The Effect of Temperature on the Phototactic Mobility of Euglena Gracilis

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ABSTRACT

Euglena gracilis is a mixotrophic algae that can gain energy through photosynthesis and phagocytosis. Previous research has extensively studied the relationship between temperature and motility; however, much of the effect of temperature on positive phototaxis is still unclear. Therefore, the objective of our study is to investigate the effect of temperature on the phototactic mobility of *E. gracilis* towards light (positive phototaxis). We predicted that an increase in temperature, until out of optimal range, would increase the rate of positive phototaxis. To test this prediction, we exposed *E. gracilis* to different incubation temperatures: 12°C, 17°C, 25°, and 35°C. Our data was analyzed using a one-way ANOVA test. It was found that there is no statistical difference in the mean cell density at different temperatures ($p=0.728$). Thus, the null hypothesis was not rejected.

INTRODUCTION

Euglena gracilis are single-celled protists that live in both salt and freshwater aquatic environments (Wang et al, 2018). These protists are mixotrophs, as they have characteristics of both heterotrophic and autotrophic organisms (Esteban et al., 2010). In such, they have the ability to capture and acquire nutrition in their environment by phagocytosis, and when exposed to light, they can utilize its chloroplasts to produce their own food by photosynthesis (Esteban et al., 2010). To help with photosynthesis, *E. gracilis* has a stigma, a red eyespot, that helps filter light and contributes to the phototactic response (Hader & Iseki, 2017).

The phototactic response of *E. gracilis* is defined by the organism's ability to sense light by moving towards (positive phototaxis) and away (negative phototaxis) from light (Jékely, 2009). This phototactic response is dependent on the motility of the organism, as well as the photosensitivity and temperature of the surrounding environment (Richeter et al., 2014). Above all, phototaxis are advantageous to *E. gracilis* and other organisms with autotrophic characteristics, as they can orient themselves most efficiently to receive light for photosynthesis

(Jékely, 2009). Thus, this interesting phenomenon holds great survival value for these photosynthetic organisms, that are more often exposed to light-limiting conditions

Furthermore, *E. gracilis* is an important oxygen producer in aquatic ecosystems, as oxygen is released from photosynthesis (Richter et al., 2014). Aside from being an effective oxygen producer, *E. gracilis* plays a significant role in the foundation of the freshwater ecological food web. As a phytoplankton species that also acts as a food source, they are responsible for providing energy to organisms in higher trophic levels, such as zooplankton, fish, etc (Chittenden et al., 2010). In addition, previous studies have found that *E. gracilis* is an important food source for the salmon population on the Pacific Coast (Chittenden et al., 2010). Therefore, a decrease in the phototactic ability of *E. gracilis* can greatly affect the coastal pacific environment as a whole, given they are primary keystone species within the larger food chain.

Our study aims to investigate the effects of temperature on the phototactic mobility of *E. gracilis*. Temperature is an important abiotic variable that can influence many chemical and biological processes of organisms (Moore, 2006). Likewise, studies have shown that temperature influences the behaviour of salmon and other marine organisms more than any other abiotic variable (Beitinger & Fitzpatrick, 1979). Seasonal changes in the temperature can often induce physiological changes in salmon, thus affecting their metabolism, growth patterns, feeding rates, and reproduction (Beitinger & Fitzpatrick, 1979). This research is important as the influence of temperature on the phototaxis *E. gracilis* can affect the rate of productivity, mobility and photosynthesis of the organism. These aspects are crucial to the growth and survival of these microorganisms, which are an important food source for salmon.

Through previous studies on other abiotic variables affecting the phototaxis response of *E. gracilis*, we predict that the positive phototaxis response in *E. gracilis* is temperature dependent. Though the growth temperature range for *E. gracilis* is diverse from 17°C to 30°C, optimal temperatures range from 19°C to 26°C for metabolic growth (Wang et al., 2018). As the temperature is increased within this optimal range, we expect an increase in the phototactic mobility and response of *E. gracilis.* However, if the temperature increases above this optimal range, we expect the phototactic mobility of the *E. gracilis* to decrease and project similar patterns as our control temperature trial. Our null hypothesis is that there will be no change in the positive phototaxis response of *E. gracilis* when temperature is increased. Conversely, our alternate hypothesis is that there will be a change in the positive phototaxis response of *E. gracilis* with increased temperature.

METHODS

Set up

The initial concentration of the provided *E. gracilis* culture was calculated, starting with the preparation of a sample of the culture for a cell count. This original 200mL culture was grown for us over a three-week period by the laboratory technicians and delivered to us in a 1L Erlenmeyer flask. 100uL of the *E. gracilis* culture and 10uL of fixative was pipetted into a microcentrifuge tube and mixed thoroughly using a pipette. Next, the hemocytometer (pictured in Figure 1) was prepared for the cell count with a clean coverslip placed on its surface. 10 uL of fixative mixture for *E. gracilis* was then pipetted into the well of the hemocytometer, with the pipette placed at the edge of the coverslip. The prepared hemocytometer was placed on the stage of a compound microscope; the focus and position were adjusted until the grid lines could be

seen clearly. The cells were viewed using the 10X magnification lense. Cell counting procedure entailed counting the cells in each of the 16 smaller grids that make up the larger grid of the hemocytometer, starting from the top left corner working towards the right. This was repeated for each row below until an overall cell count of approximately 200-300 cells was reached. When counting each of the smaller grids, the cells that fell on the top and left border lines were included. The average cell count, the dilution factor of the hemocytometer at the correct magnification, and a fixative correction factor were used to calculate the cell concentration (cell/mL) of the *E. gracilis* culture.

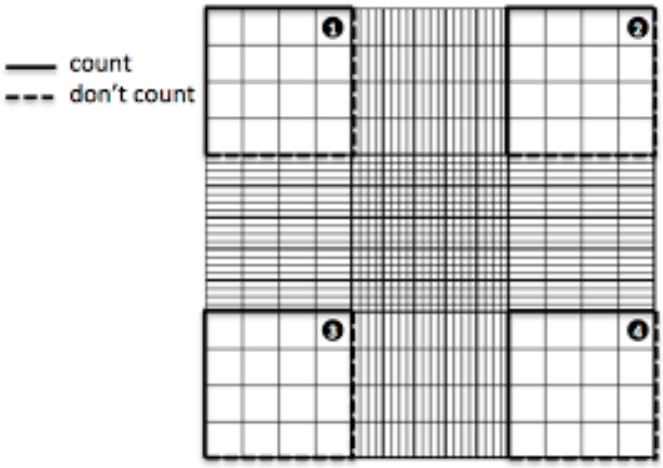

Figure 1: The grid as observed under the hemocytometer.

As seen in Figure 2 below, each of the samples were prepared for experimentation using a 10mL pipette to transfer 40mL of *E. gracilis* from the original 1L Erlenmeyer flask to each of the four correctly labelled 500mL tubes provided for each treatment. The first tube was placed in a 12°C cold room, the second in a 17°C cold room (control), the third in a 25°C incubator and the final tube in a 35°C incubator. Each 500mL tube was left in its respective temperature-

controlled environment for 2-6 hours, or until the thermometer reading was consistent with the treatment temperature.

While the 500mL tubes were reaching their correct treatment temperatures, 12 blacked out petri dishes were prepared (pictured at the bottom of Figure 2 and Figure 3). The entire bottom half of the petri dish was covered with black plastic using clear tape, and the lid of the dish was replaced with a circle of black plastic cut with a circumference slightly larger than that of the original lid. Subsequently, a hole in which light could enter the blacked out petri dish was created in the lid. The hole was approximately the size of a standard whole punch (10% of the petri dish diameter), which could be cut out of the black plastic lid using scissors or a hole punch.

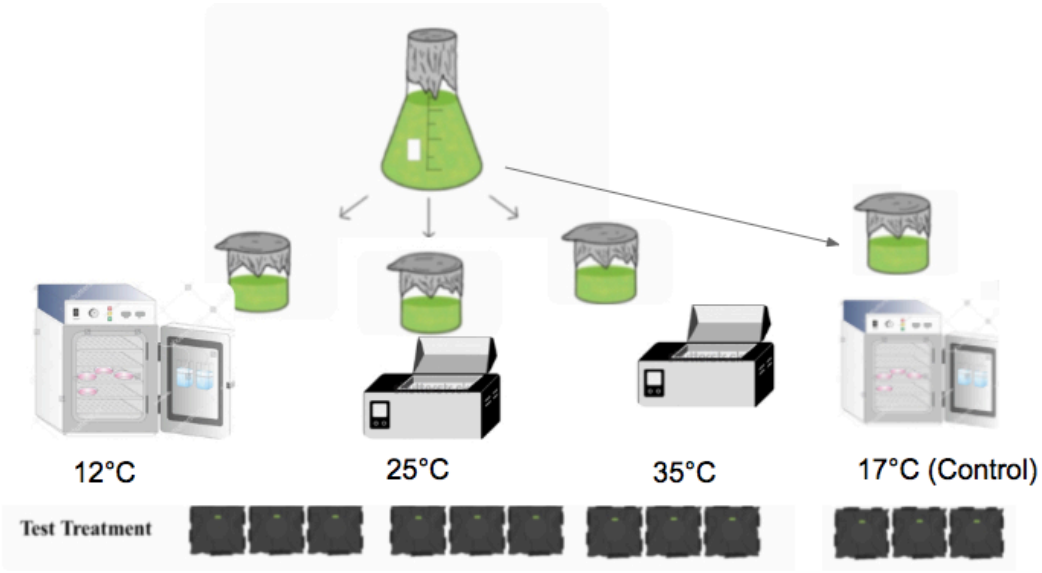

Figure 2: Visual flowchart outlining the major procedural steps and treatment set up.

For each treatment, a pipette was used to transfer 10mL of *E. gracilis* from the 500mL tube into one of the blacked-out petri dishes. This was repeated three times for each treatment, all within the correct cold room or incubator in order to keep the temperature consistent with the treatment. 12 replicates were prepared in total, 3 for each treatment and 3 for the control. After

preparing the petri dishes within the correct environments the experiment was run. An iPhone X was used as the light source with a brightness of 50 lumens. In reference to Figure 3, the flashlight was placed directly up against the cut-out hole in the lid of the petri dish, while the light source was kept stationary for a full five minutes. Directly after light exposure, a pipette was used to sample 100uL of *E. gracilis* through the hole cut in the lid, with care taken not to disturb the sample and to keep the pipette at a 90° angle to the table surface. This was done to ensure that the sample was taken directly from the area of light exposure. The samples were then placed in correctly labelled microcentrifuge tubes, and this sampling procedure was then repeated for each of the 12 replicates.

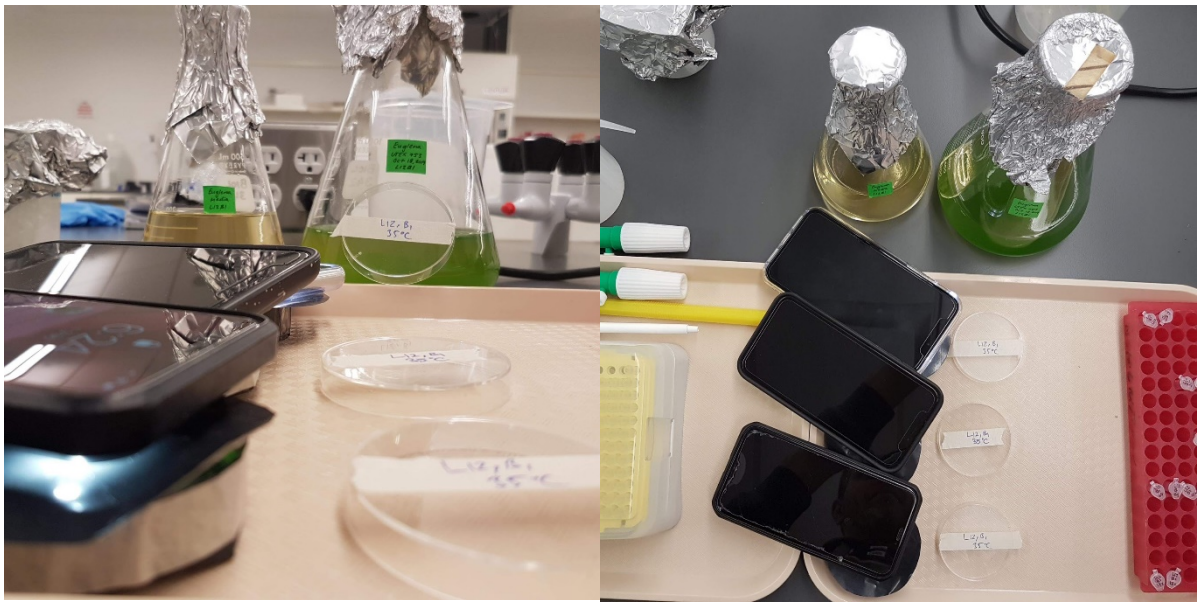

Figure 3: A visual of experimental setup at 25°C.

Once the samples had been placed in the correct microcentrifuge tubes, 10uL of fixative was added in order to prevent any cell division which could skew the cell concentration. In order to determine the cell concentration for each treatment, a cell count was performed. The procedure outlined above was repeated for each of the 12 replicates. The same dilution factor and

fixative correction factor as the initial cell count was used to calculate cell concentration of each sample. The resulting data was analyzed through the application of a one-way ANOVA, allowing for the determination of mean difference in cell density at the point of light exposure for each of the treatments. Higher cell density in the area of light, indicated a higher instance of positive phototaxis, while lower cell density indicated a lower instance of positive phototaxis.

RESULTS

The data was collected from the blacked-out Petri dish, only taken from the standard hole previously made. This hole was approximately 10% of the diameter of the entire Petri dish and was the point of entry for our light source. The number of *E. gracilis* cells were observed under the hemocytometer and the cell density at each trial was calculated by the following formula: (average *E. gracilis ce*lls at a specific temperature)*(dilution factor)*(fixative correction). The average cell density at each temperature trial was then calculated. Since the experiment contained one explanatory variable: temperature, the difference between the mean density of the *E. gracilis* cells was determined through a one-way ANOVA test. Prism 8 application was used to run this test.

The one-way ANOVA test returned an F statistics of $F_{(3,8)}$=0.445, which is lower than the F-critical (4.07), indicating that the variance between the mean cell densities of the *E. gracilis* is not significantly different. Moreover, the p-value of the test (0.728) is greater than the significant level of 0.05, suggesting that the mean cell densities are not significantly different. The lowest variance was observed at 12°C (1.3E9) while the highest was observed at 17°C (1.48E10). The low variance implies that the data collected are placed close to the mean, while the high variance suggests the data collected are scattered away from the mean.

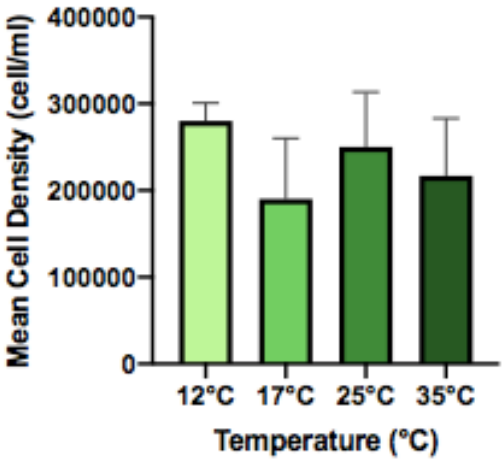

Figure 4. The bar graph represents the mean density of the E. gracilis cells as observed under the hemocytometer at temperatures 12°C,17°C, 25°C, 35°C. Three replicates were done for each trial at the Biology 342 lab at UBC. Error bars represent the standard error of the mean (SEM). According to the one-way ANOVA, the p-value = 0.728, $F(3, 8) = 0.445$, and $r^2=0.143$.

As per Figure 4, there are patterns of increased cell density observed at both 12°C and 25°C. At 35°C, mean cell density does appear to be slightly higher compared to our control temperature trial but, the magnitude of the difference is marginal. The average cell density at 17°C, the control group, was 190,000 (cells/ml). As the temperature increased to 25°C the average cell density increased to 250000 (cells/ml). At 35°C, the average cell density was 216,667 (cell/ml), which was still higher than the control group. The highest average cell density was observed at 12°C (280,000 cells/ml) while the lowest was observed at 17°C (190,000 cells/ml). The average cell density of *E. gracilis* at temperatures 12°C, 17°C, 25°C, 35°C was found to be 280,000 cell/ml (SEM=20817 cell/ml), 190,000 cell/ml (SEM=70238 cell/ml), 250,000 cell/ml (SEM=63509 cell/ml), and 216,667 cell/ml (SEM=66416 cell/ml), respectively.

DISCUSSION

Data Analysis and Biological Reasoning

According to our results, we fail to reject the null hypothesis that increased temperature has an effect on the positive phototaxis response of *E. gracilis*. However, there was still an observed trend in positive phototaxis response (Fig 4): compared to our control, cell density increased at 12°C and 25°C. Cell density did taper at 35°C, but this aligns with current literature, as the range temperature for *E. gracilis* metabolic function is between 17°C to 30°C (Wang et.al, 2018). The one-way ANOVA test revealed that the effect of temperature on the positive phototaxis was statistically insignificant; the p-value was 0.728 which is greater than 0.05. Thus, we can infer that temperature independently is not a driving factor for phototactic movement towards light. Previous research done by Bruce and Pittendrigh suggests that light acts as a trap to attract these microorganisms (1956). This suggests that a positive phototactic response may also be a result of the presence of light in general. As such, cell density at even unfavorable temperatures, like both 12°C and 35°C could be achieved. Research has also shown that when microorganisms, like *E. gracilis*, are placed in rather unfavorable temperature conditions, their phototactic movement towards light is greater (Hader et al., 2006). This can largely be contributed to the fact that more exposure to light in these conditions will allow for higher productivity, a greater metabolic rate and increased mobility. This in turn promotes growth and the cultures chance of overall survival (Hader et al., 2017). Based on enzyme kinetics, temperature plays a key role in the rate of reaction. Thus the photosynthesis rate, as compared to higher temperatures, is slower at 12°C. Due to this, an increased cell density being observed at 12°C is less surprising. Thus, *E.gracilis* could be seeking out light in hopes to increase the rate of photosynthesis and to be able to carry out this process even if the surrounding conditions are unfavorable.

In addition, recent studies have indicated that *E. gracilis* may have more temperature tolerance and independence than previously noted (Wang et al., 2018). Some studies have found that low and high incubation temperatures affect growth and phototactic motility of this species in different ways: low incubation temperatures favor protoplasmic growth, while high incubation temperatures favor cell division (Buetow, 1962). In such cases, the maximal growth rate occurs at 25°C to 30°C, while maximal accumulation of cellular material occurs at around 12°C to 16°C (Buetow, 1962). Maximal accumulation of cell material is proportional to cell density and cell growth, and thus, a larger dry weight of *E. gracilis* would be expected around temperatures of 12°C to 16°C (Buetow, 1962). Even though there was no significant difference found between the average cell densities at our varied temperatures, our results still showcased this pattern: highest average cell density was at 12°C, with 25°C being second highest. Similarly, our results showed average lower cell densities at both 17°C and 35°C, as these temperatures do not directly fit into the ideal ranges mentioned above. Since higher cell density in the area of light is indicative of an increased rate of positive phototaxis, the overall culture is responding to the external light environment and photosensitive at temperatures of 12°C and 25°C, whereas at 17°C and 35°C, this response is observed at a lower rate due to mean cell density being lower as well.. As mentioned previously, photosensitivity to the environment is a key driving factor for a phototactic response in these organisms (Wang et al., 2018).

Sources of Error

This study was subjected to multiple errors. As mentioned previously, to ensure no movement in the culture, we opted to use the black plastic as a covering for the top of the Petri dish. In doing so, the black cover on the Petri dish apparatus was not stable, which could have led to possible inaccuracies in sampling. It was also hard to maintain the phones and have the

lights be turned on simultaneously. This could have potentially led to slight variations in the exposure time of light for each *E. gracilis* culture. Lastly, in using incubators, we were unable to place the light exposure directly in the apparatus. And in having to move our samples back and forth, there could have been slight changes in temperature and disturbance to the cells in the culture. Both of these aspects can lead to larger variations in data than anticipated.

Future Research

Since we were unable to reject the null hypothesis, future studies should still be conducted on temperature and positive phototaxis, to investigate direct cause and effect. Since patterns in cell density with temperature were seen in our data, but more sporadically, a different methodology for measurement of phototaxis response should be proposed. Therefore, our study should have used a more direct approach to record phototactic response. Thus, perhaps an experiment exploring both temperature and light, and the combined effect on phototaxis should be considered for next time. This would allow focus to be solely on the interplay between temperature, light and phototaxis, and can be more suggestive of a direct effect of one variable to another.

CONCLUSION

To conclude, our research has found that there are evident patterns in the relationship between temperature and the positive phototactic response of *E. gracilis*. The trend of cell densities observed at each temperature trial is consistent with patterns observed in other current research and our prediction but however, we failed to reject our null hypothesis that temperature has no effect on the positive phototaxis of *E. gracilis.*

ACKNOWLEDGMENTS

We would like to express appreciation to Professor Jordan Hamden and our teaching assistant Tessa Blanchard for their support, valuable time, and constructive feedback during the development of our research project. Furthermore, advice and feedback on our proposal given by our laboratory technicians, Chanelle Chow and Mindy Chow, have also been a great help in refining our research aim and study. Their expertise in the laboratory has helped improve our learning, experimental design, and analysis of this study. A final thank you to the University of British Columbia and Biology 342, for providing us access to opportunities and resources to conduct this valuable research.

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APPENDIX

Table 1: One-way ANOVA Summary

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.3825E+10	3	460833333	0.4448913	0.7275330	4.0661805
Within Groups	8.2867E+10	8	1.0358E+10			
Total	9.6692E+10	11				

Table 2: Summary of statistics

Groups	*Count*	*Sum*	*Average*	*Variance*
12°C	3	840000	280000	1300000000
17°C	3	570000	190000	1.48E+10
25°C	3	750000	250000	1.21E+10
35°C	3	650000	216666.667	1.3233E+10

Distribution of Sections

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