The Effect of Temperature on the Growth Rate of *Euglena gracilis*

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Abstract

Climate change is linked to a rise in global temperatures, which affects many organisms that are sensitive to these changes. The objective of our study was to investigate the effect of temperature on the growth rate of *Euglena gracilis.* We measured the growth of *E. gracilis* populations through daily cell counts, across treatments of 13°C (n = 3), 20°C (n = 3), 25°C (n= 3), and 30°C (n = 3), for 11 days. From a one-way ANOVA ($p = 2.81*10^{-10}$, $F_{3,8} = 812.77$) and a Tukey-Kramer test, we found that growth rate significantly differed between the treatments. In order of lowest to highest, the mean growth rates were 0.13/day, 0.23/day, 0.26/day, and 0.27/day for the treatments of 13°C, 20°C, 30°C, and 25°C, respectively. Our results support our alternative hypothesis that temperature does have an effect on the growth rate of *E. gracilis,* and we were able to reject the null hypothesis that temperature does not affect their growth rate. These findings have implications for global change biology, as changing water temperatures will affect the growth and reproduction of *E. gracilis*, which will result in alterations to food webs in the ecosystems in which it plays a role.

Keywords: Euglena gracilis, algae, temperature, growth rate, cell division

Introduction

Euglena gracilis is a single-celled flagellated alga that grows by both phagocytosis and photosynthesis, depending on the environmental conditions (Dahoumane et al., 2016). It is found mainly in freshwater environments, but can occasionally be found in saltwater and soil (Richter et al., 2003). As a primary producer, *E. gracilis* is an important species in aquatic ecosystems (Zhu & Wakisaka, 2018). For instance, a number of organisms that are preyed on by salmon, such as *Corophium salmonis* and *Chironomidae* insects, rely on *E. gracilis* as a food source (Maier & Simenstad, 2009). Furthermore, *E. gracilis* converts CO_2 into O_2 during photosynthesis, increasing dissolved oxygen in the water, which salmon take up through their

gills (Kitaya et al., 2005). Consequently, the success of *E. gracilis* populations has larger effects on the ecosystems and species involved.

Temperature is one of the abiotic factors with the largest impact on the growth of algae (Metsoviti et al., 2019). *E. gracilis* reproduces by binary fission (Li et al., 2017), and cell division is closely linked with growth (Jorgensen & Tyers, 2004). Previous studies have shown that higher temperatures favour cell division, with the greatest growth occurring between 25°C and 30°C (Buetow, 1962; Buetow, 1963). Another study conducted by Kitaya et al. (2005) found that multiplication of *E. gracilis* was maximal at the slightly higher temperature range of 27°C to 31°C. However, Yamada et al. (2016) found that there is the potential for cell damage to occur and for survival rates to decrease at temperatures greater than 29°C. These studies demonstrate varied results with regards to the optimal temperature for the growth of *E. gracilis*, hence our experiment will help fill this knowledge gap.

The objective of our study is to determine how temperature affects the growth rate of *E. gracilis.* We will test growth, measured through changes in cell density, at four different temperatures (13°C, 20°C, 25°C, and 30°C). This will allow us to discover how differences in water temperature can impact the growth and life cycle of *E. gracilis*, and in turn freshwater ecosystems. Based on the majority of our sources, we predict that the populations incubated at 25°C will experience the greatest growth rates. Our null hypothesis is that temperature does not affect the growth rate of *E. gracilis,* whereas our alternative hypothesis is that temperature does have an effect on their growth rate.

Methods

We measured the population growth of *E. gracilis* at four temperatures: 13°C (n = 3), 20°C (n = 3), 25°C (n= 3), and 30°C (n = 3) for 11 days. The treatment at 25°C served as our control, because this temperature falls within the range of temperatures at which *E. gracilis* experiences maximum growth rates (Buetow, 1962; Buetow, 1963).

Determining the initial concentration and preparing the diluted stock

To begin, we obtained *E. gracilis* stock (UTEX 753) grown in culture medium prepared from the UTEX Euglena Medium Recipe at the University of British Columbia. We mixed a sample of *E. gracilis* stock with fixative in a 10:1 ratio and then used a hemocytometer (Figure 1) and a standardized method of counting (Figure 2) to obtain the initial cell count. Using the equation: cell density (cells/mL) = number of cells * dilution factor of hemocytometer square * correction for fixative, we calculated the concentration of the stock solution to be 5.5 x 10^5 cells/mL. We then prepared a diluted stock solution with a concentration of 5 x 10^4 cells/mL.

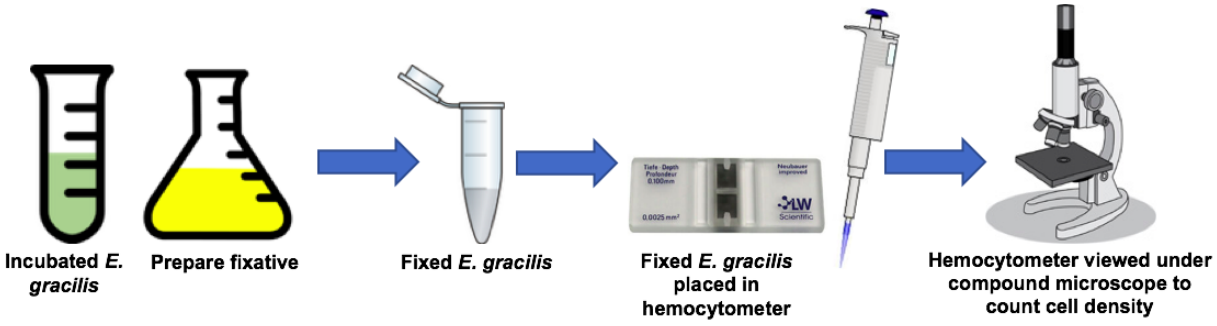

Figure 1. This schematic diagram shows the steps taken to determine the initial concentration of cultured *E. gracilis. E. gracilis* and fixative were mixed in a 10:1 ratio before the cells were counted using a hemocytometer.

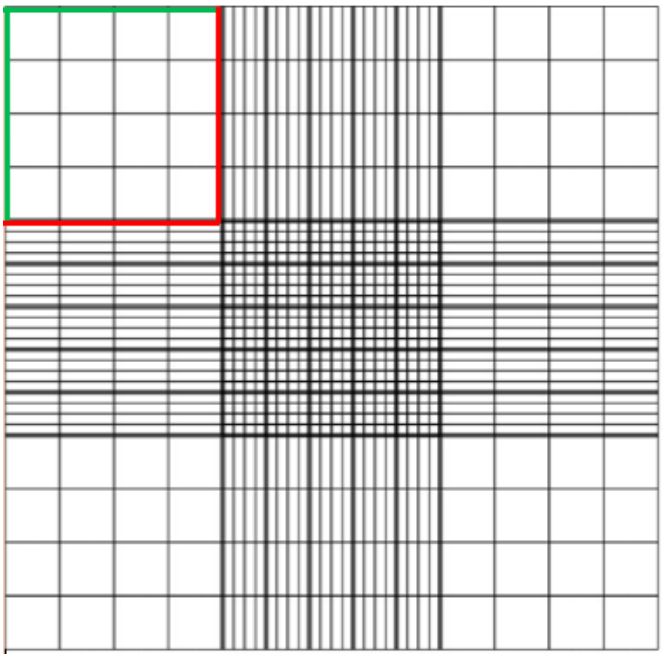

Figure 2. This hemocytometer grid demonstrates the standardized method of counting that was used. For cells on the edges of squares, only those on the edges marked in green were counted for each square. Adapted from "What is a hemocytometer?" by M. Jalan, 2019 (https://www.scienceabc.com/pure-sciences/what-is-a-hemocytometer-calculation-counting-how-to-use.html).

Preparing the populations

Using sterile technique, we divided the diluted stock equally into 12 test tubes (Figure 3). We then placed the test tubes into their respective incubators, which were set for 12:12 h light/dark cycles. We accounted for the initial lag phase by waiting 48 hours before beginning the subsequent cell counts.

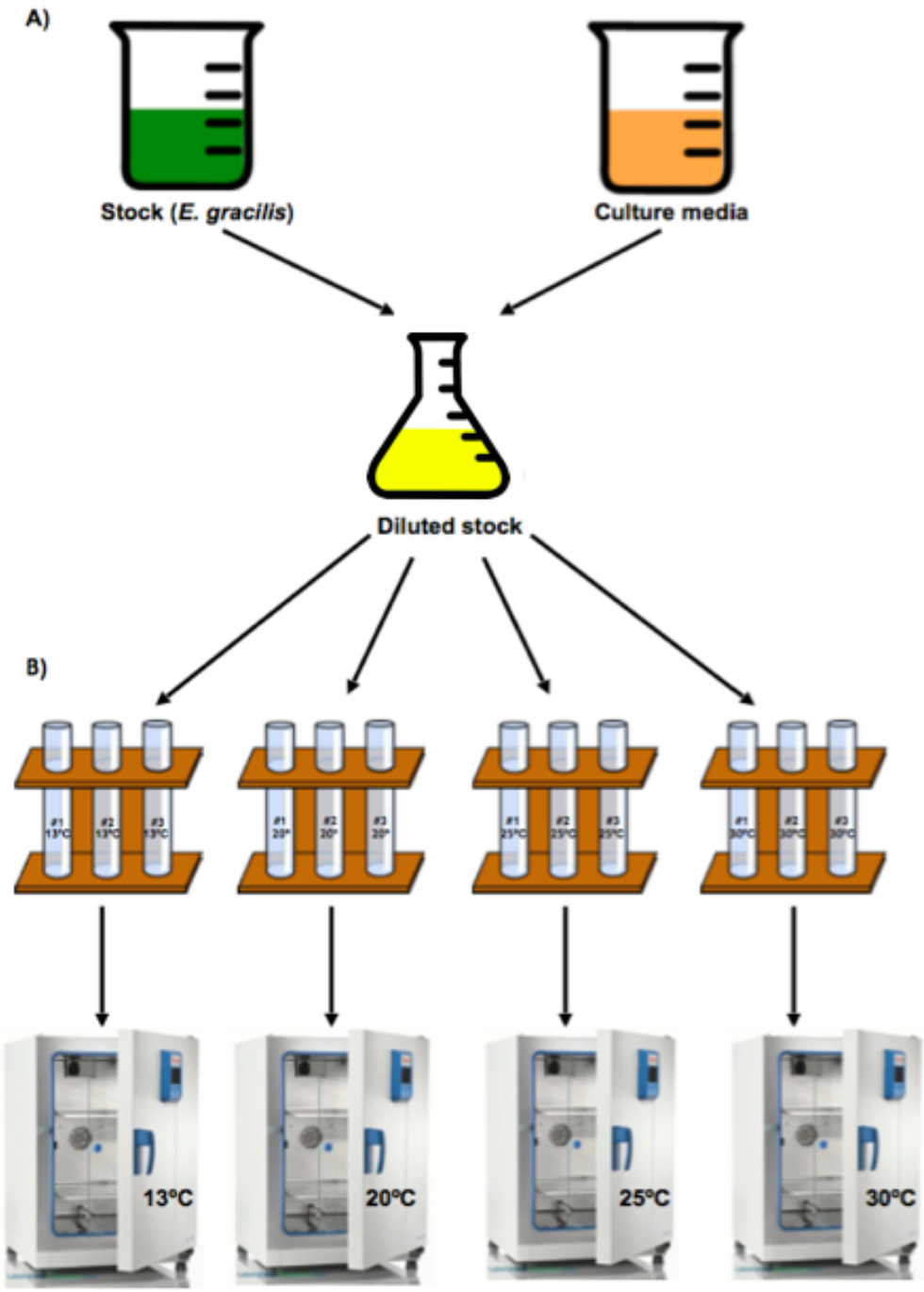

Figure 3. Diagram showing the preparation of test tubes of diluted *E. gracilis* for incubation at 13°C (n = 3), 20°C (n = 3), 25°C (n = 3), and 30°C (n = 3). In 2A, stock *E. gracilis* solution and culture medium were mixed together to make a diluted stock. In 2B, the diluted stock solution was divided equally into test tubes before being placed into 13°C, 20°C, 25°C, and 30°C incubators with 12:12 h light/dark cycles.

Observing population growth

Immediately after removing the test tubes from the incubators, we recorded qualitative observations, including any changes in colour, for the populations of *E. gracilis*. We then obtained samples from each of the test tubes and mixed them with fixative in a 10:1 ratio to prevent movement and further cell division in the samples, before placing the test tubes back into their respective incubators. To count the cells and determine the cell density of each of the samples, we used the same procedure that we used to determine the initial concentration of the stock. We did this every 24 hours over the next 11 days, excluding weekends. It should be noted that at the start of the second week, we began counting three replicates per sample instead of just one, as suggested by our mentor, to ensure that the counts were valid and reliable. We also calculated the coefficient of variation (CV) for each of our samples to ensure that it was less than 10%.

Statistical analysis

We used the calculated cell densities for each of the populations to create growth curves with average cell densities for each treatment. Additionally, we calculated the growth rate k (day^{-1}) for each of the populations using the equation: $\ln N = kt + \ln N_0$ (Shehata & Kempner, 1977). We plotted the average growth rate (day^{-1}) for each of the treatments on a bar graph. Lastly, we used R software version 3.5.1 to perform a one-way ANOVA to determine if there was a significant effect of temperature on growth rate and a Tukey-Kramer test to determine which pairs of treatments significantly differed in their growth rates.

Results

Throughout our experiment, variations in the colours of the *E. gracilis* populations were evident. Prior to our initial dilution, the stock *E. gracilis* culture solution was an opaque, medium green at the bottom and fairly colourless toward the top. After dilution of the stock solution to our starting cell density, the solution was a clear, light yellow colour. As the populations increased in cell density, all of the solutions became greener and darker in colour, with the colour being more concentrated toward the bottom of the test tubes. More rapid colour changes were observed for the 20°C, 25°C, and 30°C populations than for the 13°C populations. At the end of our experiment, the solutions containing the 20°C, 25°C, and 30°C populations were slightly darker than the original stock *E. gracilis* culture. In contrast, the 13°C populations were a light yellow-green colour and only darkened slightly in comparison to the first day.

Figure 4 depicts mean cell densities for each treatment, which are representative of those of the individual populations. Up until Day 4 of incubation, the cells incubated at 30°C grew the fastest. From Day 4 to Day 7, the 30°C populations had decreased growth rates, while the 20°C and 25°C populations showed increased growth rates. By Day 7, the cell densities of the 25°C populations had become the highest, exceeding those of the 30°C populations. On Day 8, the 20°C populations had reached the same cell density as the 30°C populations. Finally, by Day 10, the 20°C, 25°C, and 30°C populations were very close to or had reached stationary phase. In contrast, the 13°C populations were still showing a slow but steady increase in cell density on Day 11.

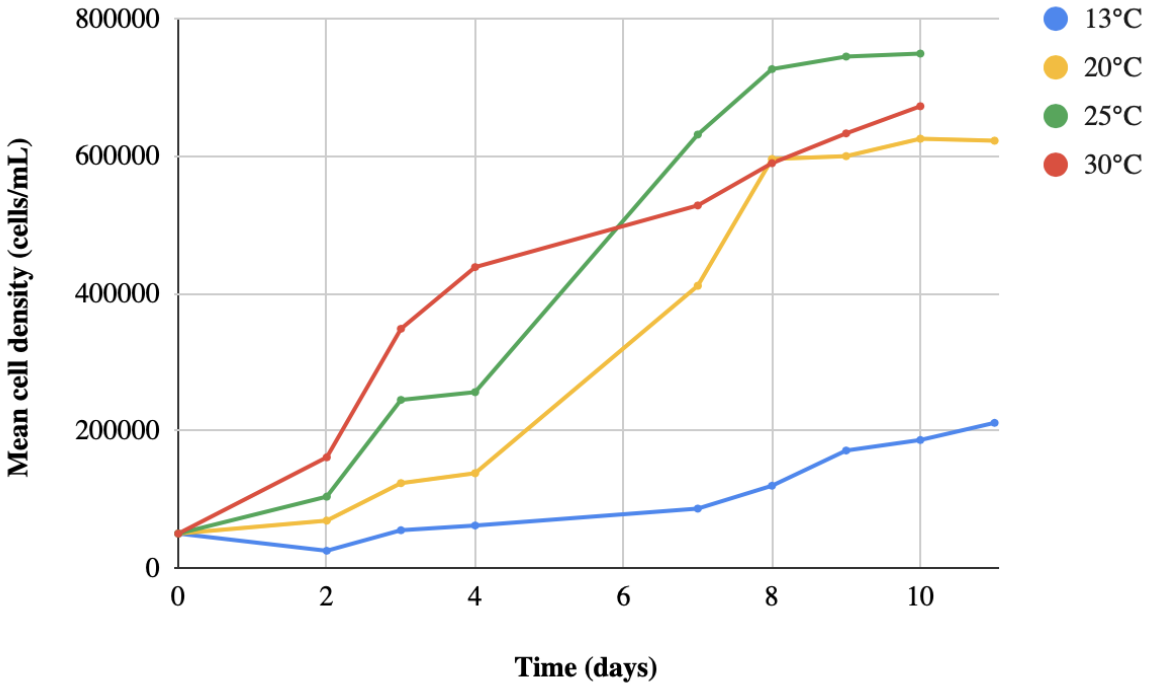

Figure 4. Mean cell densities (cells/mL) for each treatment for *E. gracilis* grown at the temperatures of 13°C ($n = 3$), 20°C ($n = 3$), 25°C ($n = 3$), and 30°C ($n = 3$) over 11 days of incubation.

The mean growth rates, in order of lowest to highest, were 0.13/day, 0.23/day, 0.26/day, and 0.27/day for the treatments of 13°C ($n = 3$), 20°C ($n = 3$), 30°C ($n = 3$), and 25°C ($n = 3$), respectively (Figure 5). In the same order of treatments, the 95% confidence intervals were [0.128/day, 0.134/day], [0.228/day, 0.230/day], [0.254/day, 0.266/day], and [0.265/day, 0.276/day]. From the one-way ANOVA, we found that temperature had a significant effect on growth rate ($p = 2.81*10^{-10}$, $F_{3,8} = 812.77$). In addition, the Tukey-Kramer test showed that the mean growth rate was significantly different between all temperature pairs. For the Tukey-Kramer test, $p < 0.001$ for the temperature pairs of 30°C and 13°C, 20°C and 13°C, 25°C and 13°C, 20°C and 30°C, and 25°C and 20°C, and $p = 0.037$ for the temperature pair of 25°C and 30°C.

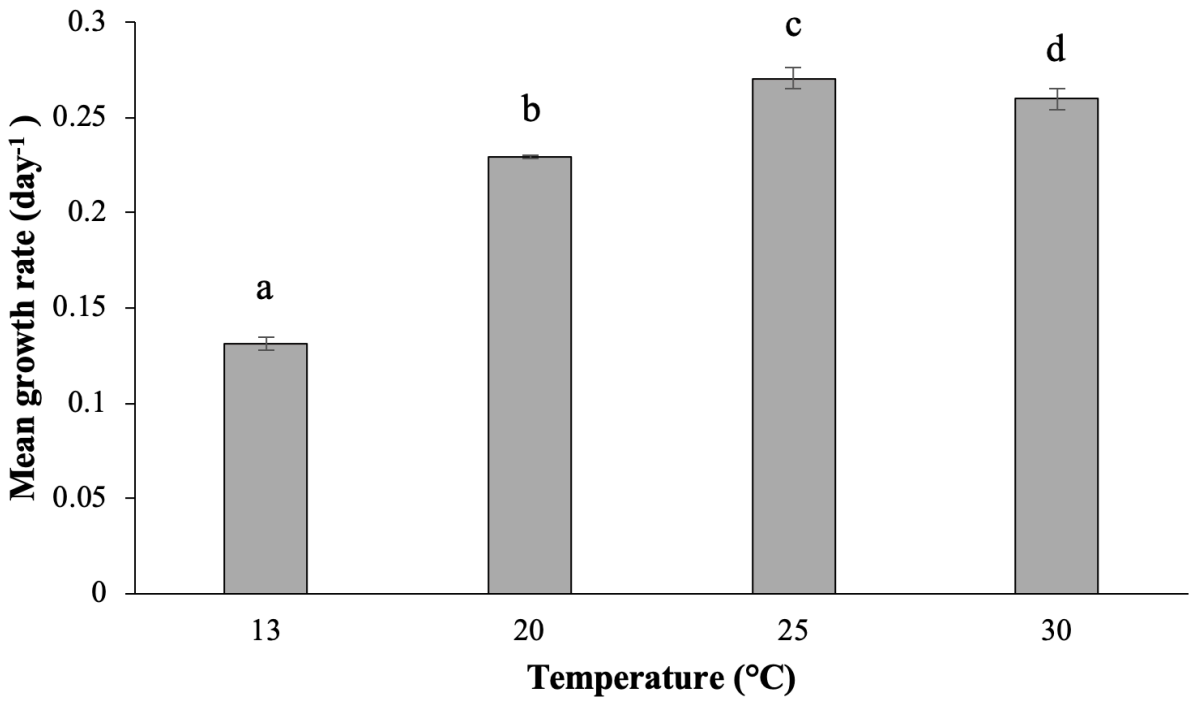

Figure 5. Mean growth rates (day^{-1}) of *E. gracilis* grown at the four temperatures of 13°C (n = 3), 20°C (n = 3), 25°C (n = 3), and 30°C (n = 3). From the ANOVA, $p = 2.81*10^{-10}$, $F_{3,8}$ = 812.77. Error bars show 95% confidence intervals. Different letters show significant difference between treatment groups as determined by the Tukey-Kramer test ($p < 0.05$ for all pairs).

Discussion

Our results reject the null hypothesis and support our alternative hypothesis that temperature affects the growth rate of *E. gracilis*. From lowest to highest growth rate, the treatments responded in order of: 13°C, 20°C, 30°C, and 25°C. These results are consistent with findings that *E. gracilis* grows more slowly at the temperatures of 13°C to 17°C than at the higher temperatures of 25°C to 30°C, since the lower range is not adequate to facilitate the reactions needed for growth (Buetow, 1962; Buetow, 1963). The 30°C treatment, which resulted in the second highest growth rate, showed a unique increase, then decrease in growth rate early in the experiment. A study by Yamada et al. (2016) found that at temperatures over 29°C, E. gracilis can suffer cell damage, in the form of chloroplast loss, which hinders photosynthesis and

decreases its ability to grow. Since the cells in our experiment initially thrived at 30°C but lost momentum soon after, we speculated that cell damage may have occurred at this temperature. If this were the case, it suggests that the growth of *E. gracilis* was dependent on photosynthesis. To better understand the reason for the unusual growth pattern of the populations incubated at 30°C, further experiments with a focus on chloroplast function and activity at various temperatures would be needed.

Our prediction was also supported, as the cells at grown at 25°C demonstrated a significantly higher growth rate than those in the other treatments of 13°C, 20°C, and 30°C. This is consistent with previous studies in which the fastest growth occurred between 25°C and 30°C (Buetow, 1962; Buetow, 1963). The 25°C growth curve increased consistently throughout our experiment, suggesting that cell damage did not occur. We can infer that 25°C is high enough for the processes necessary for growth and mitosis to occur, but not high enough to induce cell damage.

The results of this study have implications for climate change and the effects of increasing temperatures on freshwater ecosystems. As *E. gracilis* is an important food source for salmon that consume protists (Muylaert et al., 2001), as well as for primary consumers preyed on by salmon (Maier & Simenstad, 2009), changes to its population size can have considerable impacts on food availability for salmon. The freshwater temperatures of British Columbia typically range from 13°C to 15°C (Mantua et al., 2010). Since the treatment of 13°C had the lowest growth rate, we can infer that the cell densities of the *E. gracilis* populations in British Columbia waters are relatively low. This could mean that *E. gracilis* is not a main food source for salmon in British Columbia, and that the primary producers that local salmon mainly rely on

are species that thrive at lower temperatures. Based solely on our results, an increase in water temperature by just a few degrees would likely result in an increased *E. gracilis* population density, which could better support salmon populations. However, too large of a temperature increase would be detrimental to salmon, as juvenile salmon prefer temperatures between 12°C and 14°C, and mortality increases substantially above 23°C (Brett, 2011; Baker et al., 1995). In a region where salmon have tremendous ecological, cultural, and economic importance (Hyatt & Godbout, 1999), it is crucial to understand the factors that could affect their population levels.

As with any study, our experiment involved sources of error and uncertainty. One source of error is in the cell counts, which were done manually using a hemocytometer. To minimize potential error, we thoroughly mixed the fixed cells before placing them in the hemocytometer, used clicker-counters to keep track of the number of cells, obtained at least three counts per sample, and calculated the CV for each sample. If errors occurred in the cell counts, this could impact the growth curves and growth rates. Since the CV was within the acceptable range (10%) for all of our counts, we can assume that the cell counts do not pose a substantial source of error. For future studies, an image analysis software can be used to obtain more accurate cell counts.

Another source of uncertainty comes from the potential for photosynthesis, as *E. gracilis* grows by both photosynthesis and phagocytosis (Dahoumane et al., 2016). The photosynthetic activity of the cells was not measured, so its impact on growth cannot be reported with certainty. There has been evidence of cells photosynthesizing to a lesser degree under artificial light than under sunlight, due to differences in light wavelengths and intensity (Darko et al., 2014). Because artificial light was used as a light source in this study, our results may not fully reflect the growth that occurs under natural sunlight. Photosynthesis could also have been affected by

variations in the amount of light exposure, due to the removal of populations from the incubators for sampling, surrounding objects inside the incubators, and the incubators being periodically opened, which let additional light in. These factors could also have influenced the temperatures of the treatments, but we assume that they are inconsequential due to the high heat capacity of water (Brewer & Peltzer, 2019). While we had no way of monitoring the activity of people opening the incubators or placing objects next to the test tubes, we ensured that the time the test tubes spent out of the incubators was approximately the same (roughly 15 min/day) for all populations. We removed the test tube racks from the incubators in one trip, took samples as quickly as possible, and returned the racks to the incubators all at once. By keeping the light exposure reasonably consistent for all treatments, we ensured that the differences in growth rate were almost certainly a result of the temperature. To eliminate this source of variation completely, the experiment would have to be done in consistent light conditions for the entire time to ensure that light exposure and the potential for photosynthetic activity remained exactly the same for all populations.

Due to time and resource constraints, we were only able to measure growth over a timespan of 11 days. Thus, we began with a relatively high cell density of 5×10^4 cells/mL to increase the likelihood of the populations reaching stationary phase by the end of the experiment. If time was not limited, it would be worthwhile to begin with lower cell densities to see if the resulting growth rates are consistent with those found in this study. We were also limited in space, so we were only able to have three replicates for each treatment, for a total of twelve populations. As with any small data set, the results would become more reliable if the sample size was increased.

Conclusion

Based on the results of this study, our prediction was supported in that the cells grown at 25°C demonstrated the highest growth rate out of the four temperatures tested (13°C, 20°C, 25°C, and 30°C). We rejected the null hypothesis and found evidence supporting our alternative hypothesis that temperature affects the growth rate of ~~*E. gracilis*.~~ We inferred that 25°C is high enough for the processes necessary for mitosis and growth to occur at a maximal rate, but not high enough to induce cell damage. The results demonstrated in this study are consistent with several previous studies regarding temperature and its effects on *E. gracilis.*

Acknowledgements

We would like to thank Jordan Hamden for his continued guidance and support throughout the course of our experiment. Jordan's knowledge and suggestions helped to fine-tune our project. In addition, we would like to thank the lab technicians, Mindy Chow and Chanelle Chow, for culturing the *E. gracilis*, preparing necessary materials and equipment, ensuring that we were using proper experimental techniques, and providing suggestions for our statistical analysis. Without their continued efforts, our experiment would not have been possible. We would also like to thank our teaching assistant Tessa Blanchard for her help during labs that equipped us with the skills needed to conduct our experiment. Lastly, we would like to thank the University of British Columbia for providing us with the opportunity to be enrolled in BIOL 342, as well as the resources and lab space needed to design and conduct our own experiment.

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Appendix

Equipment List: Hausser Scientific Fuchs Rosenthal Ultra Plane 3720 hemocytometer, Fisherbrand® microscope coverglass, Zeiss Axiostar Plus compound microscope (R1517), Sanyo Versatile Environmental Test Chamber MLR-351H (set to 30°C), VWR Scientific Model 2020 Low Temperature Incubator (set to 25°C), Panasonic MLR-352H incubators (set to 13°C and 20°C), Thermo Scientific Finnipipette F1 pipettes with various volume limits (P10, P20, P100, P1000), Fisherbrand® micropipette tips, Fisherbrand® clicker-counters, KIMTECH Kimwipes, Eppendorf tubes, Erlenmeyer flask, test tubes, test tube racks, Fisherbrand® 10 mL non-pyrogenic serological pipette, and Fisher Scientific nitrile gloves

Chemical List: Prefer fixative by Anatech Ltd., culture medium prepared according to UTEX Euglena Medium Recipe, distilled water (dH_2O), 70% ethanol

Table 1. Euglena Medium Recipe from UTEX Culture Collection of Algae at The University of Texas at Austin used by lab technicians Chanelle Chow and Mindy Chow at the University of British Columbia to prepare the *E. gracilis* culture.

#	**Component**	**Amount**
1	Sodium acetate	1 g/L
2	Beef extract	1 g/L
3	Tryptone	2 g/L
4	Yeast extract	2 g/L
5	$CaCl_2*H_2O$	0.01 g/L
6	dH_2O	Add to 1 L

Table 2. Cell densities (cells/mL) for populations of *E. gracilis* in the four temperature treatments of 13°C (n = 3), 20°C (n = 3), 25°C (n = 3), and 30°C (n = 3) over the course of 11 days.

Population	Days of incubation								
	0	2	3	4	7	8	9	10	11
13 °C #1	50000	28531	57444	58056	93744	143550	189383	197230	207185
13 °C #2	50000	15469	50600	66611	84071	103565	168667	189860	207369
13 °C #3	50000	30938	56650	60500	81289	111980	154917	171875	219395
20 °C #1	50000	67375	132000	149875	427167	534600	556500	570185	617650
20 °C #2	50000	64281	107800	126500	409750	636350	618750	636185	621500
20 °C #3	50000	74938	130625	137500	396000	616000	624250	669185	672833
25 °C #1	50000	108969	255750	297000	605000	724625	753500	755333	No data
25 °C #2	50000	96594	220000	225500	616000	696300	704000	709500	No data
25 °C #3	50000	105875	258500	245667	672833	759000	777150	782833	No data
30 °C #1	50000	148500	363000	440000	519750	565813	599500	712250	No data
30 °C #2	50000	187000	327250	415250	532583	597865	649000	652685	No data
30 °C #3	50000	147813	354750	459250	531667	605000	650375	652667	No data

Table 3. Growth rate (day^{-1}) and mean growth rate (day^{-1}) for the populations of *E. gracilis* in the four temperature treatments of 13°C (n = 3), 20°C (n = 3), 25°C (n = 3), and 30°C (n = 3), calculated using data collected on the first and the last days of sampling for each treatment.

Population	Growth rate (day^{-1})	Mean growth rate (day^{-1})
13°C #1	0.129235374	0.13099747
13°C #2	0.129316074	
13°C #3	0.134440978	
20°C #1	0.228536268	0.22922009
20°C #2	0.229101173	
20°C #3	0.230022837	
25°C #1	0.271513571	0.27061899
25°C #2	0.265253749	
25°C #3	0.275089639	
30°C #1	0.265640597	0.25981739
30°C #2	0.256907162	
30°C #3	0.256904404	

Table 4. Output of the ANOVA in R software version 3.5.1 on the growth rates of the *E. gracilis* populations in the four temperature treatments of 13°C (n = 3), 20°C (n = 3), 25°C (n = 3), and 30°C (n = 3). Values for degrees of freedom (*df*), sum of squares (Sum Sq), mean of squares (Mean Sq), the F value, and the p value are shown.

	df	**Sum Sq**	**Mean Sq**	***F***	***p***
Treatment	3	0.03638	0.01213	812.77	$2.811*10^{-10}$
Residuals	8	0.00012	0.00001		

Table 5. Output of the Tukey-Kramer test in R software version 3.5.1 on the mean growth rates of *E. gracilis* in the four temperature treatments of 13°C (n = 3), 20°C (n = 3), 25°C (n = 3), and 30°C (n = 3). Values for the difference between means (diff), lower (lwr) and upper (upr) bounds of the 95% confidence intervals, and the p value are shown.

Comparison	**diff**	**lwr**	**upr**	***p***
30°C x 13°C	0.1288	0.1187	0.1389	0.0000
20°C x 13°C	0.0982	0.0881	0.1083	0.0000
25°C x 13°C	0.1396	0.1295	0.1497	0.0000
20°C x 30°C	-0.0306	-0.0407	-0.0205	0.0000
25°C x 30°C	0.0108	0.0007	0.0209	0.0366
25°C x 20°C	0.0414	0.0313	0.0515	0.0000