The shape of *Euglena gracilis* in response to temperature

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Abstract

Euglena gracilis is a photosynthetic single-celled eukaryote which can change its shape depending on the environmental conditions. Previous studies have shown the importance of unicellular photosynthetic organisms in the production of both atmospheric and aquatic O_2, as well as the implications of *E. gracilis'* shape on its photosynthetic capacity. This present study looks at the effects of temperature on the shape of Euglena, which has yet to be explored. To do this, we incubated *E. gracilis* culture at 10°C, 25°C, and 31°C (n=3 for each temperature treatment) for one hour before taking photos of the culture under a compound microscope. We quantified the results by counting the number of balled-up and elongated cells for all temperatures and replicates. We calculated a ratio of the counted number of balled-up cells divided by the total number of cells (balled up + elongated) for our statistical analysis. Our one-way ANOVA analysis shows that there is a statistically significant difference between the number of balled-up cells at 10°C and 31°C compared to the 25°C control treatment ($p= 0.0069$). We therefore reject our null hypothesis that each temperature group will have the same mean ratio of balled-up cells/total cells counted, showing that temperature has an effect on the shape of *E. gracilis*.

Introduction

Euglena gracilis is a photosynthetic protist that produces wax esters, unsaturated fatty acids, proteins, paramylon, and various other compounds (Wang et. al, 2018). They are able to grow in a variety of habitats that range in temperature and pH, and its cell composition can be affected by both the presence and absence of light (Wang et. al, 2018). As a phytoplankton, *E. gracilis* acts as the basis to aquatic food webs in both marine and freshwater ecosystems and plays a key role in maintaining ecological balance (Stallwitz & Hadert, 1993). Thus, research involving *E. gracilis* can lead to not only conclusions about itself, but further the foundational basis for other studies involving phytoplankton and aquatic systems.

Factors affecting the growth rates of *E. gracilis* have been significantly explored in literature, with an emphasis on the effects of temperature. It was found that although exponential growth was temperature dependent, linear growth was not (Wang et.al, 2018). Additionally, temperature also had a lesser effect on cell composition (Wang. et.al, 2018).

However, the effect of temperature on *E. gracilis*' shape remains unclear. The change in shape is one of the most distinguishable characteristics of *E. gracilis*, and has been reported to be linked with photosynthetic and respiratory reactions (Li M. et al., 2017). *E. gracilis* shapes are variable and can range from elongated to spherical (Li M. et al., 2017). Shape is required for E. gracilis to have proper electron flow and photosynthetic rhythm (Lonergan, 1983). Following disruption of the photosynthetic rhythm, larger impacts on the cell's internal biological clock and other regulatory processes may be impacted as well (Lonergan, 1983). This change in shape has been hypothesized to be a response to a variety of factors, including light, temperature, pH and cation concentration and could be a result of stress (Li M. et al., 2017). Thus, we seek to focus on one factor that has been hypothesized to play a role in the change of *E. gracilis* cell shape- temperature.

The purpose of our study is to determine the effect of temperature on the cell shape of *E. gracilis.* After incubating at three different temperatures (10°C, 25°C, and 31**°**C), each euglena cell will be classified as balled or elongated. This will allow us to determine how temperature as a stressor can impact the shape of *E. gracilis*, and provide an insight on the optimal temperatures that would lead to maximum photosynthetic capacity. Due to *Euglena*'s maximum growth rate being reduced at an upper limit of 30°C and accumulation of cellular material being limited at 13.3 °C (Buetow, 1962), we expect that temperatures outside of those will act as stressful conditions, and lead to *E.gracilis* changing its shape from elongated to spherical (balled-up). Thus, based on previous literature, we predict that all euglena cells will be elongated at 25°C, while all cells at 10°C and 31°C will be balled-up.

Methods

Culture Preparation

We received a flask of ~50mL *Euglena gracilis* culture from Mindy Chow at the University of British Columbia. We then transferred 36mL of the stock culture into nine 10mL test tubes (4mL per test tube). In order to observe the morphological response of *E. gracilis* to temperature, we incubated the culture at three temperatures at the upper and lower limits of *E. gracilis*' thermal range (10°C and 31°C), as well as its optimal temperature (25°C). Three test tubes containing culture (n=3) were placed in an incubator at either 10°C, 25°C, or 31°C (Figure 1). The test tubes were left in the incubators for approximately 1 hour to allow the *E. gracilis* culture to acclimate to the temperature of the incubator.

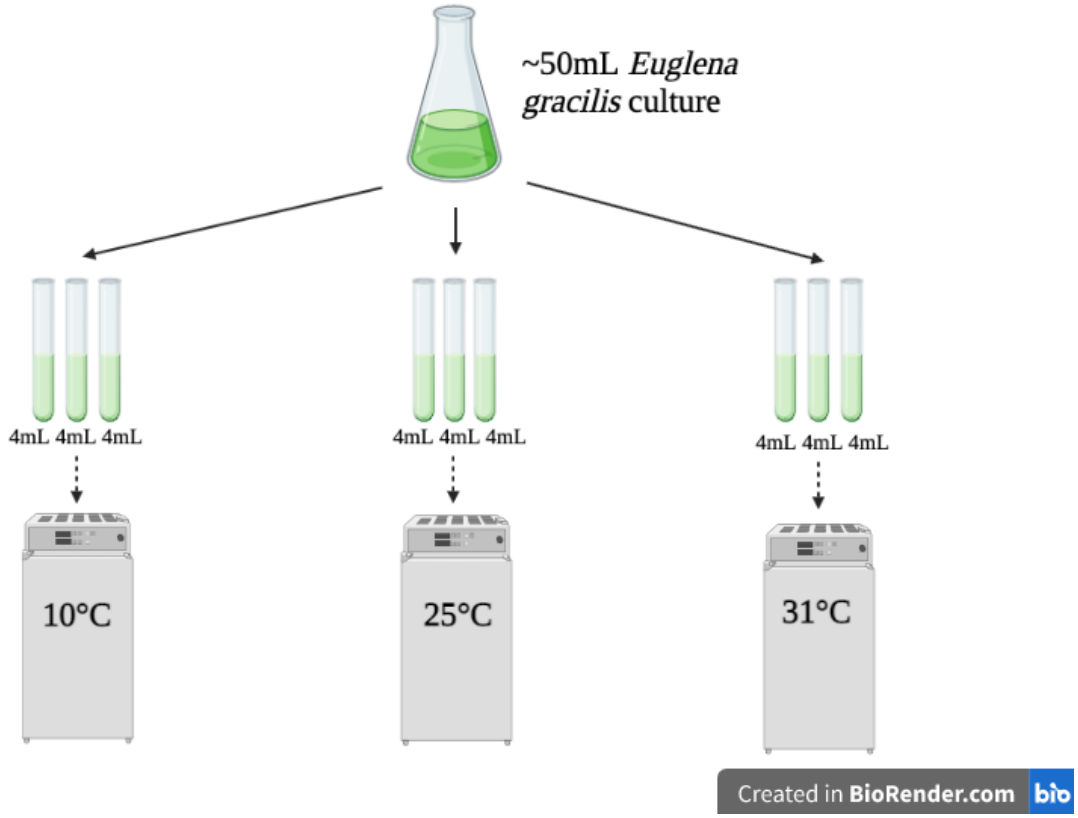

Figure 1. General schematic of culture preparation. This schematic was created using BioRender.

Quantification and Observation

After one hour, slides were prepared by obtaining 10μL of sample from each test tube at each temperature (9 slides total). To reduce acclimation of the cells back to room temperature, only the replicates for a single temperature were prepared and observed at a time. The slides were observed using a compound microscope calibrated through Kohler illumination. Two images of each slide were taken using an iPhone 11 camera at different locations on the slide to ensure the presence of Euglena for quantification and analysis. To quantify our results, we counted the number of balled-up and total cells for each photograph. The total number of cells includes balled-up and elongated cells.

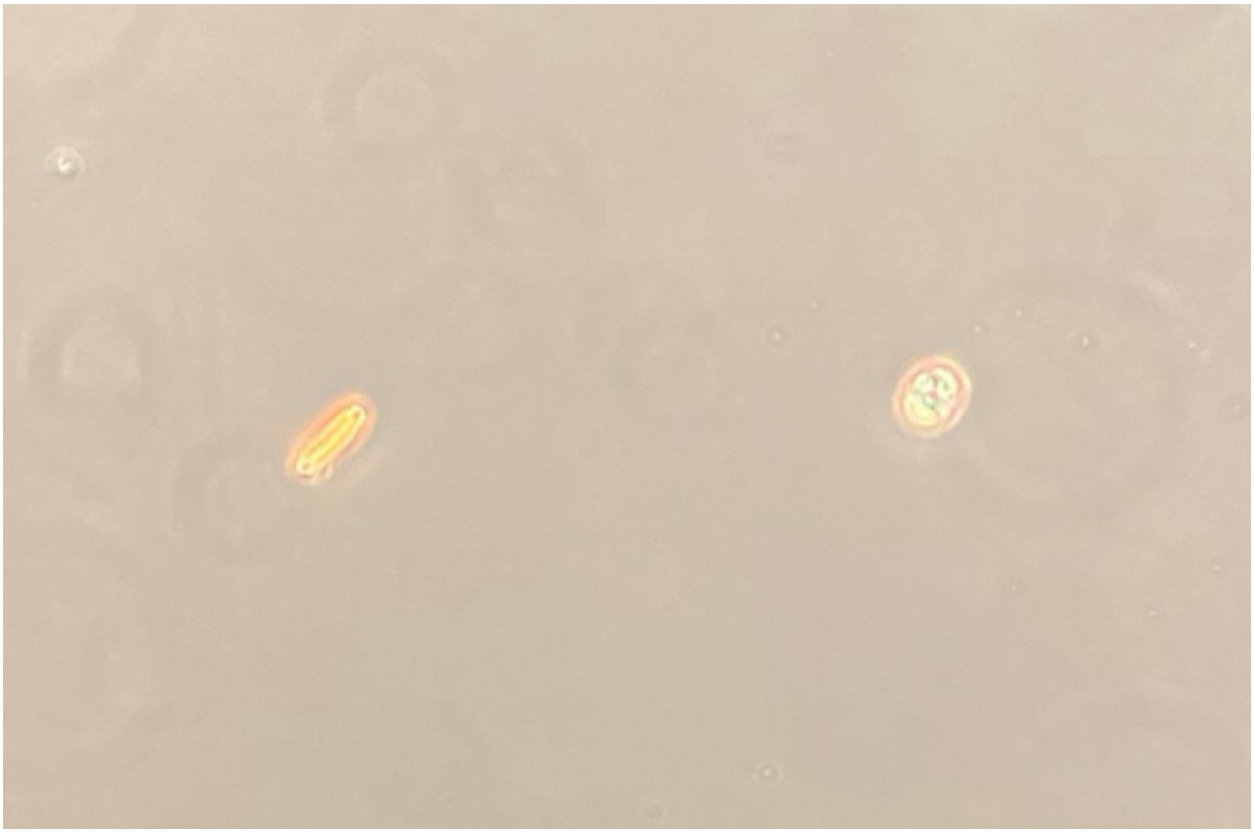

Figure 2. Elongated and Round Cells. Cells that appeared the same or similar in shape to the cell on the left side of the figure were counted as elongated, while cells that appeared the same or similar in shape to the cell on the right side of the figure were counted as balled-up.

Statistical Analysis

For statistical analysis, we calculated the ratio of the number of balled-up cells to the total number of cells for each temperature, replicate, and image. Due to our variables being categorical, a one-way ANOVA test was done to test for a significant difference between the three means, and a post-hoc Tukey HSD test was used to confirm which categories of treatment was statistically significant.

Results

We counted the number of balled-up and elongated cells for each temperature and replicate (Figure 3) which were used to produce a ratio we could use for statistical analysis (Figure 4). To do this, we divided the number of balled-up cells by total cells (balled up + elongated cells).

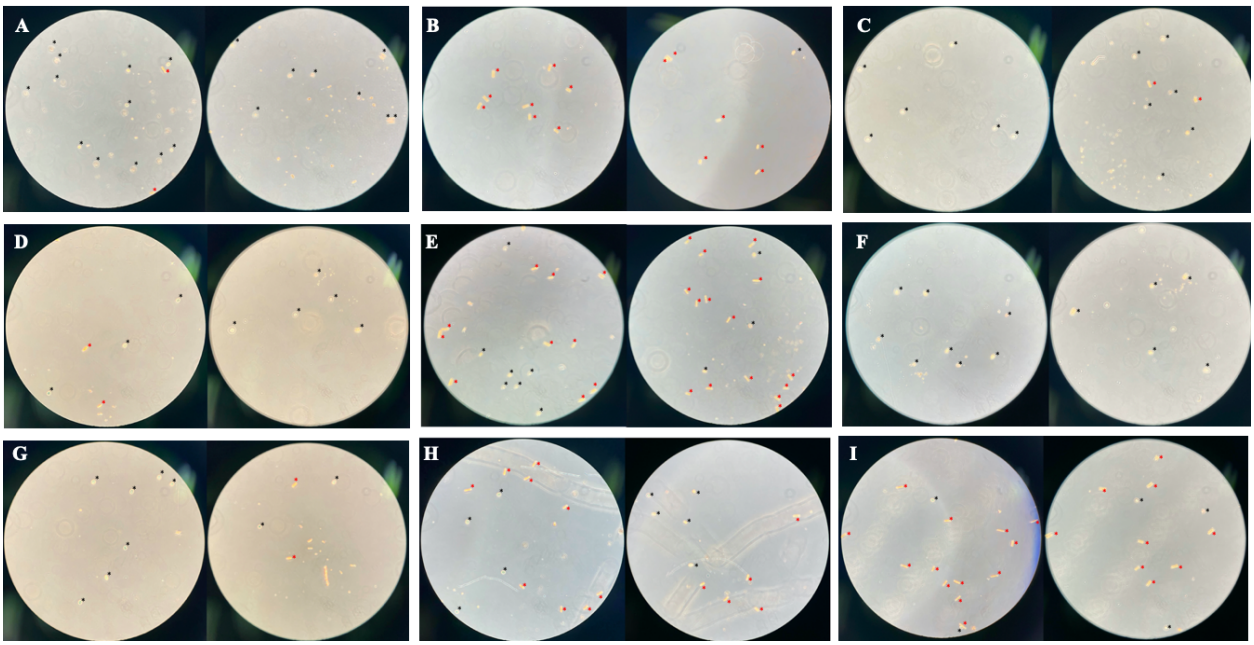

Figure 3. Counted cells of temperature treatments. Photographs were taken using an iPhone 11 camera. Slides were prepared with culture from each temperature treatment (n=3 for each temperature) and placed on a compound microscope after incubating for one hour at 10°C (A, D, G), 25°C (B, E, H), and 31°C (C, F, I). Black asterisks are used to indicate the balled-up cells counted. Red asterisks are used to indicate the elongated cells counted.

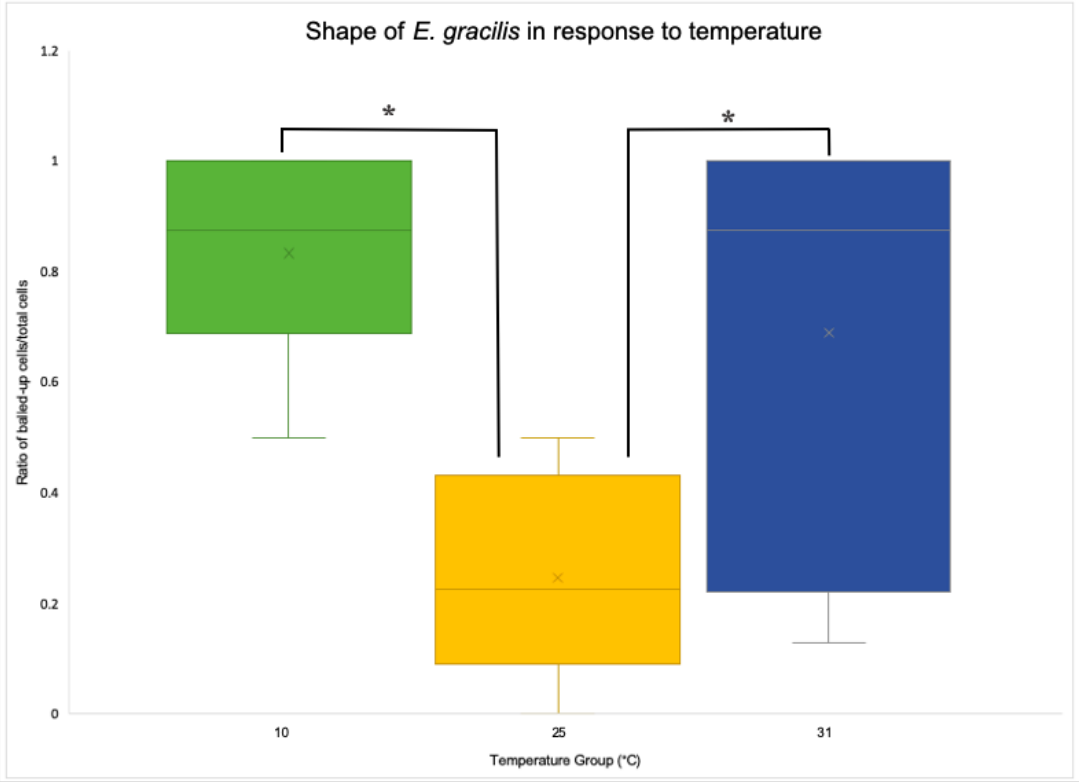

Figure 4. Shape of *E. gracilis* in response to temperature. The cell shape ratio of Euglena gracilis in 3 different temperature treatments: 10°C (n=3), 25°C (n=3), and 31°C (n=3). The box represents the interquartile range. The X within the box represents the mean ratio of balled-up to total number of cells. The line within the box represents the median value. The bottom and top whiskers represent the minimum and maximum cell shape ratios respectively of each temperature treatment.

A one-way ANOVA was used to determine if the difference in the mean ratio between each temperature group was statistically significant (Vasavada). This analysis found the p-value to be 0.0069 (Table 1). Additionally, we used a post-hoc Tukey HSD test to determine which temperature treatments were significantly different from each other (Vasavada). This analysis found the p-values for the comparison of 10°C and 25°C, 31°C and 25°C, and 10°C and 31°C to be 0.0068537, 0.0398594, and 0.6460135 respectively (Table 2). These results indicate that E. gracilis tends to become balled-up at both 10°C and 25°C, but not at 31°C.

Table 1. One-Way ANOVA Results. All values were calculated using One-way ANOVA (ANalysis Of VAriance) with post-hoc Tukey HSD (Honestly Significant Difference) Test Calculator for comparing multiple treatments (Vasavada). Sum of squares, degrees of freedom, and mean square are all values used in the calculation of F statistic and p-value which are both used as determinants of statistical significance.

	Sum of squares (SS)	Degrees of freedom (v)	Mean square (MS)	F statistic	p-value
Treatment	1.1205	2	0.5603	7.0590	0.0069
Error	1.1905	15	0.0794		
Total	2.3111	17			

Table 2. Post-hoc Tukey HSD Results. All values were calculated using One-way ANOVA (ANalysis Of VAriance) with post-hoc Tukey HSD (Honestly Significant Difference) Test Calculator for comparing multiple treatments (Vasavada). The Tukey HSD Q statistic is a value determined by the degrees of freedom for the comparison and the comparison and the number of treatments. The Tukey HSD p-value is used as a determinant of statistical significance summarized in the Tukey HSD inference column.

Treatments pair	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inference
10°C vs 25°C	5.1008	0.0068537	**p<0.01
10°C vs 31°C	1.2607	0.6460135	insignificant
25°C vs 31°C	3.8401	0.0398594	*p<0.05

Discussion

The results of our statistical analysis have led us to reject the null hypothesis (Ho) that there is no difference in mean ratio of cell shape between temperature treatments. Our results therefore support the alternate hypothesis (Ha), that there is a significant difference in the ratio of cell shape due to the change in temperature. The p-value from our one-way ANOVA tells us that the mean ratio of balled-up/total cells was different between all of the temperatures (10°C, 25°C, and 31°C). A post-hoc Tukey HSD test was also performed, which revealed that the significant differences occurred between both of our treatment temperatures, compared to the control: 10°C and 25°C, 31°C and 25°C. This means that at the control temperature of 25°C, most *E. gracilis* were random in which cell shape they assumed; no clear pattern emerged for the amount of cells that were either balled-up or elongated. In contrast, the cells which were incubated at the more extreme temperatures of *E. gracilis*' thermal range did have a noticeable difference in cell shape. At 10°C, most cells were found to be balled-up in shape. The same observation was seen at 31°C. While the criteria for our prediction was not fully met (all cells at the treatment temperatures of 10°C and 31°C would be balled-up and only the cells at the control temperature of 25°C would be elongated), the trend in the results of our experiment show support for our prediction to be possibly true. This leads our group to believe that the variable of temperature does in fact influence the cell shape of *E. gracilis*.

While our results support our alternate hypothesis, we should also consider the possible effects for other variables that could have resulted in a similar outcome. The most notable variable to consider would be the effect of light. Previous studies have described that the presence of light can be a deciding factor for the shape of a cell. Lonergan (1983) describes how in dark conditions, *E. gracilis* can be observed to be more round in shape. In contrast, in a bright situation, *E. gracilis* is observed to be more elongated (Lonergan, 1983). This is due to the photosynthetic capacity of *E. gracilis.* The reason we have to consider this is because of the limitations in the available equipment. The only equipment that we had access to for microscopy

imaging was a compound brightfield microscope. The bright light, which is necessary for producing a visible image through the magnification lenses, could have caused some *E. gracilis* to elongate their cell shape during imaging. A method to work around this in the future would be to try and flash freeze our slide samples prior to imaging (Li S. et al., 2017). Flash-freezing our slide samples would ensure that the cell shape of *E. gracilis* would remain representative of the temperature conditions, reducing the variability of the effect of light causing *E. gracilis* to elongate. Another possible limitation of our experiment was the consistency of incubation time. While our experimental design only allowed for one hour of incubation time, a study by Ozasa et al., (2017), found that an incubation time of three hours is sufficient for *E. gracilis* to adapt to their environmental conditions. To add to this, the exact incubation time also differed between all treatments, with a maximum of 15 minutes difference, due to the time it took to prepare and image the slides in between. Another limitation we had for our experiment was our ability to distinguish cells from random debris due to the varying focus of the captured image. This may have caused some error in our quantification. In order to improve the rigor of our experiment and to increase the confidence in the results, future experiments would benefit from an increased replicate number. Our experiment only included 3 replicates from each temperature treatment. Increasing the replicate number would increase the statistical support of our result. Thus, future studies could benefit from improved experimental designs and equipment.

When considering the impact of our research, we were excited to discover that the implications for *E. gracilis* for use in industries such as for nutrition, energy, medicine, and more are quickly expanding (Gissibl et al., 2019). In addition to this, the National Oceanic and Atmospheric Administration estimates that up to 50-80% of our atmospheric oxygen is produced by floating plants, algae, and some bacteria that can photosynthesize, including our model organism*, E.gracilis* (NOAA, 2021). Spindari et al. (2019) have also previously pointed out the significance of *E. gracilis* in the health of our ecosystem in relation to its oxygen producing capabilities. And as pointed out earlier, one of the markers for the photosynthetic capability of *E.*

gracilis is their elongated shape (Lonergan, 1983). Since our experiment looked at the effect of temperature on cell shape, future experiments could include a measurement of oxygen production in conjunction to measuring the ratio of *E. gracilis* cell shape. This could affect the ability of *E. gracilis* and other similar photosynthetic organisms to produce the oxygen we all rely on.

Conclusion

Based on the results of our study, it was found that both 10°C and 31°C incubation temperatures caused a significant change in cell shape when compared to the 25°C control temperature. This supports our prediction and we are able to reject the null hypothesis, concluding that temperature does play a role on cell shape. Understanding the effects of temperature on Euglena shape can further benefit studies regarding future *Euglena* use and can help further our understanding on aquatic ecosystems.

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