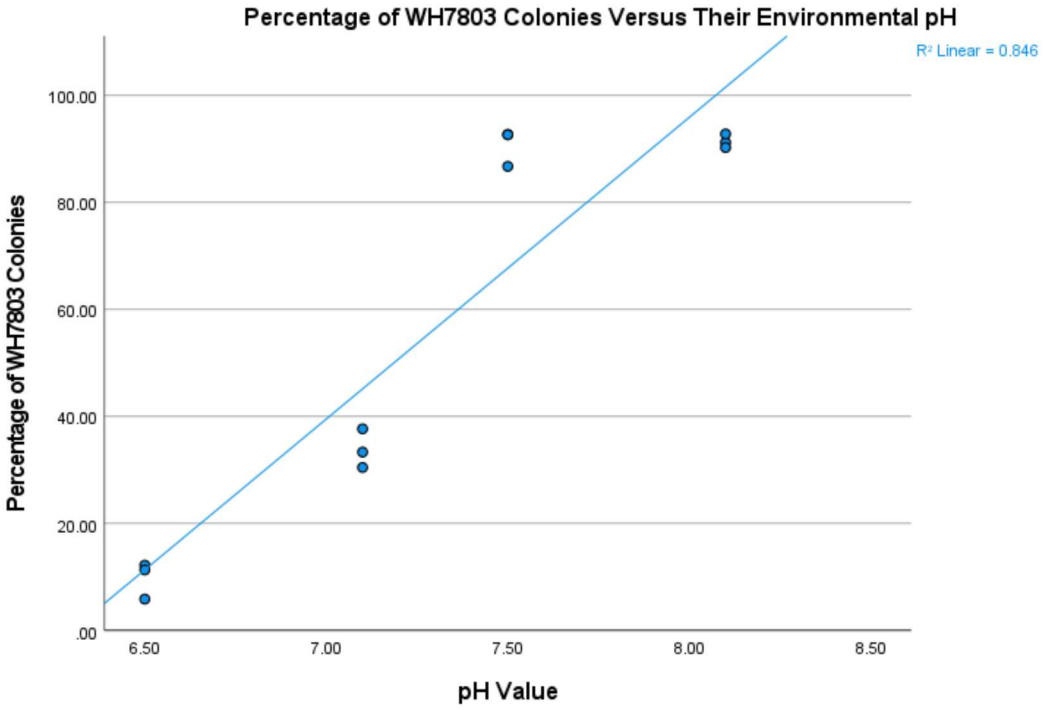

Figure 7. *Scatterplot depicting the results of T168, where the percentage of WH7803 colonies are plotted against their environmental pH. Each data point corresponds to a plate count. The line of best fit is a linear proportion.* $R^2 = 0.846$.

The statistical analysis performed on the data, as shown in Figure 8, was a correlation analysis. The values from this table provide information on the relatedness of the variables. Firstly, the Pearson Correlation value was 0.920, a value close to "1," indicating a high relatedness between the two variables. Finally, the significance was evaluated as less than 1% of

error, which was within the realm of the projected 5% allowed error, indicating that the results were significant.

Correlations

		pH Value	Percentage of WH7803 Colonies
pH Value	Pearson Correlation	1	.920**
	Sig. (2-tailed)		<.001
	N	12	12
Percentage of WH7803 Colonies	Pearson Correlation	.920**	1
	Sig. (2-tailed)	<.001	
	N	12	12

**. Correlation is significant at the 0.01 level (2-tailed).

Figure 8*. Correlation statistical analysis of the percentage of WH7803 colonies versus their environmental pH. Correlation: r = 0.920, sample size: n = 12, significance: p = 0.001 < 0.005.*

Discussion

The statistical analysis results indicated that the two variables, the pH of the environment and the relative abundance of the *Synechococcus spp. WH7803* colonies were linked. In other words, the visible trends correlated with the statistical analysis. In the introduction of this paper, the proposed hypothesis stated that the lowering of the pH would have an effect on the abundance of the *WH7803* in saltwater samples. Based on the statistical evidence obtained, the results of this experiment support this hypothesis. Furthermore, the prediction presented stated that as the pH decreased, so would the abundance of the cyanobacteria. Based on the observable trends determined from the results, and the positive linear correlation between the variables, the prediction is also supported by this experiment's findings.

The results found in this experiment closely resemble previous findings. Traving et al. (2014) also aimed to discover a link between the *WH7803* cyanobacteria and their environmental pH. However, in their case, rather than examining changes in abundance, they studied how acidification would affect both the growth of the *WH7803* and their interactions with cyanophages that infect them. The results found by Traving et al. (2014) mirrored this experiment's results, especially regarding the cyanobacteria's growth. Figure 9 shows the growth rate results yielded by Traving et al. (2014). The graph shows an obvious trend of increased growth rate at the pH value of 8 (the ocean's current pH). The results obtained by Traving et al. (2014)mirrors this experiment's findings. The cyanobacteria had more success in the pH 8.1 in this experiment than in the lower pH values.

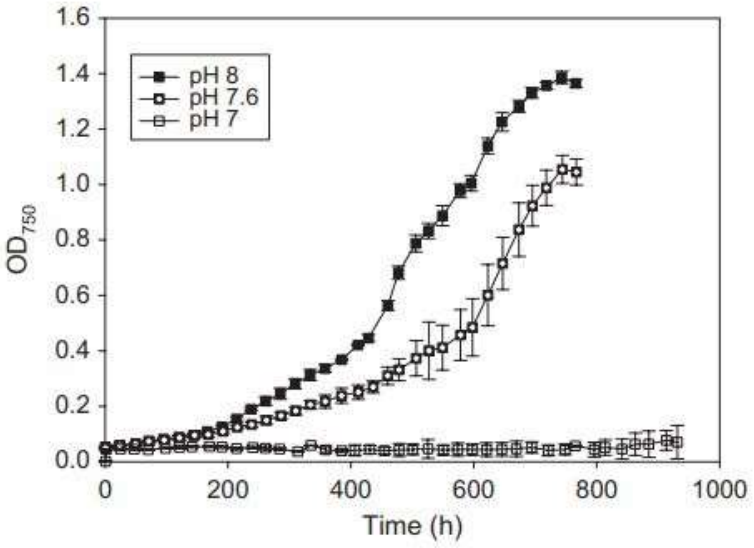

Figure 9. *Line graph depicting the growth rate of WH7803, at three different pH values: 8 (filled boxes), 7.6 (bold boxes) and 7 (empty boxes). The data follows each specimen's cell density (OD_{750}) versus the time (hours). Image source: Traving et al. (2014).*

Another study conducted by Fernández-Juárez et al. (2023) aimed to model how climate change as a whole would affect a different type of bacteria (diazotrophs), particularly for their productivity, growth and ability to adapt to their surroundings. The researchers examined the effect of pH, salinity and temperature on the bacteria. Relating to the change in pH, Fernández-Juárez et al. (2023) tested a more extreme variation in pH levels, yet the findings paralleled the results of this paper, where higher pH values were shown to have more favourable effects on the cell growth of the different cyanobacteria, as shown in Figure 10.

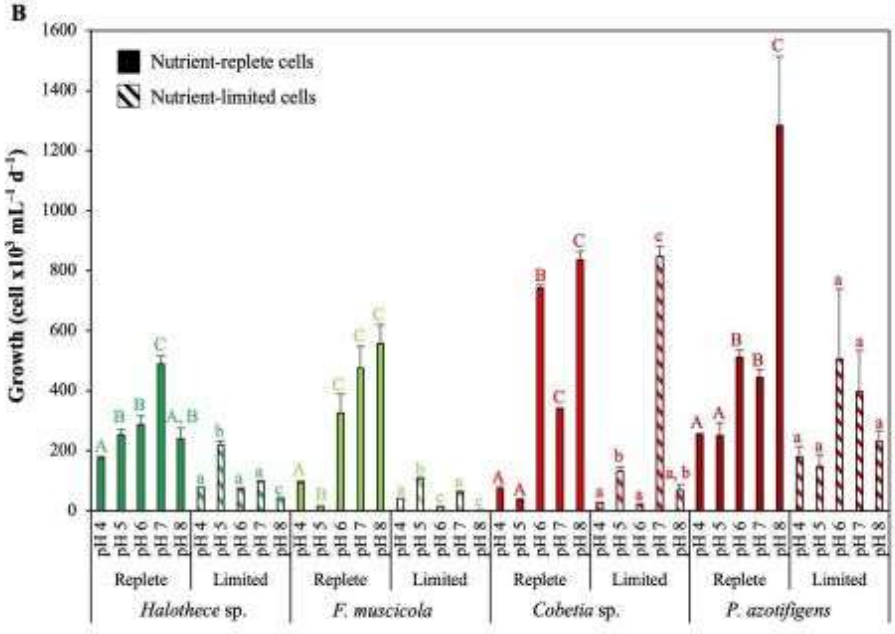

Figure 10. *Histogram representing the effect of pH on the growth (cell x 10^3 $mL^{-1}d^{-1}$) of four different types of cyanobacteria. The data was further separated into nutrient-replete groups (solid bars) and nutrient-limited cells (hashed bars). Image source: Fernández-Juárez et al. (2023).*

After completing the bacterial sampling in this experiment, the tested bacterial cultures' pH values were reassessed. The results (shown in Figure 11) showed that the final pH of each of

the solutions had returned to 7.1. This indicates that the *WH7803* cultures may have changed their environment during the incubation period. While this information provides an insight into how the cyanobacteria adapt to environmental changes, this also provides a source of error for the final results. In future experiments, it may be beneficial to monitor the cultures' pH values more closely It may be beneficial to add HCl or NaOH to each culture daily, in order to maintain a stable pH value. Another source of error is the small sample size. Due to time and supplies restrictions, only 12 plates could be used for data. In future experiments, a larger sample size would be ideal.

Figure 11. *pH paper tests of each bacterial culture treatment. This final pH was tested after all the sampling required for this experiment had been completed.*

In conclusion, the goal of this experiment was to examine the relationship between pH and *Synechococcus spp. WH7803* abundance. After designing an experiment to test these

variables, it was determined that both the hypothesis and the prediction presented at the beginning of this paper were supported by the data. A future experiment that could build on this study could be looking further into the relationship between the *WH7803* and the companion bacteria seen on the plates (see Figure 6). Through trial and error, it was discovered that the *WH7803* could not grow without this companion bacteria, suggesting that there may be some type of mutualistic relationship between the two bacteria. Further research into this area could provide some insight into the interactions between the bacteria, and perhaps even their affect on ocean productivity. The knowledge of how humans effect the ocean and how cyanobacteria could be so negatively affected by man-made issues could be the first step in kickstarting effective solutions to climate change. While cyanobacteria are small, their impact on their environment is anything but. Without them, life on Earth would be unrecognizable.

Acknowledgements

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