Prochlorococcus-Mediated Restoration of Hypoxic Ecosystems Across Varying Levels of Turbidity in Aquatic Environments

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Abstract

The current approaches to manage hypoxia caused by high turbidity levels only prevent or lessen the quantity of sediment discharge; little research is done on the areas that are already hypoxic, which causes these areas to become low-nutrient because aquatic plants' opportunity to photosynthesis is reduced. The goal of this study is to utilize the Cyanobacteria *Prochlorococcus marinus* to provide a bioremediation solution to locations where sediment flow has caused hypoxia. When exposed to high turbidity conditions, *Prochlorococcus marinus* will create an equivalent or comparable amount of oxygen to when it is not present in low turbidity conditions. In order to simulate aquatic habitats, fifteen tanks were set up with varied turbidity levels and the common aquatic plant *Phaeophyceae* (brown algae). Turbidity levels were shown using the inhibition of different amounts of UV light (25%, 50%, 75%). Analysis through a When and ANOVA statistical test was performed, the results indicated that there was a significant effect of time (p-value day < 0.0001) and the presence of *Prochlorococcus marinus* (p-value treatment < 0.0001) on oxygen production levels. Ultimately supporting the hypothesis that when *Prochlorococcus marinus* is exposed to high levels of turbidity-mimicked conditions via UV light inhibition, it does affect ecosystems by producing equal or similar levels of oxygen. With this in mind, future research should focus on applying *Prochlorococcus marinus* to actual marine ecosystems that currently face hypoxia to replicate the results of the current study.

Keywords Sediment Runoff, Bioremediation, Eutrophication Dynamics, Prochloroccocus Marinus, Turbidity, Cyanobacteria, Photosynthetic Efficiency, Hypoxia, Bioenergetic Potential

Introduction

Sediment runoff has been part of Earth's processes for millennia. The geological activity goes as follows: as rainwater enters the ocean, its speed slows and the sediment particles eventually spread out onto seabeds and coral reefs (Carilli, 2014). This natural process, indeed harmful, takes thousands of years for there to be enough accumulation that harms the environment (Snelgrove, 2013). More recently, however, anthropogenic sources have hindered this delicate balance. The Natural Oceanic and Atmospheric Administration reports that 80% of ocean pollution comes from land (NOAA, 2023). The biggest contributor is what is called a nonpoint source resulting from runoff, which encompasses a variety of anthropogenic sources such as septic tanks, vehicles, farms, and ranches. The Environmental Protection Agency reports that 40% of all impaired ocean waters or turbidity stems solely from nonpoint sources of pollution generally resulting from land runoff, precipitation, atmospheric deposition, drainage, seepage, or hydrologic modification (EPA, n.d). Nonpoint source (NPS) pollution, unlike pollution from industrial and sewage treatment plants, comes from many diffuse sources (EPA, n.d). The heightened levels of pollution on land eventually lead to higher levels of sediment runoff which enters the ocean through water sources. The biggest detrimental effect is a higher level of turbidity or the measure of water clarity (USGS, 2018). An increase in turbidity levels has been shown to have harmful effects on water ecosystems as lesser intensities of sunlight UV penetrate the water surface reaching the aquatic plants or Submerged Aquatic Vegetation (SAV; ICPRB, 2017). Less sunlight results in less plant growth due to less photosynthesis, and because plants produce less oxygen, there is less oxygen available for aquatic organisms (ICPRB, 2017). These hypoxic regions, where there is low or depleted oxygen in a water body, create "dead zones" that are unable to sustain life in an ocean environment. (NOAA, 2022). The frequency of "dead zones" has only been further exacerbated in recent years, in 1950 only 10 documented cases were identified to at least 169 in 2007 only over 50 years (NGS, n.d).

In recent years many solutions have been proposed in response to turbidity levels, a relevant case being the Great Barrier Reef (GBR) in Australia. An estimated 17 million tonnes of waste pollutants each year go into rivers which are eventually deposited into the Great Barrier Reef (GBR) which is five to nine times greater than 200 years ago (TNCA, 2019). As a result, turbidity levels near these sites were found to be 0.3 - 0.4 times higher than the average which was around five NTU (Nephelometric Turbidity Units) (Macdonald, 2015). To combat this, the Australian Government has pledged to spend nearly 8.2 billion dollars by 2025 on cleaning up the Great Barrier; nearly eight times the amount set up the year prior where one billion dollars was set aside for nine years from 2022-2023 to 2029-2030 (APH, 2022).

Traditional methods of cleaning and lowering turbidity levels include vegetative buffer strips (using natural vegetation to create buffers that trap the sedimentation), sedimentation basins (a basin that allows suspended particles to settle out before reaching the ocean), erosion control measurements (carefully managing the amount of erosion that occurs before reaching the ocean), and regulatory measures (regulating the amount of anthropogenic activity that causes). Although all traditional solutions mitigate or prevent sedimentation runoff from reaching the ocean or other water sources to avoid hypoxia, however, most fail in several aspects. Initially, these methods can only prevent or mitigate the turbidity levels in an area and fail to address environments that are already affected by high levels of turbidity which currently or will

experience hypoxia. The other aspect is cost efficiency, just like in the aforementioned case study, large amounts of funds are required to continually maintain these conditions. For example, every year the Australian government spends about 20-21 million dollars on maintenance alone (APH, 2023). Current methods have tried to use organisms to improve water quality such as filtration and removal of pollutants through the use of aquatic plants, however, they lack any fundamental value in environments that have high levels of turbidity. However, a new approach using certain cyanobacteria may provide new insight into finding cost-effectiveness, and unlike traditional methods that struggle with high turbidity hypoxia regions, certain cyanobacteria with unique characteristics may be the key to addressing these challenges.

Prochlorococcus marinus, or Prochlrococcus as it is commonly known, is a type of cyanobacteria defined as marine unicellular cyanobacterium and is the most abundant photosynthetic organism on Earth (SD, 2020). Members of this genus are classically thought to be adapted to high-oxygen and nutrient-poor ocean conditions, with a principle divergence between high-light and low-light ecotypes (NIH, 2021). This organism was first discovered and isolated by scientists in 1989 from the bottom of the euphotic zone in the Sargasso Sea (NIH, 1999). Prochlorococcus is characterized by a genus of very small (0.6 μm) marine cyanobacteria with unusual pigments (chlorophylls a2 and b2) (NIH, 1999). These bacteria are responsible for causing 5% of global photosynthesis (Pennisi, 2017). Due to its high number of gene strains (80,000; Pennisi, 2017), there are several ecotypes (i.e., different environmental conditions that organisms are fit for) these bacteria can survive in - ranging from sunlit water surfaces to 200 meters depth (Pennisi, 2017). As such using its unusual pigmentation (chlorophylls a2 and b2), the photosynthetic bacteria enacts photosynthesis to produce nearly 20% of all oxygen produced in the biosphere, a higher percentage than all of the tropical rainforest combined (NOAA, n.d). The utilization of this resilient cyanobacteria may prove to be invaluable in areas experiencing hypoxia due to high levels of turbidity, which are not only limited to aquatic plants but also common algae that exist in marine environments for example: Phaeophyceae (brown algae).

Phaeophyceae or brown algae class of about 1,500 species of algae in the division Chromophyta, common in cold waters along continental coasts (Britannica, n.d.). There are 16 species of Phaeophyceae (brown algae), comprising about 285 genera and about 1800 species (Yoon, 2009). Species range from simple microhairs to giant kelps that can reach 60 meters in length. Large multicellular phaeophyceae or giant kelps play an important role in coastal marine ecosystems. These multicellular organisms, with distinct multicellular structures including holdfasts, stipes, and blade(s) later carry specialized transport cells (referred to as trumpet hyphae depending on their size) to transport metabolites (Yoon, 2009). Large kelps are large biomass that grow extensively and form marine forests that are home to a variety of species.

This experiment aimed to determine whether the use of Prochlorococcus under different dust concentration conditions (25%, 50%, 75%) simulated with different solar irradiance suppression could produce the same amount or amount of oxygen with similar results when compared to conditions without hypoxia and low turbidity. Confirmation of these findings may provide insights for environmentalists to find solutions to protect marine ecosystems currently facing high levels of hypoxia and turbidity because these factors provide plants with low solar

radiation penetrating water reducing photosynthetic rates (ICPRB, 2017). Prochlorococcus is the most abundant photosynthetic bacterium on Earth and can replace plants that lose their energy levels in low-growth areas primarily by reversing hypoxia in the water or preventing increased turbidity (NIH, 2017). From the perspective of a government agency, the use of Prochlorococcus may be an alternative to biological management. Current methods revolve around preventing and reducing erosion to keep sediments dry. These have been highly successful for their goals: of mitigation and prevention, but essentially fail to address areas already experiencing high levels of disturbance and subsequent hypoxia.

The Alternative hypothesis (H1) states that when Prochlorococcus marinus is exposed to high levels of turbidity-mimicked conditions via UV light blockage, it will positively affect ecosystems by producing equal or similar levels of oxygen (ml/g) to low turbidity level treatments that are not exposed to Prochlorococcus, thus serving as a viable alternative for restoring low-light/growth aquatic ecosystems impacted by sediment runoff. According to the Null hypothesis (H0), the oxygen levels (ml/g) produced by experimental groups introducing Prochlorococcus marinus will be equal or comparable to each other. There would be no significant differences in oxygen concentration between low and high turbidity level environments.

Methods

Fifteen individual tanks were set up for this experiment, with each one having a volume of 1,200 ml and measuring 226 ml meters high by 82 ml meters wide. Each tank was bedded with a substrate layer consisting of 0.5 cm of artificial ocean sand at the bottom, followed by 1 cm of aquatic sand soil. After which, the tanks were populated with 3 grams of Phaeophyceae (brown algae) per tank, and 1,000 ml seawater sourced from the South Korean East Sea coast was gently poured into each tank. The initial temperature of the seawater was between five to seven degrees Celsius as such the tanks were left to acclimate for a total of three days before the experiment began.

Bacteria introduction of the Prochlorococcus consisted of an initial inoculation period in 1L of Pro99 Growth Medium and 100 ml of Prochlorococcus starter culture for three days. The treatment phase consisted of adding the Prochlorococcus culture into tanks six through fifteen for a total of 122 ml per tank after which each tank was gently mixed to assimilate the Prochlorococcus with the new environment.

All tanks were kept under UV LED shelves to mimic sunlight which they would have typically received in their normal environments. Before beginning day one of the experiment to simulate low light conditions the initial lumens were calculated (6,000 lux) after which medical clothes were employed to cover the tanks at varying percentages (25%, 50%, 75%) to emulate the varying degrees of turbidity in an ocean environment. Tanks seven through nine were covered at 25% (4,500 lux), tanks ten through twelve were covered at 50% (3,000 lux), and tanks thirteen through fifteen were covered at 75% (1,500 lux). Tanks four through six were also covered by 50%, however, the aforementioned did not include the bacterial treatment as such acting as the control while tanks one through three were not covered to simulate a normal healthy ocean environment.

Seawater chemistry measurements included oxygen levels (measured twice daily using a dissolved oxygen probe - morning and night, in ml/g). Tank measurements consisted of measurements being taken every morning and every night and included observation of LED lights on the two shelves where the tanks were placed (set for 12-hour day/night periods), temperature (room temperature was set between 20.5-22.5 degrees Celsius while water temperature was kept between 19-20 degrees celsius), pH (kept between 7-7.5 using pH strips), and salinity (maintained between 25-28 ppt using a salinity probe). All aforementioned measurements were taken daily (day and night) during the experimental period lasting 21 days (Day 1- Day 21) during which all conditions were carefully observed and controlled adjusting if needed.

All data collected (day/night) was inputted into RStudio where code was created to compare all the data. To test the effect of time and treatment on each response variable, a linear regression model (y=mx+b; lm = response ~ Day*Treatment) was used, and an ANOVA statistical test was enacted on the linear model. These results correlated with each line plot. To test the overall effect of treatment on each response variable, an ANOVA statistical test was used with Treatment as a fixed factor and each environmental measurement as the response variable (aov(response~ Treatment)). These correlate with each boxplot with treatment on the x-axis. To test the overall effect of tank and treatment on each response variable, an ANOVA statistical test was used with Treatment and Tank as fixed factors and each environmental measurement as the response variable (aov(response ~ Treatment*Tank)). These correlated with each boxplot with a tank on the x-axis.

The independent variable during this study was the level of sunlight exposure (UV LED) which was manipulated through the different percentages of coverage (25%, 50%, 75%). The dependent variables were the response variables (day/night) which included: Oxygen level (ml/g), Temperature (celsius degrees), Salinity (ppt), pH, and Light intensity (lux).

Results

The results of the two-way ANOVA testing the effect of treatment and tank content on important response variables (oxygen, temperature, salinity, and pH) revealed robust statistical patterns. The treatment factor revealed a highly significant effect on oxygen concentration (F value = 174.261, $P < 0.0001$; Table 1). In contrast, the tank factor had no significant effect on all response variables, with a P-value of 0.829 for oxygen. The residues exhibited a range of variations, ranging from large variations for oxygen to moderate variations in temperature, salinity, and pH.

Table 1. Tank vs. All four response variables (Oxygen, Temperature, Salinity, pH).

Response	Factor	Df	Sum Sq	Mean Sq	F value	P-value
Oxygen	Day	20	382.760	19.138	2279.640	< 0.0001
	Treatment	4	748.200	187.050	22280.500	< 0.0001

	Day	80	278.480	3.481	414.650	< 0.0001
	Residuals	525	4.410	0.008		
Temperature	Day	20	128.736	6.437	107.667	< 0.0001
	Treatment	4	0.107	0.027	0.448	0.774
	Day	80	7.470	0.093	1.562	0.0025
	Residuals	525	31.387	0.060		
Salinity	Day	20	1.450	0.072	2.315	0.0011
	Treatment	4	0.178	0.044	1.419	0.226
	Day	80	1.948	0.024	0.778	0.918
	Residuals	525	16.437	0.031		
pH	Day	20	2.000	0.100	3.536	< 0.0001
	Treatment	4	0.079	0.020	0.701	0.592
	Day	80	2.875	0.036	1.271	0.068
	Residuals	525	14.848	0.028		

Considering the analysis of tank and response variables, it was clear that the “day” factor had a significant effect on all response variables, which were found to be highly significant, especially for oxygen (F value = 2279.640, P < 0.0001; Table 2). The treatment factor, although significantly effective on oxygen concentration (F value = 22280.500, P < 0.0001; Table 2), showed significant effects on temperature, salinity, and pH.

Table 2. Day vs. All four response variables (Oxygen, Temperature, Salinity, pH).

Response	**Factor**	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**P-value**
	Treatment	4	748.200	187.050	174.261	< 0.0001
Oxygen	Tank	1	0.100	0.050	0.047	0.829
	Treatment	4	0.100	0.030	0.024	0.999
	Residuals	620	665.500	1.070		
	Treatment	4	0.110	0.027	0.099	0.983
Temperature	Tank	1	0.010	0.009	0.032	0.858
	Treatment	4	0.130	0.032	0.117	0.977
	Residuals	620	167.460	0.270		
Salinity	Treatment	4	0.178	0.044	1.391	0.236

	Tank	1	0.009	0.009	0.269	0.604
	Treatment	4	0.018	0.004	0.139	0.968
	Residuals	620	19.808	0.032		
pH	Treatment	4	0.079	0.020	0.630	0.641
	Tank	1	0.180	0.180	5.731	0.017
	Treatment	4	0.049	0.012	0.389	0.816
	Residuals	620	19.495	0.031		

When an ANOVA statistical test was performed, the results indicated that there was a significant effect of time (p-value day < 0.0001) and the presence of Prochlorococcus marinus (p-value treatment < 0.0001) on oxygen production levels. Ultimately supporting the hypothesis that when Prochlorococcus marinus is exposed to high levels of turbidity-mimicked conditions via UV light blockage, it does affect ecosystems by producing equal or similar levels of oxygen (Fig. 8).

Through the careful design of the experimental design, factors were controlled which in turn allowed the experiment to solely focus on the fact that it was the Prochlorococcus that influenced the results. The boxplots comparing the Tank vs. the three response variables (Temperature, pH, and Salinity; Fig. 1-3) further emulate this as it demonstrates that there was no overall effect of the tank on any of the four response variables. Indicating that the experimental conditions remained consistent across all tanks and providing confidence that an average of the values could be taken among the three tanks for each treatment type so that it is representative of the entire treatment (Fig. 1-3).

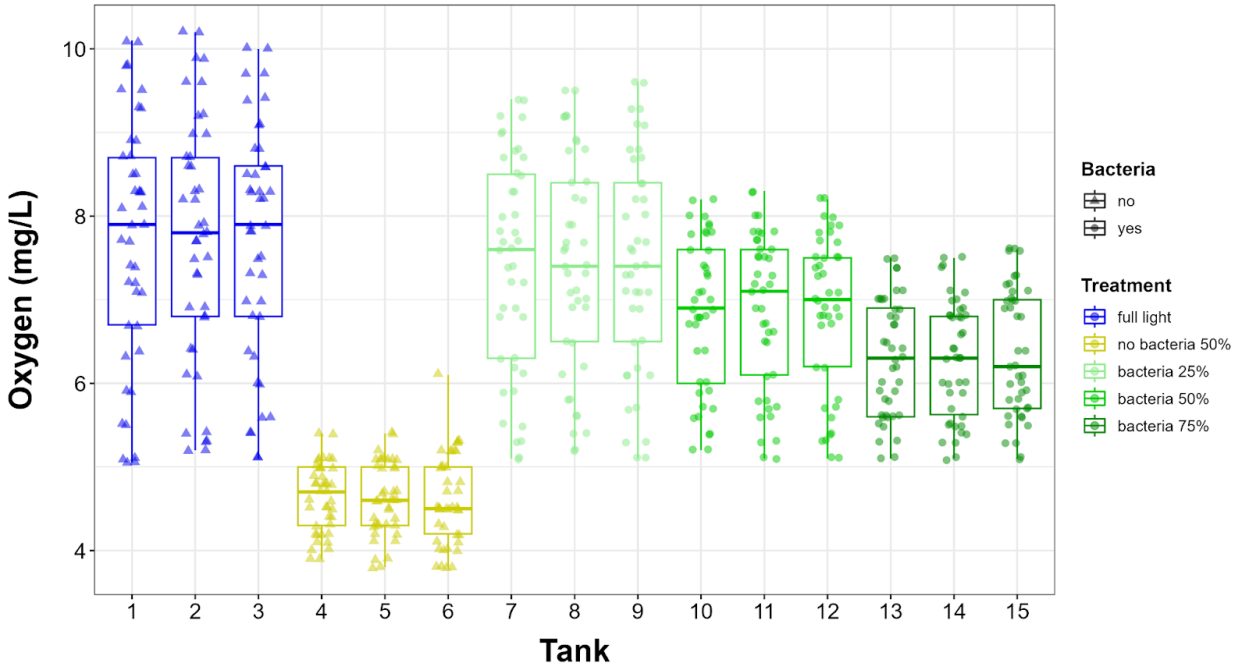

Figure 1. Boxplot of oxygen (ml/g) levels over time (days). Bacteria presence in each treatment is indicated by shape and treatment is indicated by color.

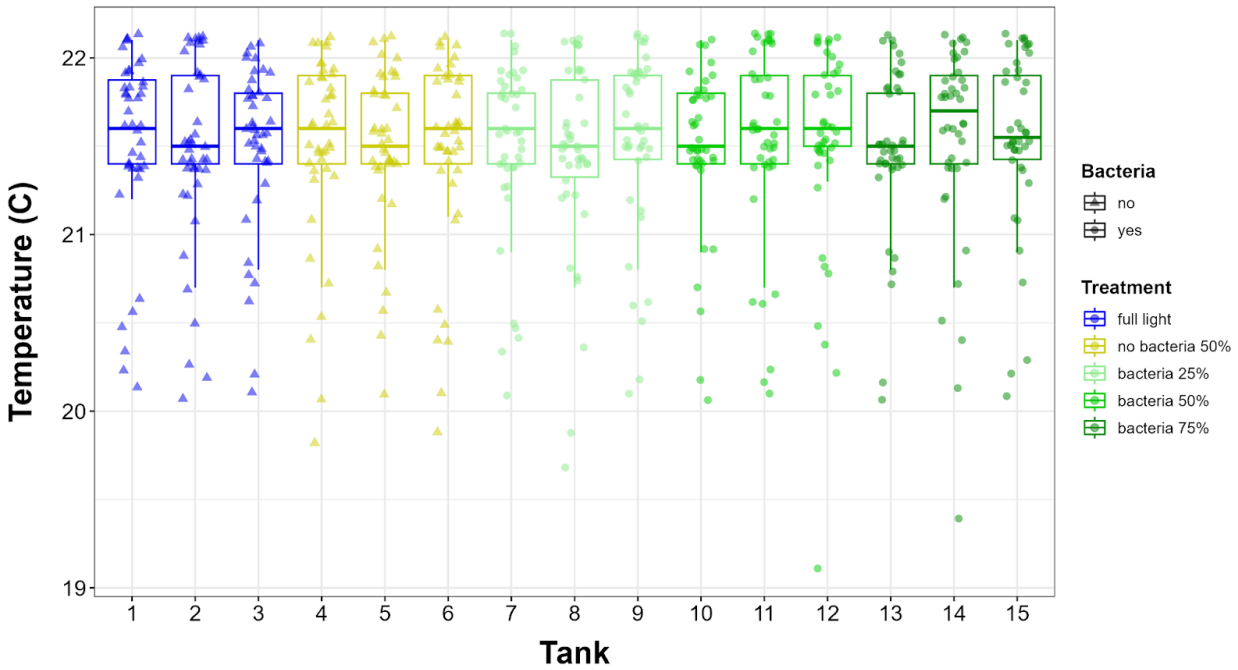

Figure 2. Boxplot of temperature (celsius degrees) levels over time (days). Bacteria presence in each treatment is indicated by shape and treatment is indicated by color.

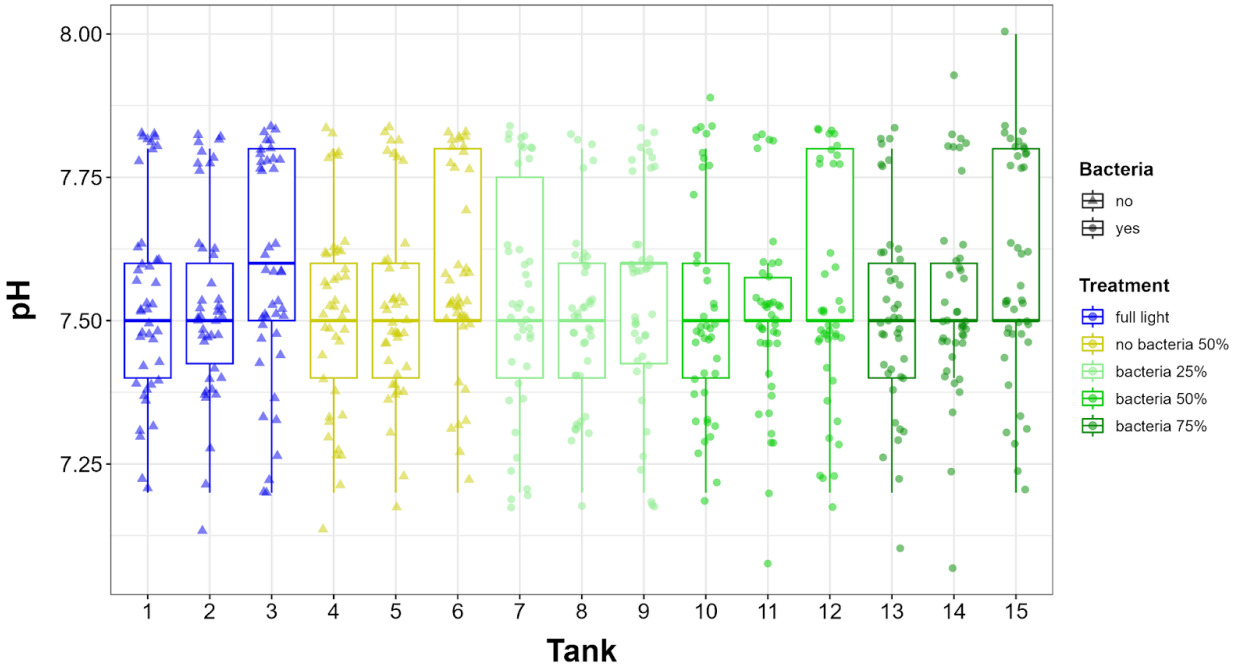

Figure 3. Boxplot of pH (pH) levels over time (days). Bacteria presence in each treatment is indicated by shape and treatment is indicated by color.

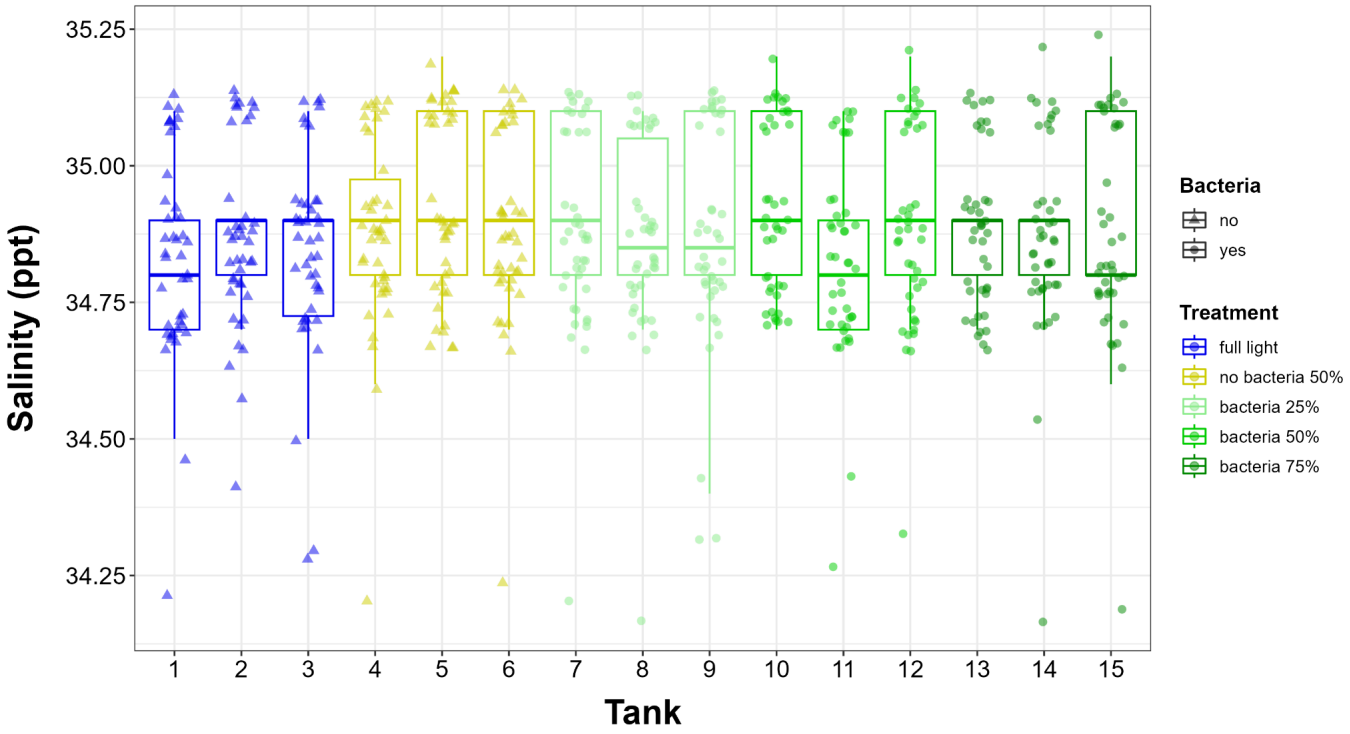

Figure 4. Boxplot of salinity (ppt) levels over time (days). Bacteria presence in each treatment is indicated by shape and treatment is indicated by color.

The line graphs comparing Day vs. Temperature, pH, and Salinity (Fig. 4-6) further provided similar reasoning to the boxplots comparing the Tank vs. the four response variables (Temperature, pH, Salinity);(Fig. 1-3). Figures five through seven demonstrated that the experimental conditions did not change over time. This implies that the experiment was constant and consistent throughout the 21-day experiment. Further strengthening the claim that the Prochlorococcus increased the oxygen (ml/g) within the (green) treatment tanks (tanks one through three and seven through fifteen; Fig. 4-6).

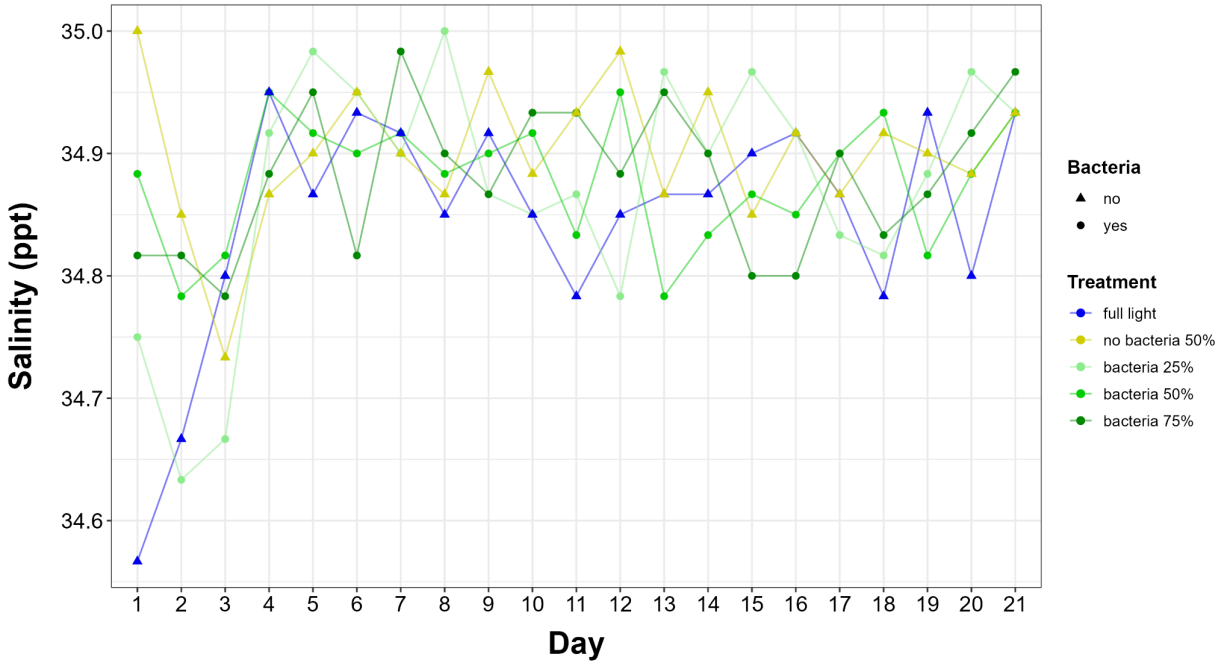

Figure 5. Linegraph of salinity (ppt) levels over time (days). Bacteria presence in each treatment is indicated by shape and treatment is indicated by color.

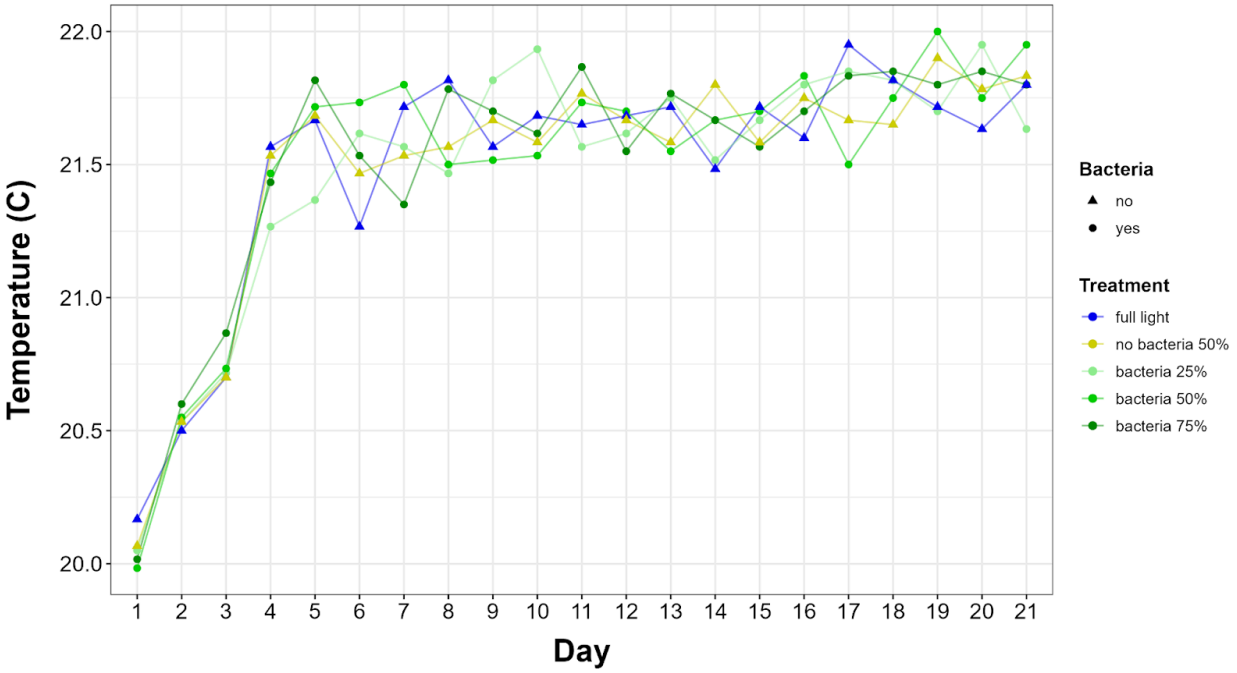

Figure 6. Linegraph of temperature (celsius degrees) levels over time (days). Bacteria presence in each treatment is indicated by shape and treatment is indicated by color.

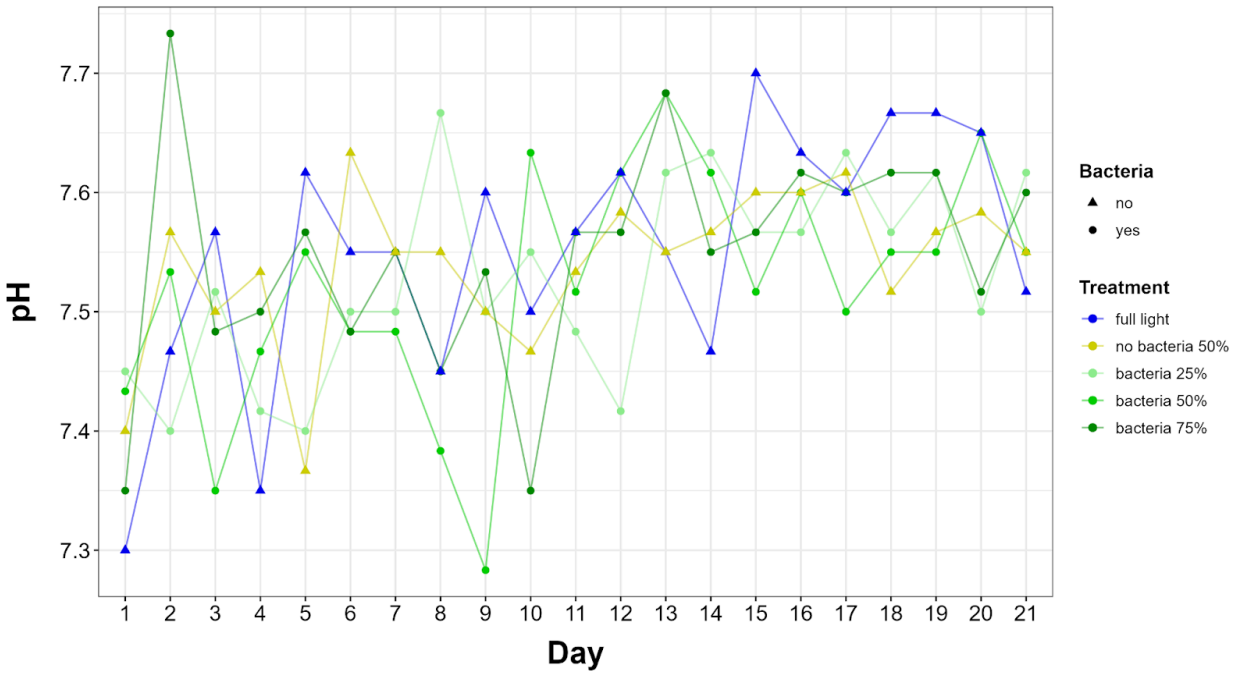

Figure 7. Linegraph of pH (pH) levels over time (days). Bacteria presence in each treatment is indicated by shape and treatment is indicated by color.

Like the above interpretations, the line graph comparing day and oxygen (Fig. 7) supported the conclusion that the addition of Prochlorococcus affected oxygen production in all

three treatments in addition to the absence of bacteria and 50% light showed a significant decrease in oxygen production. f showed a steady increase independent of time, supporting the conclusion that bacteria contribute to oxygen production that nearly satisfies full light conditions (25% in full light treatment vs. bacteria); again (Fig. 7). This is evident as early as day 6, and only increases with time (Fig. 7). The treatment box with oxygen (Fig. 6) is similar to the above interpretation, and supports the claim that bacteria included in this treatment are beneficial for oxygen production as it also indicates that affect bacterial treatment is evident regardless of time (Fig. 6).

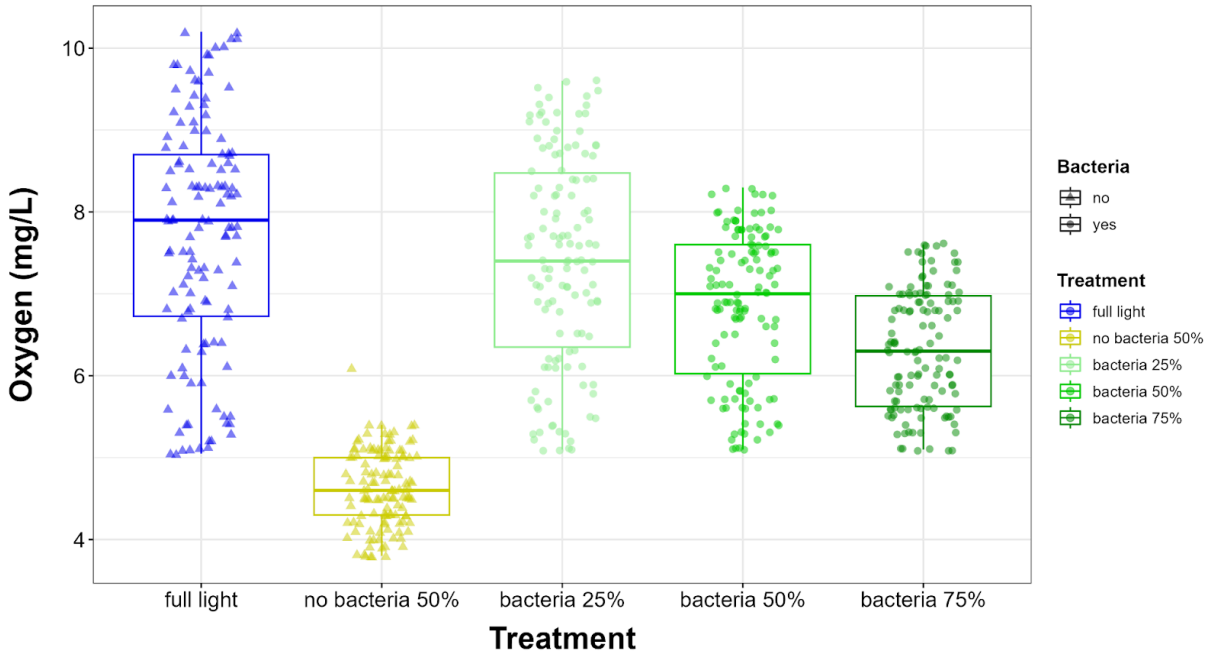

Figure 8. Boxplot of oxygen (mg/L) production over time (days). Bacteria presence in each treatment is indicated by shape and treatment is indicated by color.

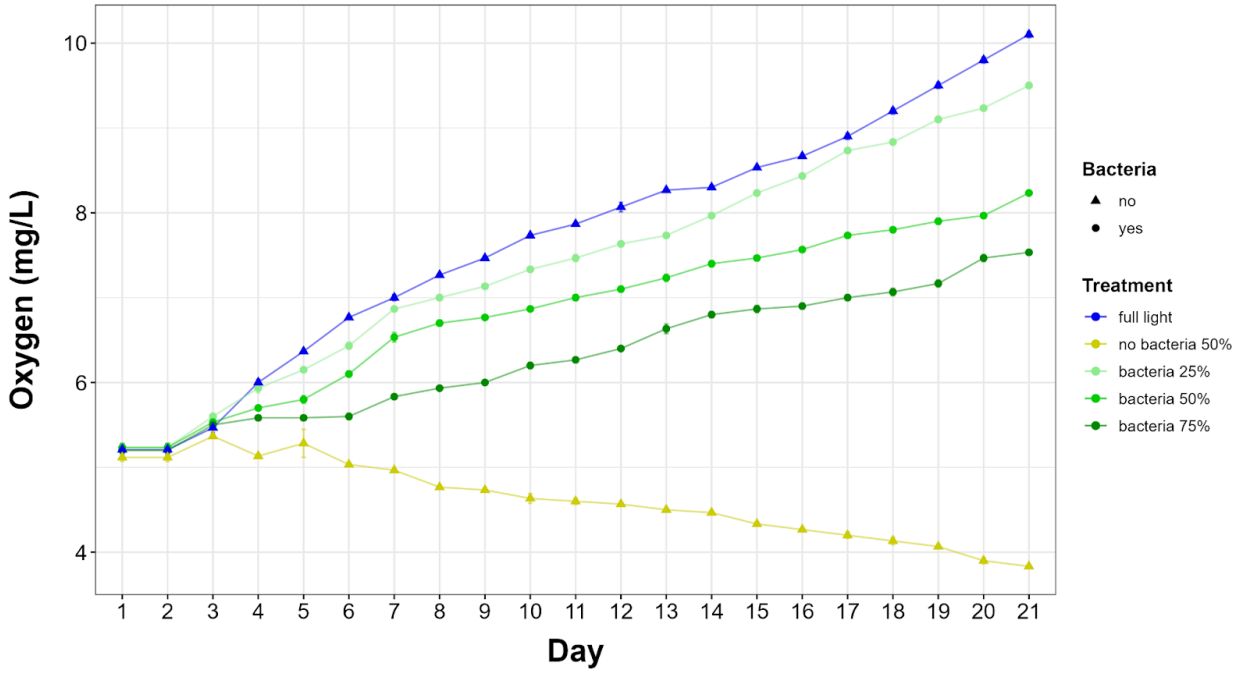

Figure 9. Linegraph of oxygen (mg/L) production over time (days). Bacteria presence in each treatment is indicated by shape and treatment is indicated by color.

Discussion

The purpose of this experiment was to determine if the Cyanobacteria Prochlorococcus marinus could be a viable solution to resuscitating aquatic ecosystems that face hypoxia due to high levels of turbidity. If found true the method of using photosynthetic organisms, particularly Prochlrococcus marinus may prove to be a viable solution for environmentalist and governmental agencies who spend large amounts of money each year on prevention and mitigation such is the case with the Australian Government which used 8.2 billion dollars by 2025 on cleaning up the Great Barrier nearly eight times the amount set up the year prior where one billion dollars was set aside for nine years from 2022-2023 to 2029-2030 (APH, 2022). Current methods against hypoxia and high levels of turbidity merely stop at the prevention and mitigation of sediment runoff from reaching these aquatic ecosystems neglecting areas that already are experiencing these detrimental conditions. However, with this study, there may be an innovation and possible solution to tackling these low-light/oxygen areas for the Great Barrier Reef. The original hypothesis stated that when Prochlorococcus marinus is exposed to high levels of turbidity-mimicked conditions via UV light blockage, it will positively affect ecosystems by producing equal or similar levels of oxygen (ml/g) to low turbidity level treatments that are not exposed to Prochlorococcus.

However, the results of the study cannot be immediately implemented as a permanent fix solution. The current experiment done was in a closed ecological system the approximation to material closure which requires methods for regenerating air, and water and producing food

(SD, 2015). As such the experiment has its limitations in only simulating an aquatic environment; it does not account for natural factors (i.e. waves and the frequent water circulation) found in a normal marine ecosystem. These limited environmental factors pose the question of the scale of application that this study may apply to a larger scale (river, lake, or ocean) whether the same results can be achieved based on scalability, and if the effectiveness of Prochlorococcus can stay the same.

Future work should focus on applying Prochlorococcus marinus to actual marine ecosystems that currently face hypoxia to see whether the same results can be achieved in comparison to this study. Regardless, a strong correlation is observed between oxygen production (mg/L) at low turbidity sites (tanks one to three) and the presence of Prochlorococcus marinus (tanks seven to fifteen) indicating that when exposing Prochlorococcus marinus to high levels of turbidity-simulated conditions. This study has begun to discuss Prochlorococcus and its potential use as an alternative for restoration against hypoxia due to high turbidity.

Acknowledgements

First and foremost I would like to acknowledge my sponsor Mr. Scott Bittner for his assistance and guidance throughout this experiment. His help was invaluable in allowing me to progress through this journey and produce something incredible. In addition, Humphrey High School made this project possible by providing me with the resources and opportunity to apply for JSHS. Finally, I extend my gratitude to my family for assisting me in gathering the materials needed for this experiment. Whether from going out to the oceans to gather the needed materials to help me build the tanks together. Without their help, I would not have been able to create such an experiment.

References

1. *Dissolved Oxygen (DO) Turbidity*, www.potomacriver.org/wp-content/uploads/2017/09/WaterWays-ChemistryInfoCardsHS.pdf. Accessed 23 Feb. 2024.
2. *EPA*, Environmental Protection Agency, cfpub.epa.gov/watertrain/moduleFrame.cfm?parent_object_id=2788. Accessed 23 Feb. 2024.
3. "How Much Oxygen Comes from the Ocean?" *NOAA's National Ocean Service*, oceanservice.noaa.gov/facts/ocean-oxygen.html. Accessed 23 Feb. 2024.
4. Macdonald, Rachael K. "Turbidity and Light Attenuation in Coastal Waters of the Great Barrier Reef." - *ResearchOnline@JCU*, 1 Jan. 1970, researchonline.jcu.edu.au/46029/.
5. "Marine Sediment." *Marine Sediment - an Overview | ScienceDirect Topics*, www.sciencedirect.com/topics/earth-and-planetary-sciences/marine-sediment. Accessed 23 Feb. 2024.
6. *Meet the Obscure Microbe That Influences Climate, Ocean Ecosystems, and Perhaps Even Evolution*, 9 Feb. 2024, ocean.si.edu/ocean-life/plankton/meet-obscure-microbe-influences-climate-ocean-ecosystems-and-perhaps-even.
7. *Nature News*, Nature Publishing Group, www.nature.com/scitable/blog/saltwater-science/runoff_how_activities_near_and/?error=cookies_not_supported&code=e58b4f82-24ed-47b8-964e-bff805500ff4. Accessed 23 Feb. 2024.
8. Partensky, F, et al. "Prochlorococcus, a Marine Photosynthetic Prokaryote of Global Significance." *Microbiology and Molecular Biology Reviews : MMBR*, U.S. National Library of Medicine, Mar. 1999, www.ncbi.nlm.nih.gov/pmc/articles/PMC98958/.
9. "Turbidity and Water Completed." *Turbidity and Water | U.S. Geological Survey*, www.usgs.gov/special-topics/water-science-school/science/turbidity-and-water. Accessed 23 Feb. 2024.
10. US Department of Commerce, National Oceanic and Atmospheric Administration. "Low or Depleted Oxygen in a Water Body Often Leads to 'dead Zones '- Regions Where Life Cannot Be Sustained." *NOAA's National Ocean Service*, 14 Mar. 2019, oceanservice.noaa.gov/hazards/hypoxia/.
11. "Which Rivers Are Polluting the Great Barrier Reef?" *The Nature Conservancy Australia*, 27 May 2019, www.natureaustralia.org.au/what-we-do/our-insights/perspectives/great-barrier-reef-pollution/.