The effect of acidification on the abundance of *Synechococcus sp. WH7803*

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Abstract

The purpose of this study was to evaluate how the acidification of the ocean would affect the abundance of cyanobacteria. To investigate this, the environmental pH of *Synechococcus spp. WH7803*, a strain of cyanobacteria, was manipulated to four different pH levels: 6.5, 7.1, 7.5 and 8.1. The results showed a significant positive linear correlation between the environmental pH and the percentage of cyanobacteria.

Introduction

The acidification of the ocean is a growing, human-caused concern. 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 as low as 7.5 by the year 2100. As Figure 1 shows, the ocean rests in a state of chemical equilibrium. However, as more CO_2 is put into the atmosphere through the burning of fossil fuels, the equilibrium reaction is pushed to form more H_2CO_3 (a weak acid) in the water, resulting in the gradual lowering of the pH of the ocean.

The ocean's producers play a significant role in the ocean's carbon cycle. Their conversion of dissolved CO_2 into organic carbon is a substantial carbon sink in the ocean (The Royal Society, 2005). 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. 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 if there is a link between the two. It was hypothesized that the lowering of the pH would have an effect on the abundance of *the Synechococcus sp. WH7803*, a type of cyanobacteria. Furthermore, it was predicted that as the pH decreased, so would the abundance of the cyanobacteria.

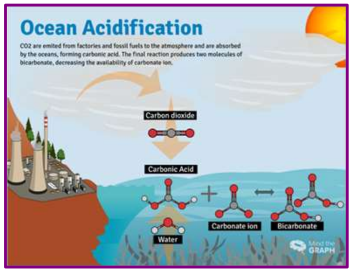

Figure 1 (left). The chemical reactions present in the ocean due to an excess of CO_2 in the atmosphere. $CO_2(g)$ reacts with H_2O to produce carbonic acid, carbonate and bicarbonate in an equilibrium reaction. Image source: https://www.legacyias.com/wpcontent/uploads/2020/10/Ocean-Acidification.png

Methods

The *Synechococcus sp. WH7803* culture used in this experiment was grown aseptically in a mixture of seawater and SN media, recipe 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 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.

To test the cyanobacteria's growth in different conditions, 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 one of the following pH levels: 6.5, 7.1, 7.5 and 8.1. 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.

Once the treated cultures had incubated for 168 hours, the samples were prepared for plating. Two 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.

Once the results were recorded, a correlation analysis was evaluated as being the preferable statistical analysis, run on the IBM SPSS program. The environmental pH was the independent variable and the percentage of the counted colonies of WH7803 was the dependent variable. Significance of the data would be determined if $p < 0.05$.

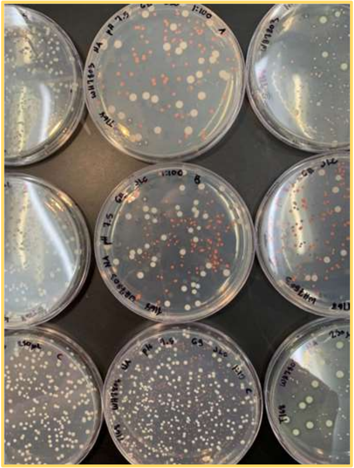

Figure 2 (above). Bacterial plates after incubation. The culture from these plates were at a pH of 7.5 and a dilution of 1:100. The pink colonies are *WH7803* and the white colonies are an unknown companion bacteria.

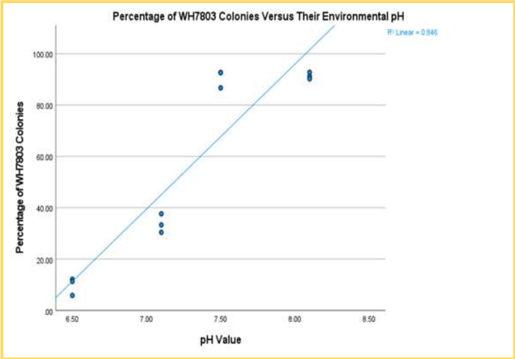

Figure 3 (above). Scatterplot depicting the results of T168, where the percentage of *WH7803* colonies are plotted against their environmental pH. Each data point corresponds to a plate count. The line of best fit is a linear proportion. $R^2 = 0.846$.

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 4 (left). Correlation statistical analysis of the percentage of *WH7803* colonies versus their environmental pH. Correlation: $r = 0.920$, sample size: $n = 12$, significance: $p = 0.001$

Results

The bacterial plates had striking results (Figure 2). Both the *Synechococcus spp. WH7803* and the companion bacteria (identity currently unknown) were counted, and the percentage of *WH7803* was calculated and used for the statistical analysis.

The graph that was generated from the results (Figure 3) showed a general trend of the data (a positive linear correlation. However, in order to determine the true significance of the data, a statistical analysis was also performed on the results.

The correlation analysis performed on the data, as shown in Figure 4, provided 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.

Discussion

The statistical analysis results indicated that the two variables (the pH of the environment and the relative abundance of the *Synechococcus sp. WH7803* colonies) were linked. Based on the statistical evidence obtained, the results of this experiment support the presented hypothesis. Furthermore, 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 observed in this experiment resemble previous findings. Traving et al. (2014) also aimed to discover a link between the *WH7803* cyanobacteria and their environmental pH. The results found by Traving et al. (2014) mirrored this experiment's results, especially regarding the cyanobacteria's growth. The cyanobacteria had more success in the pH 8.1 in both experiments than in the lower pH values.

In conclusion, the goal of this experiment was to examine the relationship between pH and *Synechococcus spp. WH7803* abundance. It was determined that both the hypothesis and the prediction presented at the beginning of this study 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. 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 causes 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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