

The Effects of Wavelength on the Growth of *Licmophora abbreviata*

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Abstract

The aim of this experiment was to investigate the effects of differing light wavelengths on the growth of *Licmophora abbreviata*. The experiment was conducted by growing 4 identical populations of the organism in separate test tubes; 3 of these populations had their test tubes covered by filters that restricted available wavelengths of light. Over a period of two and a half weeks, samples were taken from each test tube five times, and the number of cells were counted under a hemocytometer in order to track population growth. The results indicated that there was no significant difference between the growth rates under different wavelengths.

Introduction

The experiment investigates *Licmophora abbreviata*, a marine organism near the base of the salmon food chain. *Licmophora* is an autotrophic algae, and therefore its population growth is affected by the light energy it receives. Being photosynthetic, diatoms are crucial in regulating levels of dissolved oxygen and carbon dioxide (~20% of global carbon fixation is attributed to diatoms). Irregular diatom growth curves can impact an ecosystem's oxygen and carbon dioxide levels, which can be detrimental for that ecosystem's inhabitants, including salmon. (Frauke et al, 2012) One example of irregular diatom growth is algal blooms called 'red tides'. Red tides can occur when diatom growth becomes explosive, dominating the local area. This results in a major drop in oxygen and sunlight levels within the area, thus suffocating other organisms. In addition, diatoms produce a by-product called domoic acid, which can cause seizures in vertebrates if in a high enough concentration. (Shi et al, 2016) Therefore, red tides can also be accompanied by a sharp increase in domoic acid concentration, leaving local vertebrates (such as salmon) vulnerable to seizures.

Diatoms are also a key player in the salmon food chain. Juvenile salmon (and sockeye salmon of all ages) rely on zooplankton as a primary source of nutrition, and zooplankton in turn rely on diatoms for nutrition. Thus, a decrease in diatom numbers will cause a decrease in zooplankton numbers, which in turn may lead to a drop in salmon numbers. Understanding the factors that influence diatom growth is important, as regulation of their growth is not only

essential for the health of a local ecosystem, but also for the health of coexisting salmon. This is why the experiment aims to test the effect of different light wavelengths on *Licmophora abbreviata* population growth.

Previous research showed that *Licmophora abbreviata* best absorbs energy from sunlight using the pigments chlorophyll and fucoxanthin (Yocum & Blinks, 1950). The former is a green pigment that absorbs blue and red light, and the latter is a brown pigment that absorbs blue and green light. Further research suggests that *Licmophora abbreviata* is dominated by these two pigments (fucoxanthin in particular), indicating that it will grow best under blue light, which both pigments absorb (Guiry, 2017). For this reason, it is hypothesized that *Licmophora abbreviata* will grow best under blue light and worst under red light, with growth under green light falling somewhere between the two.

Methods

Firstly, a stock solution containing *Licmophora abbreviata* specimens was obtained. This solution was then diluted to a concentration of 1×10^4 cells per mL, vortexed, and separated into twelve samples of equal volume contained in capped test tubes.

These tubes were separated into four groups of three replicates each. In three of these groups, each test tube was wrapped in acetate paper, with the wavelength differing between each group: 680nm (red acetate paper), 520nm (green acetate paper), and 410nm (blue acetate paper). The fourth group was left unwrapped as a control.

Light intensities through each of the three kinds of acetate paper were measured. This was accomplished by using a phone app which measures Lux levels by utilizing the phone camera. First, the level of light within the laboratory was measured. Second, each acetate paper was placed over the camera of the phone and the new Lux was measured. In this way, the percent change in light intensity could be calculated for each colour of acetate paper.

All test tubes were then placed on a rack in an incubator and left to grow under light. Five times over the next two and a half weeks, the tubes were quickly removed, so that 100 μ L samples could be extracted from each and placed in labeled Eppendorf tubes along with 5 μ L of fixative (iodine potassium iodide). These samples were then refrigerated until their cell density could be counted using a hemocytometer. Once multiple cell density values were measured, they were compared to calculate changes in cell density and overall cell population for each replicate.

Results

Given the low initial rates of growth, and the observed contamination in later stages, we decided to narrow our focus down to the interval between November 3rd and November 7th, during which growth appeared fairly steady for all treatments. We found the average growth rates for each replicate within this period, then took the means for each treatment to find the average growth rates of the treatments (Figure 1). A one-way ANOVA test for these growth rates yielded a p-value of 0.339964. This demonstrates that our results are not statistically significant.

On days three, six, and seven the control group had an average cell density of 6.4×10^4 , 2.69×10^5 , and 3.77×10^5 cells/mL respectively; the red-light group had an average cell density of 2.89×10^4 , 1.28×10^5 , and 1.71×10^5 cells/mL respectively; the green-light group had an average cell density of 2.34×10^4 , 9.97×10^4 , and 1.39×10^5 cells/mL respectively; and the blue-light group had an average cell density of 4.60×10^4 , 1.29×10^5 , and 3.11×10^5 cells/mL respectively. The accompanying trendline for these values is represented in Fig. 2.

In terms of average overall growth from day three to day seven, the control group showed a 590% growth, the red-light group showed a 592% growth, the green-light group showed a 594% growth, and the blue-light group showed a 676% growth.

Discussion

Based on the gathered data, we cannot reject our null hypothesis. Even within the 'best' period of growth displayed by the *Licmophora* populations, the standard errors were large enough that any perceived differences between the wavelength treatments could have been due to error. The only exception is the control treatment, which grew demonstrably faster than the other. This was likely due to it being unaffected by the decreases in light intensity caused by the acetate paper used for other treatments.

We hypothesized that blue light would facilitate growth the most and red light the least. Our blue light populations did seem to grow the best on average, but surprisingly the populations grown under red light performed better than those grown under green light. In our hypothesis we predicted that *Licmophora* exposed to green and blue light would have the most growth, due to diatoms being photosynthetic and using pigments such as chlorophyll and fucoxanthin, with fucoxanthin (which absorbs blue and green light) being dominant. Since chlorophyll can utilize red light, but fucoxanthin cannot, the surprisingly large growth we observed under red light may be due to a greater relative concentration of chlorophyll in our diatoms than expected (Guiry, 2017). Previous studies have also shown that most species of diatoms are able to sense and adapt to blue and/or green light, however, some species of diatoms contain a red light sensing phytochrome (Fortunato et al., 2016). It's possible that the diatoms placed in red light were able to adapt by producing more chlorophyll than fucoxanthin, which led to them being more successful than expected under these conditions. That said, even if these factors did have an impact, it was not enough to make the differences in growth significant.

Two of the populations grown under restricted light conditions exceeded the total final population of the control group. This may be due to adaptation similar to that mentioned above,

but is more likely an error; data collected over time shows the control group was generally more successful than all the color-specific groups, and only fell behind during our later data collection days. Given that we had issues with contamination later on, it seems likely that this, or some other error, affected the control group after November 7th, causing a population decrease that deviated from its previously steady growth curve. Before this, the control group showed greater overall population growth than those restricted to specific colors.

There are two main sources of error within our research that should be addressed if this experiment is to be replicated. As stated earlier, the first source of error was a contamination in the *L. abbreviata* samples. This led to significant portions of data needing to be omitted, thus leaving us with less data to analyze and base our conclusions off. With less data to analyze, the possible error in a conclusion becomes larger. To best avoid this in future experiments, sterilization protocols should be adhered to stringently. The second source of error manifests itself in the acetate paper. Acetate paper significantly decreases the intensity of the light passing through, and this decrease in light intensity varies depending on what colour of acetate paper is being used. This means that in addition to *L. abbreviata* samples being exposed to different wavelengths of light, they were also being exposed to different intensities of light. In order to avoid this in future studies, after each acetate paper's decrease in light intensity should be calculated each *L. abbreviata* sample should be incubated at some distance from the light source that would offset the respective acetate paper's decrease in light intensity.

Conclusion

Our hypothesis stated that we expected *L. abbreviata* to grow best under blue light, and worst under red light, with growth under green light lying somewhere between the two. However, results demonstrated that the growth of *Licmophora abbreviata* does not seem to be greatly affected by which wavelengths of light it receives.

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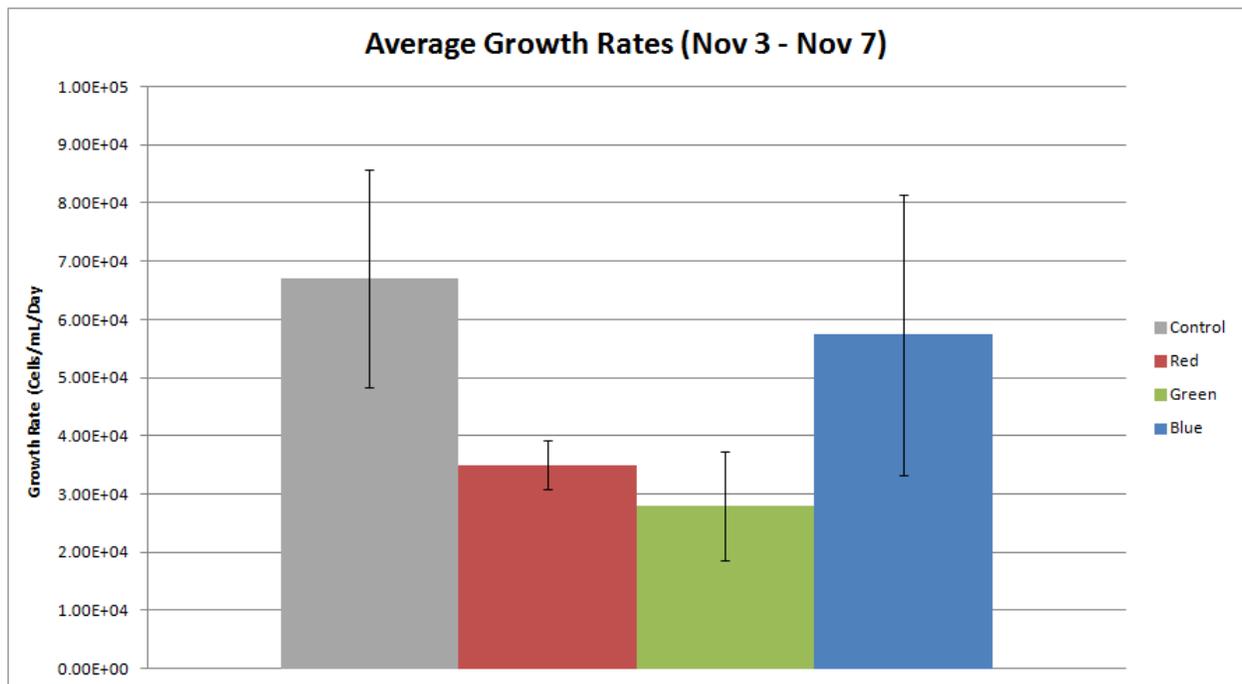
Figures

Figure 1. Average cell density growth over time for *Licmophora* exposed to the three treatments, from November 3 to November 7 2017.

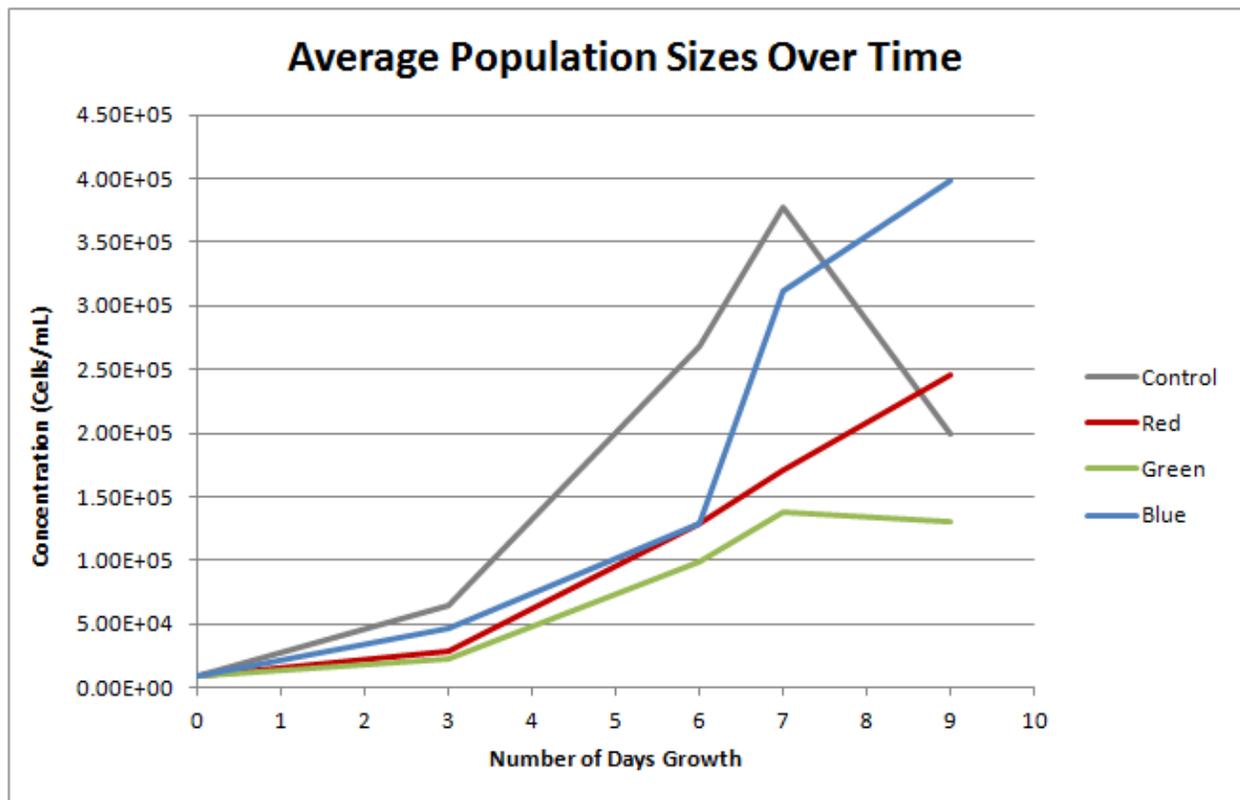


Figure 2. Average cell density growth over time for *Lichophora* exposed to white light (control), blue light, red light, and green light.

Bibliography

Guiry, M.D. (n.d.) *Phaeophyceae: Brown Algae*. Retrieved from <http://www.seaweed.ie/algae/phaeophyta.php>

Fortunato, A.E., Jaubert, M., Enomoto, G., Bouly, J.P., Raniello, R., Thaler, M., ... Falciatore, A. (2016). Diatom Phytochromes Reveal the Existence of Far-Red-Light-Based Sensing in the Ocean. *Plant Cell*, 28(3), 616-628. Retrieved from <http://www.plantcell.org.ezproxy.library.ubc.ca/content/28/3/616.full>

Frauke, A.D., Rogato, A., d'Alcala, M.R., Falciatore, A. (2012). Exploring the Molecular Basis of Responses to Light in Marine Diatoms. *Journal of Experimental Botany*, 63(4), 1575-1591. Retrieved from <https://academic.oup.com/jxb/article/63/4/1575/449618>

Yocum, C.S., & Blinks, L.R. (1954). Photosynthetic Efficiency of Marine Plants. *Journal of General Physiology*, 38(1), 1-16. Retrieved from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2147479/>

Wilhelm, C., Jungandreas, A., Jakob, T., & Goss, R. (2014). Light Acclimation in Diatoms: From Phenomenology to Mechanisms. *Marine Genomics*, 16, 5-15. Retrieved from http://www.sciencedirect.com/science/article/pii/S1874778713000810?_rdoc=1&fmt=high&origin=gateway&docanchor=&md5=b8429449ccfc9c30159a5f9aeaa92ffb

Shi, P., Shen, H., Wang, W., Yang, Q., & Xie, P. (2016). Habitat-Specific Differences in Adaptation to Light in Freshwater Diatoms. *Journal of Applied Phycology*, 28(1), 227-239. Retrieved from <https://search-proquest-com.ezproxy.library.ubc.ca/docview/1761626623?accountid=14656>