Investigating the effects of temperature on *Euglena gracilis* growth rate

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Abstract:

Algal blooms are occurring more frequently due to anthropogenic activities, in which rising water temperatures is one of the leading causes (Paerl et al., 2001). In the present study, the relationship between temperature and growth rate of *Euglena gracilis*, a single-celled alga, was investigated to further correlate rising temperatures with more frequent algal blooms in freshwater systems. *E. gracilis* was prepared and incubated at three different temperatures (17℃, 20℃ , and 30℃). The cells were then counted on a hemocytometer slide under a microscope at days 1, 4, 7, 10, and 12 following the day of initial preparation and a growth rate curve was plotted for each of the treatments and replicates. The growth rate was found to be the greatest at 30℃ and lowest at 17℃ ($p<0.01$). This demonstrates that rising temperatures play a role in increasing the rate of algae division and ultimately resulting in algal blooms.

Introduction:

E. gracilis is a single celled freshwater algae species that belongs in the genus *Euglena*. They are primarily found in stagnant freshwater such as ponds and lakes. These cells are characterized by an elongated shape that ranges from 15 to 500 μm long. The organelles consist of a single nucleus, contractile vacuole, chloroplasts, flagella, and a flexible pellicle. *E. gracilis* are a facultative mixotroph in which they primarily undergo photosynthesis to generate energy but are also capable of feeding heterotrophically on other algae via phagocytosis. *E. gracilis* also reproduce asexually through binary fission (Britannica, 2020).

E. gracilis are often an important component in the freshwater ecosystems they are found in, as they are the primary producers that produce dissolved oxygen in the waters that is essential for respiration. They also act as a decomposer that consumes other organisms or dead organic

matter to break it back down into chemical nutrients (Porter, 1977). Positioned at the bottom of the food chain, *E. gracilis* acts as a food source for a variety of algae-feeding organisms such as crustaceans and small fish (Porter, 1977). *E. gracilis* are able to thrive even in harsh environments with water pH as low as 3 and low light environments (Kitaya et al., 2005).

E. gracilis , however, can also cause toxic algal blooms in the freshwater ecosystems. Algal blooms, in which rapid growth and accumulation of algae occur, can bring adverse effects to the ecosystem. The decomposition of dead algae can deplete oxygen in the water and kill other organisms that undergo respiration. Algal blooms cause water quality deterioration and food web alterations (Paerl et al., 2001). *Euglena sanguinea*, another single-celled alga or another species of alga, in particular, is known to produce alkaloid toxin euglenophycin during blooms, which is toxic to fish and inhibits growth of bacteria in the waters. Euglenophycin is produced in at least six species of euglenoid algae and six of seven strains of *E. sanguinea* (Zimba et al., 2017). Algal blooms occur naturally but they can also be caused due to anthropogenic activity (Paerl et al., 2001).

Climate change is one of the main causes of the algal blooms (Paerl et al., 2001). Small freshwater systems are more vulnerable to the rising temperatures than oceans, due to a lack of cooling due to mixing. Studies have shown that low incubation temperatures promote protoplasmic growth in *E. gracilis* whereas high incubation temperatures favour cell division (Buetow, 1962). The maximal growth rate occurs at 29℃, whereas the maximal accumulation of cellular material occurred at temperatures ranging from 13.3℃ to 17℃ (Cook, 1966).

In this study, we aim to determine the relationship between temperature and the growth rate of *E. gracilis*. The temperatures in which *E. gracilis* will be incubated are 17℃, 20℃, and 30℃. This mimics the trend in the rising freshwater temperatures in mid northern latitudes; the current mean surface water temperature of lakes in mid-northern latitudes is 17℃ (Hren & Sheldon, 2012), and the projected rise in the water temperature is 2.4℃ by 2050 (Meehl, 2007). We predict the growth rate will be greatest at 30℃ and lowest at 17℃. The closer the temperature is to 30℃, the higher the growth rate of *E. gracilis* will be, as previous studies suggest that 30℃ has been seen to be the optimal growth temperature for *E. gracilis* .

Methodology:

We used a stock solution of *E. gracilis* that would be diluted with media to get 100,000 cells per mL. We mixed 100 microliters of the original cultured solution with 20 µL of fixative, provided in the lab, in an Erlenmeyer flask. Once mixed, we took 20 µL of that mixture and inserted it onto a hematography slide to count the cells. We counted cells until we reached about 150 cells and divided them by how many boxes they occupied to get a ratio (Figure 1). This step was repeated twice so we could calculate the average of total cells.

Once our diluted solution was made, it was divided into 9 separate 5 mL test tubes to be put into incubators labeled 17℃, 20℃, and 30℃. Using 30 degrees Celsius as the control, we had 3 replicates of each treatment, and we sampled from the tubes every other day for 2 weeks.

For our sampling, we labeled 40 centrifuge tubes with the appropriate temperature and sample number. After sterilizing the test tubes over an open fire, we micropipette 500 µL of our

E. gracilis solution and added 100 µL of fixative into each of the centrifuge tubes. We then used the micropipette to mix the solution by pipetting a little of the solution in and out of the pipet about 3 times. Once all the sampling was done, we put the tray of centrifuges into a fridge until we came in to count the cells.

At the end of two weeks, we took the centrifuge trays out of the fridge and set up our compound microscopes. We used a micropipette to mix the solution in the centrifuge, because all the *E. gracilis* had settled to the bottom and pipetted 20 µL onto a hemocytometer for *E. gracilis*. We slid the counting chamber under the microscope and started to count the amount of *E. gracilis* cells we saw (Figure 1). We stopped counting *E. gracilis* cells between 150-160 cells and had to keep track of how many boxes they occupied. Once we finished counting, we put all of our data into an excel sheet and did an one-way ANOVA test to calculate the total cell count and growth rate.

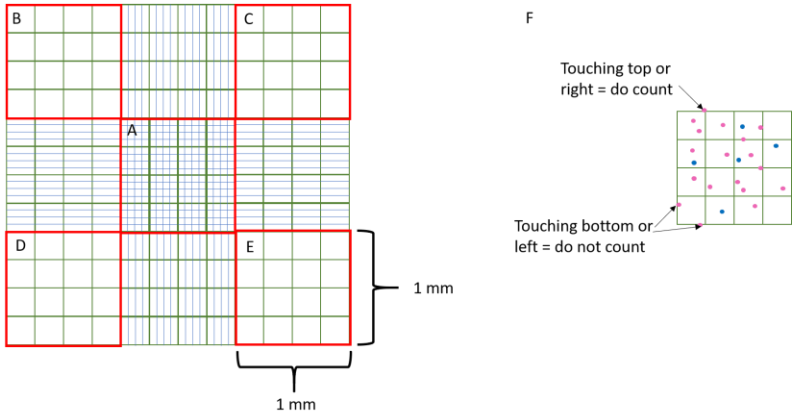

Figure 1. Hemocytometer grid for counting.

Results:

The data for cell density (cells per mL) for each sample of *E. gracilis* has been graphed below in Figure 2. In Figure 2 we can see that the 30℃ treatment samples initially had the largest growth rate (slope), however after day seven the growth rate flattened or became negative for each sample at around 500,000 cells per mL. Because this trend was seen in each of the 30℃ treatment samples, and not just one or two, we believe that this trend is due to these samples achieving such a fast initial growth rate that the rate of death and decay of older cells surpassed the rate of growth. Due to this we have chosen to analyze the data for the 30℃ treatment samples only up to day seven. From the figure we can also see that the 17℃ and 20℃ treatment samples had similar initial growth rates, however by around day seven the 20℃ treatment samples had a much higher cell count and growth rate than the 17℃ treatment samples. Finally, like the 30℃ treatment samples the growth rate of the 20℃ treatment samples also seemed to stall on day seven to ten, however because they continue to rise from day ten to day twelve this data was included in the statistical analysis.

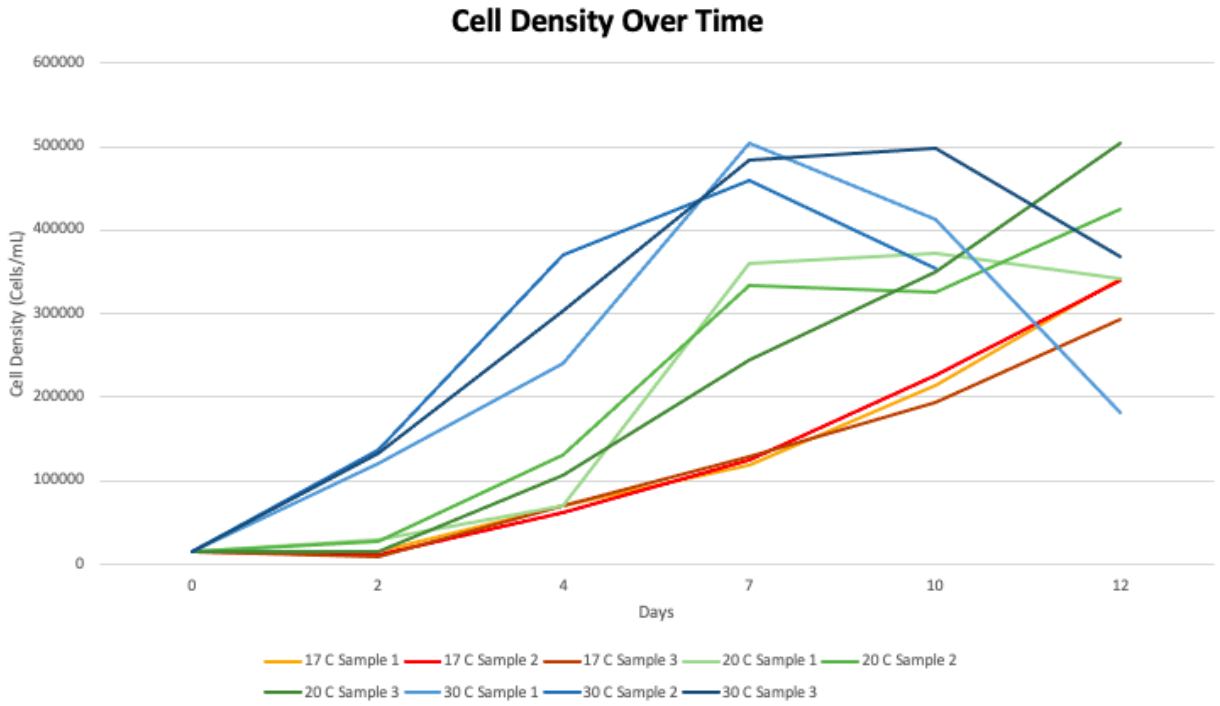

Figure 2. Depicts cell density (cells per mL) of *E. gracilis* grown in temperature treatments of 17℃, 20℃, and 30℃, each with three replicates over the twelve day sampling period.

A mean growth rate for each sample was calculated through a linear regression of the cell density over time. Note as mentioned above only data up to day seven was used for the 30 ℃ treatment, because as seen in Figure 2. After day seven the samples from the 30 ℃ treatment all showed negative growth rates, which has been attributed to the rate of cell death and decay being larger than that of cell replication. The average growth rate and 95% confidence interval for each of the treatments is as follows: 63055±5707 cells per day for 17 ℃, 92304±10514 cells per day for 20 ℃, and 157743±984 cells per day for 30 ℃. This can be seen in Figure 3. where the 30 ℃ treatment has the highest growth rate as well as the smallest 95% confidence interval, while the 17 ℃ treatment had the lowest growth rate. Additionally, the 20 ℃ treatment had a growth rate in between the other two samples but was closer to the growth rate of the 17 ℃ treatment, and had the largest 95% confidence interval. A one-way ANOVA test was then performed on all of the growth rates for each sample giving the result $p = 0.003875$ and $F_{2,6} = 5.143$. Since this is a significant result, further Turkey-Kramer tests were done to test for significance between each temperature. The returned a result of $p < 0.01$ for all pairs of treatments of 17 ℃, 20 ℃, and 30 ℃. Note full results of the ANOVA and Tukey-Kramer tests can be found in Figures 6,7 in Appendix A.

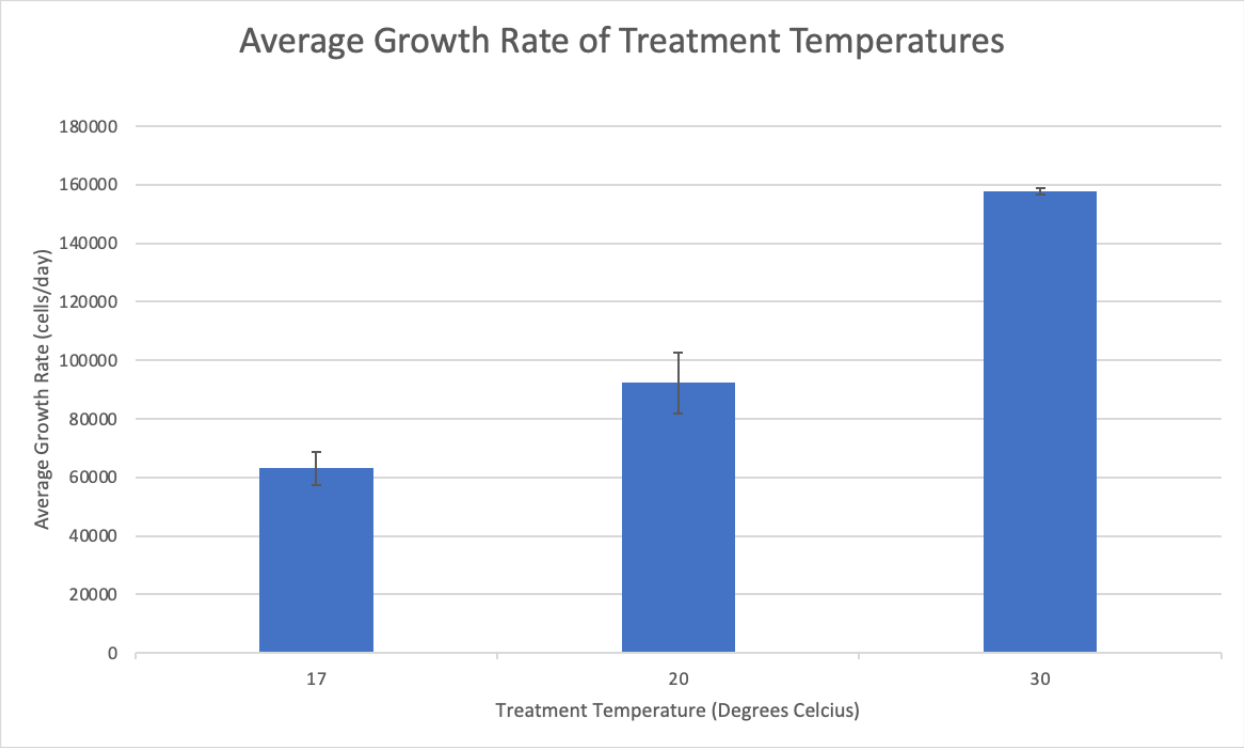

Figure 3. Average growth rate (cells/day) of *E. gracilis* grown in temperature treatments of 17℃, 20℃, and 30℃, each with three replicates over the twelve-day sampling period. As well as the 95% confidence interval for each growth rate.

Discussion:

The growth rate of *E. gracilis* is dependent on temperature and the relationship is positive because as temperature increases, the number of cells per day also increases. We reject the null hypothesis that there is no correlation between the growth rate of *E. gracilis* and temperature. Further statistical analysis shows that the different temperature treatment groups are significantly different from each other in regard to growth rate. Therefore, we support the alternate hypothesis and prediction that *E. gracilis* will have a higher growth rate at a temperature near 30℃ since that has been seen as the optimal growth temperature for *E. gracilis*.

The results correlate to what other scientists have found. Higher temperatures between 25°C to 30°C promote cell division of *E. gracilis* while colder temperatures between 13°C to 17°C are not sufficient for the reactions driving growth, resulting in a slow growth rate (Buetow, 1962). Also, it was found that the maximal growth rate of *E. gracilis* occurs at 29°C (Cook, 1966). Ko, et al. (2019) uncovered similar results that are consistent with our study, showing that lower temperatures cause lower growth rates. Interestingly, 30°C did not result in the highest growth rate for their study. 30°C treatment had a unique positive spike then a decrease in cell counts. They speculate that cell damage inhibited the ability for *E. gracilis* to grow because their cell counts only lost momentum in growth. Our study also had troubles with *E. gracilis* growth rates at 30°C but our growth rates shifted to a negative slope from Day 7 onwards with the overall amount of cells being greatly reduced. We believe our cells reached a plateau of growth at Day 7 and then cell death and possible disintegration occured since we were seeing cell count numbers that were drastically lower than the previous days.

When conducting the experiment, there were many sources of errors that could have arised. For example, when creating our diluted solution of *E. gracilis*, there could have been contamination. Furthermore, pipetting and sampling from our diluted solution of *E. gracilis* into test tubes and centrifuge tubes may not have been completely sterile. We did our best to keep conditions sterile, but we have no way of determining if the samples were indeed sterile. Another factor could be the time that the test tubes were sitting at room temperature during collection. We tried to sample and return the test tubes to the incubators as quickly as possible but in order to ensure that they all had the same exposure to room temperature, we collected all the test tubes from their respective incubators at the same time. One other possible error could be the activity

of people opening and closing the incubators. We don't know how often people used the incubators but we hope it is similar and consistent in use. Most importantly, human error with hemocytometer cell counts could have been a source of error. We counted 150-160 cells in the hemocytometer grid and then determined how many boxes were occupied to make counting easier and consistent for our group members but that might have caused too much variation and error. Previous studies don't provide information on how they specifically did their hemocytometer cell counts. But to minimize this error, the cell count for each sample was done in duplicate, with the counts being done manually by two different people. Although this could have altered the results, both individual cell counts of 30°C after Day 7 showed that *E. gracilis* cells decreased. Furthermore, we thoroughly mixed the fixed cells before placing them in the hemocytometer. Clicker-counters were also used to help keep track of the number of cells in the hemocytometer slide. If errors occurred in the cell counts, growth curves and growth rates could be greatly impacted. A possible suggestion would be to use an automated cell counter or image analysis software to reduce variation and obtain more accurate cell counts.

Testing occurred over a small range of temperatures (17 ℃, 20 ℃, and 30 °C). For future studies, observing a larger range of temperatures would be of interest to see the contrast growth rate of *E. gracilis* at colder and warmer temperatures. It would also be beneficial to do cell counts every day for an overall understanding of growth rate but due to time constraints, this experiment sampled every other weekday. With limited time to prepare samples and count, each temperature treatment was sampled in triplicates and cell counts were done in duplicate. Ideally, a larger sample size with possibly more replicates for temperature sampling and cell counts in triplicate would increase the confidence and reliability of the measurements.

Conclusion:

Based on the results of this study, our prediction was supported in that the cells grown at 30°C demonstrated the highest growth rate out of the three treatments (17°C, 20°C, and 30°C). We reject the null hypothesis and therefore support our alternative hypothesis that temperature does affect the growth rate of *E. gracilis*. As temperature increases, cell division is favoured. Our findings are important as small freshwater systems are more vulnerable to rising temperatures and climate change is one of the main causes of the algal blooms. The results of our study are aligned with several previous studies regarding temperature and its effects on *E. gracilis*.

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Appendix A:

Sample	Cell Count	Sample	Cell Count	Sample	Cell Count
Day 0	14736				
17 C Day 2 1	15562.5	20 C Day 2 1	29062.5	30 C Day 2 1	120190.476
17 C Day 4 1	69750	20 C Day 4 1	69824.1758	30 C Day 4 1	240849.057
17 C Day 7 1	118239.5	20 C Day 7 1	360333.334	30 C Day 7 1	503367
17 C Day 10 1	214090.5	20 C Day 10 1	372000	30 C Day 10 1	412019.139
17 C Day 12 1	342240	20 C Day 12 1	341909	30 C Day 12 1	182477
17 C Day 2 2	10312.5	20 C Day 2 2	27562.5	30 C Day 2 2	137510.87
17 C Day 4 2	62250	20 C Day 4 2	131771.535	30 C Day 4 2	369428.572
17 C Day 7 2	124186.5	20 C Day 7 2	333824.561	30 C Day 7 2	460500
17 C Day 10 2	225786.5	20 C Day 10 2	325200	30 C Day 10 2	353078.5
17 C Day 12 2	339835.5	20 C Day 12 2	425210.5	30 C Day 12 2	
17 C Day 2 3	9375	20 C Day 2 3	14625	30 C Day 2 3	133500
17 C Day 4 3	68950	20 C Day 4 3	107398.299	30 C Day 4 3	302571.429
17 C Day 7 3	128865	20 C Day 7 3	244680	30 C Day 7 3	483892
17 C Day 10 3	193901.5	20 C Day 10 3	349492	30 C Day 10 3	498000
17 C Day 12 3	293102.5	20 C Day 12 3	504238.5	30 C Day 12 3	368219.869

Figure 4. Table of the cell density (cells per mL) of each treatment sample (17℃, 20℃, and 30°C, n=3) of *E. gracilis* through the 12 day sampling period.

Growth Rate of Each Treatment Sample (cells per day)			
	17 ℃	20 ℃	30 ℃
Sample #1	65188	84434	158655
Sample #2	66682	89924	156921
Sample #3	57295	102554	157654

Figure 5. Growth rate of each sample, calculated through a linear regression of the cell density over time for each treatment sample (17℃, 20℃, and 30°C, n=3) of *E. gracilis* through the 12 day sampling period.

SUMMARY				
Groups	*Count*	*Sum*	*Average*	*Variance*
17	3	189165	63055	25441209
20	3	276912	92304	86331900
30	3	473230	157743.333	757674.333

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.4104E+10	2	7051845409	187.997769	3.8751E-06	5.14325285
Within Groups	225061567	6	37510261.1			
Total	1.4329E+10	8				

Figure 6. Output from an ANOVA test in excel with alpha 0.05, for each treatment temperature (17℃, 20℃, and 30°C, n=3) of *E. gracilis*, the first table shows the average and variance in each treatment temperature. The second table shows the degrees of freedom, F critical value, sum of square (SS), mean square (MS) and p value.

	Q statistic	p-value
17 Vs 20	8.272	0.003
20 Vs 30	26.78	0.001
17 Vs 30	18.51	0.001

Figure 7. Output of the post-hoc Turkey Kramer test calculated with Excel, comparing the difference in growth rate between each treatment temperature (17℃, 20℃, and 30°C, n=3) of *E. gracilis.* The table depicts each treatment pair as well as their corresponding Q and p-value.