Effect of Light Exposure on the Growth Rate of Euglena gracilis

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Abstract

This study aims to investigate the effect of different lengths of light exposure on the growth of Euglena gracilis. E. gracilis is an important part of the ecosystem as they are essential in providing nutrients for the salmon in Salish Creek. Longer lengths of daily light exposure were predicted to increase the growth rate of E. gracilis. To test this prediction, E. gracilis was exposed to three treatments with various lengths of light exposure (no light cycle, 8-hour light cycle, full light cycle). Cell count of each treatment sample was recorded over a ten-day period. The ANOVA test results showed a relationship between daily length of light exposure and the growth rate of E. gracilis. As a result, we reject the null hypothesis. Thus, longer light exposure would result in increased growth of E. gracilis. The major source of error in our experiment stems from the usage of different microscopes and micrometers in the process of cell-counting and not leaving a few days in the initial period for the lag phase of the cells. Additional uncertainties and limitations such as inconsistent incubator light intensities may have also affected the growth of E. gracilis.

Introduction

Euglena gracilis is a single-celled alga that resides in freshwater and has the capability to survive in very extreme environments such as acidic streams and heavy metal polluted rivers (Rodríguez-Zavala et al., 2010). It has been found that E. gracilis is a unique and valuable microalgal species because it intracellularly holds up to 59 different bioactive ingredients such as vitamins, minerals, amino acids, and unsaturated fatty acids that can be used by humans (Kim et al., 2020). In addition, E. gracilis has a substantial role in converting carbon dioxide gas to oxygen, improving water quality, and acting as a feed for fish in bioregenerative ecosystems (Kitaya et al., 2005). Benefitting species such as salmon rely on microalga, like E.gracilis, to maintain a healthy environment as they provide oxygen and nutrients to the ecosystem. Since E. gracilis is such a valuable microalga in the ecosystem, it is important to consider the different

environmental factors that would affect its growth rate. One factor that influences the growth of the E. gracilis is the duration of light exposure that they receive.

In a previous study, it was found that the autotrophic growth of E. gracilis was initially exponential for approximately 2-4 days and became linear as the days of light exposure increased (Wang et al., 2018). After closely analyzing the linear growth rate, it was also determined that the growth rate of the species decreased in lower light and increased with greater light (Wang et al., 2018). Another past study focused on the varying light exposures, specifically comparing the no light to light, in a wild type gracilis (Shao et al., 2019). This study's results suggest that the wild-type E. gracilis had a higher growth rate under light throughout the duration of the experiment, whereas the growth was much slower in the dark (Shao et al., 2019).

This experiment investigated if light exposure is correlated with the growth of E. gracilis by setting up three treatments with varying light exposure over a period of 10 days. Through research and the understanding of previous studies of the effect of light on E. gracilis, it was predicted that an increase in light exposure would promote growth rate. This study was conducted due to the lack of research in the area of light exposure and its relation to the growth of E. gracilis.

Methods

The general procedures of the experimental methods are shown in Figure 1. Initial preparation of the sample solution was prepared from E. gracilis wild-type stock solution and growth medium solution. The initial concentration of the wild-type solution was 2.58×10^5 cells/mL, which was determined by the average of three cell counts of the solution. 41.86 mL of

growth medium solution was added to dilute 58.14 mL of wild-type solution to produce 100 mL of sample solution with the concentration of 1.50 x 10^5 cells/mL. 10 mL of sample solution was added to 9 test tubes each. Three test tubes of sample solution were placed in each treatment for a total of three replicates per treatment.

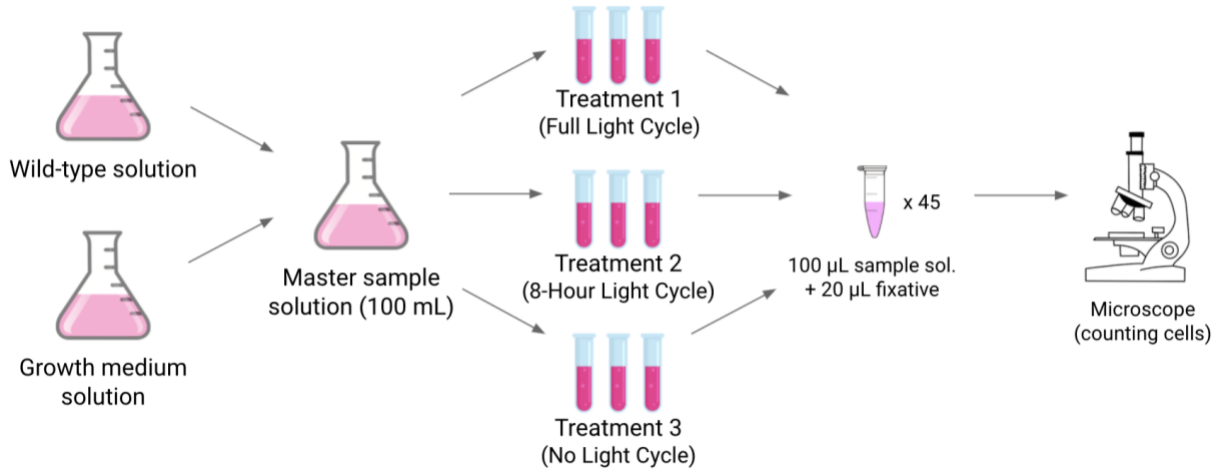

Figure 1. General overview of the experimental methods.

For the experiment, three different treatments were used to investigate the effects of light exposure on the growth rate of E. gracilis. An incubator set at 25˚C with the light present for 24 hours per day at the average light intensity of 8697 lux was used for the full light cycle for treatment 1. For treatment 2, the 8-hour light cycle, the incubator used was set at 25˚C with light present for 8 hours per day at the average light intensity of 1723 lux and no light present for the remaining 16 hours per day. For treatment 3, the no light cycle, the incubator was set at 25˚C with no light present for 24 hours per day with the light intensity of 0 lux.

Sample collections were done every two to three days during the experimental phase, specifically on day 0, 3, 5, 7, and 10. 100 µL of sample solution was taken from a test tube and added to a Eppendorf tube along with 20 µL of fixative and mixed by micropipetting the fluid contents up and down. This process was repeated for each of the replicates in each of the

treatments for a total of nine samples per sample collection and an overall total of 45 samples collected over the course of the experiment. Samples were stored in a refrigerator set at 4˚C prior to counting the cells to determine cell concentration.

Cell count was done on day 10 after the last sample collection. A Zeiss microscope with 10x objective lens and haemocytometer were used to perform the cell counts. This was done by placing a coverslip over the haemocytometer and adding approximately 20 µL of sample from an Eppendorf tube in between the coverslip and haemocytometer. The sample was resuspended by micropipetting up and down prior to being placed on the haemocytometer. The haemocytometer was then observed under the microscope in order to count the cells and determine cell concentration. Cell count was calculated using the following formula: [approximate 150 cell count] / [number of 01 x 0.25 mm gridded squares in hemocytometer] x [8 x 10^4]. This was repeated for each of the 45 samples collected over the course of the experiment.

Google Sheets was used to construct the graph of the growth curves of E. gracilis in each of the treatments. For the statistical analysis of the data, a one-way ANOVA test at the significance level of 0.05 was used to determine if there is a significant difference between the means of growth rate of the treatments.

Results

Experiment results are presented in Figure 2 and Figure 3. On day 3, cell counts of all three groups are at a similar level but slightly decreased from day 0. On day 5 sample, the trend of decreasing cell count continues, the full light cycle group takes the largest dip from day 3 and has the lowest cell count. 8-hour light cycle group cell count decreased more than no light

group. Day 7 data shows all groups in a growth trend with full light treatment having the highest cell count followed by the no light treatment and 8-hour light treatment groups. On day 10, the growth trend of all three groups continues. Cell count of 8-hour light and full light groups increased substantially more than the no light group. Interestingly, the 8-hour group surpasses the full light treatment group with the highest cell count among the three groups at 3.25E+05 cells/ mL. Linear growth rates of all three groups were derived from the cell count data across the 10 days period. A one-way ANOVA test on growth rates at the significance level of 0.05 was performed. P-value was found to be 0.005595, which is less than the significance level. The null hypothesis is rejected. To further understand the impacts of duration of light exposure on growth rate, Tukey's HSD with an alpha value of 0.05 was performed. The P value between the no light group and 8-hour light cycle group is 0.01177. The P value between the no light group and 24-hour light cycle group is 0.0074. There is no significant difference in growth rate by increasing the light exposure length from the 8-hour light cycle to the 24-hour light cycle. The experimental data shows that light exposure length positively impacts the growth of Euglena gracilis.

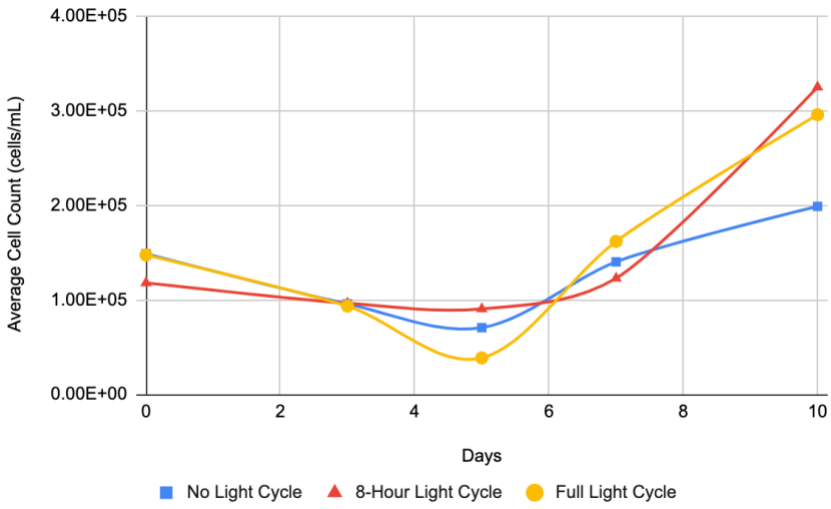

Figure 2. Graph summarizes the relationship between the three different trials for light. The no-light cycle is represented by squares, the 8-hour light cycle is represented by triangles and the full-light is represented by the circles. Data was graphed based on the average amount of cells counted of all 3 replicates for each day.

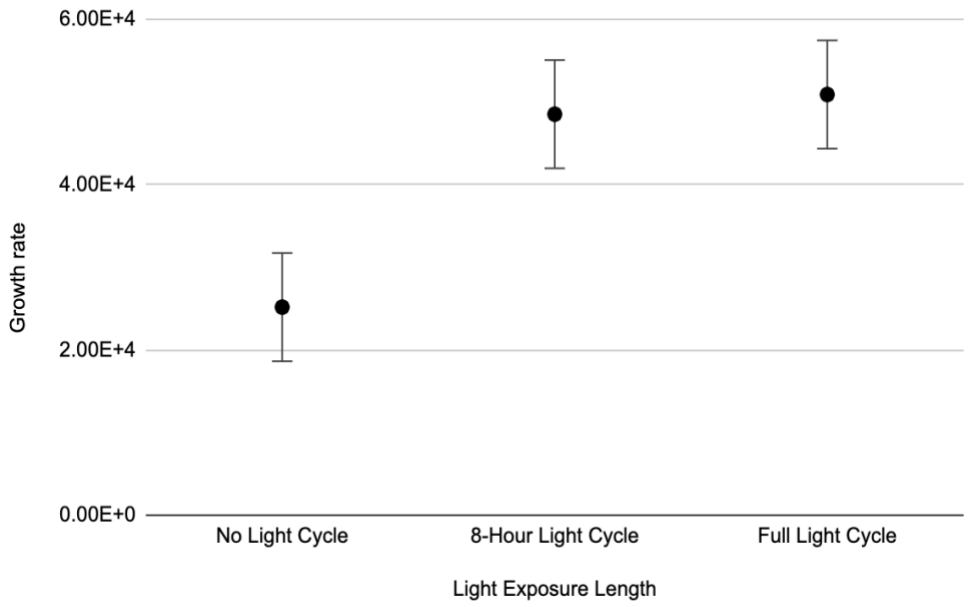

Figure 3. Effects of light exposure length on growth rate

Discussion

The goal of this experiment was to determine if the length of exposure to light would affect the growth of E. gracilis. An ANOVA test was performed on the three trials of data and the P-value was found to be 0.005595, thus rejecting the null hypothesis. The data did support our initial hypothesis that light exposure length would affect the growth of E. gracilis. A plausible explanation of this can be inferred through Figure 2. Initially, the cell count of E. gracilis was around 1.5×10^5 on day one, dropping slightly on days three through five, and rising again after day five until day 10. This could potentially show a very long lag phase for the cells to get used to their environments (up until day four/five), before the exponential phase when the growth of Euglena Gracilis is evident in the figure. A previous study by Georges Freyssinet

from the Laboratoire Associé au Centre National de la Recherche Scientifique (Laboratory of the National Center for Scientific Research), found that Euglena generally has a lag period of around 3-4 hours when a dark-sample of Euglena is exposed to light (Freyssinet 1976). In our experiment, we did not consider the lag period and proceeded with cell counting which may have slightly affected the results. A study by scientists from the University of Almeria discovered that light intensities over 1630 μE m−2 s−1, a photoinhibition effect was observed on the growth of microalgae (Grima et al., 1995). The light intensities in our experiment were tested and found to be 1723 lux for the 8-hour incubator and 8697 lux for the higher lux incubator. This may have surpassed the optimal light intensity for the growth of Euglena and suppressed the photosynthesis capabilities resulting in inaccuracies.

The results in this experiment may be less reliable as the data was collected by anecdotal means. The main factor of uncertainty arises from the usage of four different microscopes to count the cells. Each microscope was set to match the given grid used for cell counting and may have been set up differently resulting in inaccuracies. The microscopes were also used by different people when counting which may induce an additional problem when judging the cells. When counting cells, cells that are in very close proximity to each other may have been counted as single cells under some microscopes whereas they could have been clearly seen as two distinct cells under different microscopes. When the samples were being incubated, there were many other groups using the same incubators for their experiments. When the other groups take their sample outside of the incubator, the E. gracilis in the incubator would be exposed to a different intensity of light for a period of time. This introduces uncertainty as the no-light sample could potentially be exposed to light for a length of time, or

the 8-hour light sample could be exposed to light during its no-light cycle. The samples for E. gracilis were also taken out of the incubator at different times of the day for sampling. If the E. gracilis were taken out early in the morning, rather than late afternoon, the trials taken in the afternoon may accumulate more growth than expected as they are in exposure to light for longer, or the opposite for the no-light trials.

As Salish Creek is known to house cutthroat trout and Coho salmon, light exposure to E. gracilis and other algae in the water is crucial in providing nutrients for the fish. E. gracilis is an important primary producer that the salmon feed on and the amount of light would affect the amount of E. gracilis present. Low amounts of light in the Creek, or high intensities of light would both reduce the amount of nutrients available for the salmon to intake. Thus, it is important for a sufficient amount of light for the E. gracilis and other marine algae to grow.

Some limitations in this experiment include incubators preset at 8-hour cycles for the half-light experiment rather than the 12-hour exposure that was desired. The light intensities for each trial were also very different and unchangeable. The light intensity for the full-light incubator was 8697 lux which is very high when compared with the light intensity of the 8-hour incubator which was measured to be 1723.

For future experiments, giving E. gracilis a few days prior to testing would eliminate the inaccuracy from the lag period and using the same light-intensity for the different incubators would reduce the number of changing variables. Due to the limitations of time, different microscopes and four different people counted the number of cells which may not be as consistent compared with a single person performing all the counting.

Conclusion

The length of light exposure is important for the growth of marine algae. In this experiment, the length of light exposure of 8-hours, 24-hours, and no light were tested versus the growth amount of E. gracilis. The experiment did support our initial hypothesis that light exposure length would affect the growth of E. gracilis. Further investigation can be done to get a better understanding of the effect of light exposure on *E. gracilis*.

Acknowledgements

We would like to thank the University of British Columbia for the opportunity to conduct this experiment through the BIOL 342 course. Specifically, we would like to express our gratitude to Professor Celeste Leander for providing us with the opportunity, knowledge, and skills to perform this experiment, Teaching Assistants Melanie Warren and Tessa Blanchard for providing us support and helping monitor our experiments, and Lab Technician Mindy Chow for providing the supplies for this experiment.

This study was researched and written at the University of British Columbia, Point Grey Campus (Vancouver), which is situated on the traditional, ancestral, and unceded territory of the xwməθkwəy̓əm (Musqueam) People.

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