Effects of Altered Light Cycles on the Growth Rate of *E.gracilis*

Robert Thompson, Jaime Deng, Andy Li - BIOL 342

University of British Columbia

December 8th, 2024

Abstract

This study was intended to investigate the effect of varying light/dark cycles on the growth and reproduction efficiency of the *Euglena gracilis* algae; based on the hypothesis that if *E. gracilis* require different survival strategies to grow their population in light compared to in darkness and it costs them time and energy to switch between these survival strategies, they will reproduce more efficiently when exposed to longer periods of light and darkness. To test this hypothesis, *E. gracilis* cultures would be exposed to three treatments: a 6-hour light cycle (6 hours of light followed by 6 hours of darkness), a 12-hour light cycle, and a 24-hour light cycle. Additionally, there would be a control group with no light exposure. However, due to unforeseen circumstances, the experiment could not be carried out as planned—alternative treatment cycles consisted of: 6 hours light with 18 hours darkness, 12 hours light with 12 hours darkness, and 24 hours light with 0 hours darkness (the control group remained unaffected by this change). During the growth of the samples, haemocytometer measurements were taken to calculate the population density at each point in time. It was found that the cultures subjected to the 12-hour light cycle exhibited the highest growth rates compared to the other groups. These findings neither support nor reject the original hypothesis due to the differences between the proposed experiment and the one that was actually carried out.

Introduction

Euglena is a genus of flagellated algae that can be commonly seen in ponds and other small bodies of water [1]. Species of *Euglena* can be found in either freshwater or saltwater, and are often abundant in quiet inland waters where they may bloom in numbers sufficient to turn the surface of the water green or red. [2] In this study, *Euglena gracilis* was chosen to work with; it is noteworthy among algae due to the fact that it can function as either an autotroph or a heterotroph depending on environmental conditions and nutrient availability.

In the wild, *E. gracilis* are likely to experience a 12-hour light/dark cycle; this is also the light cycle most commonly employed to grow *E. gracilis* in controlled environments. While it is well-established that photosynthesis in algae occurs most efficiently under certain light intensities, the effect of extended light/dark cycles has not been explored extensively.

We hypothesized that *E. gracilis* may have a more efficient growth rate when exposed to longer light and dark periods. The rationale behind this hypothesis was based on the idea that each transition between light and dark conditions would require *E. gracilis* to spend time and energy acclimating to the new conditions, potentially limiting growth efficiency. Existing literature suggests that *E. gracilis* is most photosynthetically active during shorter intervals, but the reason for this is unclear. This study therefore aimed to determine whether *E. gracilis* can thrive in longer cycles—if extended cycles reduce the time spent in acclimation between light and dark conditions, increasing the growth efficiency for the algae.

Methods

It should foremost be noted that the following procedure partially emerged as a result of technical difficulties that occurred late into the project's timeline. It is notably different from the *intended design of the experiment, rendering it incompatible with our original hypothesis. For the sake of clarity, the original procedure has been omitted and replaced by the one which was carried out instead.*

12 cultures of *E. gracilis* were prepared in test tubes at a concentration of 1×10^5 cell/mL and a volume of 20 mL. The test tubes were incubated at 30 degrees Celsius and subjected to different light cycles throughout their growth.

Each of the 3 experimental groups had a different amount of light exposure per 24-hour period of time. This means that the 6-hour group had 6 hours of light each day, while the 12-hour group had 12 hours of light each day. The 24-hour group was under constant light exposure—having 24 hours of light each day. Additionally, there was a control group that was kept in constant darkness. Each group consisted of 3 samples to account for biological variability and increase the sample size of the experiment.

100µL samples were taken from each group on Monday, Wednesday, and Friday for a period of two weeks. Sterile technique was used and the samples were fixed with 10µL IKI. Then, each sample was refrigerated at a temperature of 4 degrees Celsius for storage. After the experiment concluded, the population densities of *E. gracilis* cells in the samples were counted—this information was used to calculate and compare the growth rates for each population at each point in time.

For this experiment, the environmental conditions were controlled. Factors such as temperature, substrate, and nutrient levels were kept as consistent as possible across the testing groups to ensure that any differences in growth are primarily due to differences in light exposure.

Results

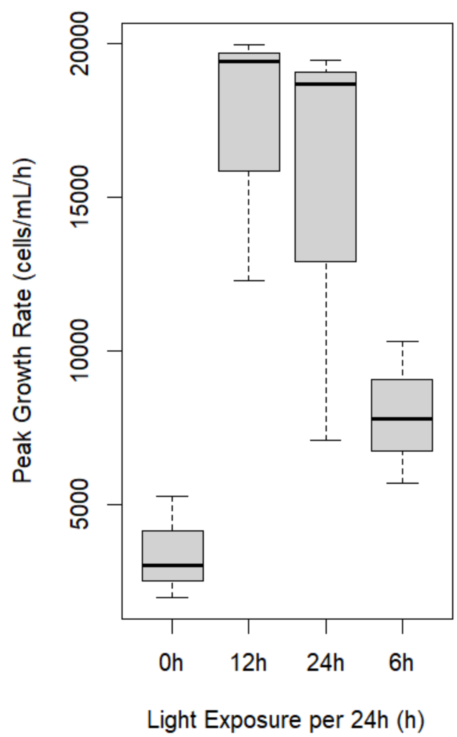

Figure 1—Graph showing the peak growth rates of *E. gracilis* during their exponential phases under different light cycles

Metric	**T1** (Day 0)	**T2** (Day 2)	**T3** (Day 4)	**T4** (Day 7)	**T5** (Day 9)	**T6** (Day 11)
Count	12	12	0	12	12	12
Mean	442521	902917	X	946000	1050271	818431
Std Dev	197254	470359	X	359475	382848	405722
Variance	3.89E+10	2.21E+11	X	1.29E+11	1.47E+11	1.65E+11
Min	185167	305250	X	319000	558250	140250
Max	962500	1463000	X	1336500	1622500	1246667

Figure 2—Descriptive statistics of cell densities for all treatment groups at five time points. All values are measured in cell/mL

Note: Although 6 days of samples were taken, the samples from the fourth day (T3) were lost prior to counting. Therefore, measurements taken at T3 are not included in the results.

The growth rate measurements indicate that the growth of *E. gracilis* was considerably influenced by the different treatments. The 12-hour group showed the fastest growth, followed closely by the 24-hour group. In addition, the 24-hour group showed a significant degree of variance across samples. The control and 6-hour groups showed the slowest growth as well as the least variance out of all groups.

Across all groups, the most growth was observed within the first two days of the experiment. Additionally, nearly every population experienced a slight decline in population at or near the end of the two-week period in which measurements were taken. Only one population, belonging to the 6-hour group, continued to grow steadily for the full duration of the experiment.

Discussion

The observed growth of *E. gracilis* under different light/dark cycles indicates that light exposure does indeed play a critical role in their growth and development. Observations of the graph trends reveal that the 12-hour cycle resulted in the highest growth. The 24-hour group also experienced rapid growth in some cases, but their growth was inconsistent compared to the 12-hour group. The 6-hour group exhibited significantly slower growth than the 12-hour and 24-hour groups, and the control group showed the least growth, confirming the importance of light for the growth of *E. gracilis*.

Analysis of Variance (ANOVA) testing revealed this difference to be statistically significant with a p-value of 0.015. These findings, while not inherently fallacious, remain irrelevant with respect to the original hypothesis due to the unequal amounts of light exposure across groups. To properly test this hypothesis, groups would need to have been exposed to the same amount of light, with the critical variable being the duration of light and dark periods if and only if those two periods are equal in length to each other.

Descriptive statistics highlighted other relevant traits—while the 12-hour cycle group had the highest mean cell densities for the first two measurement periods, it was surpassed by the 24-hour group after 4 days. Variance between groups increased in the first 2 days as the cells entered their exponential growth phase and decreased after 4 days when most groups had reached the stationary phase.

The fact that most experimental groups experienced a decline in population at or near the end of the experiment suggests the existence of a point where factors other than light availability, such as nutrient depletion or metabolic stress, may limit further growth. The two colonies in the 6-hour group which did not experience this decline ultimately reached a cell density almost double that of the colony which did decline.

Although the trends observed in the data are clear, we identified sources of uncertainty and variation that could have influenced the results. The primary cause for concern is the fact that the groups were observed to have different cell densities on day 0 even before they started growing. It is unclear why this occurred, as all groups were prepared from the same culture medium, which was recorded as having a concentration of 1 x 10^5 cells/ml. It seems improbable that errors in counting could singlehandedly produce as much variance as was observed. A separate source of uncertainty arose from the fact that it was impossible to sample groups while keeping them in darkness. Disruption of the light/dark cycles could have caused fluctuations in growth rates, but we believe such effects would be minor due to the short duration of the disturbances. Future studies could reduce uncertainty by increasing the sample size and/or ensuring even more careful control over starting conditions.

Conclusion

While our findings cannot be applied to the primary hypothesis and prediction, they broadly align with contemporary understanding of photosynthetic organisms—that they will experience quicker growth when exposed to more light. The 12-hour group exhibited the highest growth rate, followed by the 24-hour group, while the 6-hour group and control group showed significantly lower growth rates. In the context of this specific species, *E. gracilis*, this may indicate that it fares better as an autotroph than as a heterotroph. Overall, these results demonstrate the importance of light exposure for algal growth and provide a broad insight into optimizing growth conditions for *E. gracilis* and other algae in laboratory environments. The topic of why the 12-hour group sometimes outperformed the 24-hour group and the issue of varying cell densities upon initial measurement put forth prime avenues for future investigation.

Acknowledgements

We would like to express our gratitude to Celeste Leander, our professor, for her support throughout this project. Her knowledge in experimental design and in the laboratory have provided substantial aid in the completion of this study. Additional thanks also go to Mindy for her assistance in refining the project, and to Josh for being both friendly and knowledgeable while providing assistance in the lab.

References

[1] Briggs, G. M. (n.d.). *Euglena: a unicellular algae*. Pressbooks. https://milnepublishing.geneseo.edu/botany/chapter/euglena/

[2] Richter, P. R., Strauch, S. M., Ntefidou, M., Schuster, M., Daiker, V., Nasir, A., Haag, F. W., & Lebert, M. (2014). Influence of Different Light-Dark Cycles on Motility and Photosynthesis ofEuglena gracilisin Closed Bioreactors. *Astrobiology*, *14*(10), 848–858. https://doi.org/10.1089/ast.2014.1176

Ecarnot, F., Seronde, M.-F., Chopard, R., Schiele, F., & Meneveau, N. (2015). Writing a scientific article: A step-by-step guide for beginners. European Geriatric Medicine, 6(6), 573–579. doi:10.1016/j.eurger.2015.08.005