Effects of Temperature Variance on *Euglena Gracilis* Growth Rate

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Abstract

Euglena gracilis, single-cell flagellate eukaryotes, are a diverse genus which can be found in both freshwater and saltwater. Salmon in freshwater depends on *Euglena gracilis* as a nutritious food source. To understand how various temperatures will impact the growth of salmon, we grew *Euglena gracilis* at different treatment temperatures. Four incubation temperature treatments were 13°C, 20°C, 25°C (optimal), and 30° C. Three replicates of each treatment, containing culture and *Euglena gracilis* were incubated for two weeks. Then, we collected data and calculated the growth rate of the species at 13°C, 20°C, 25° C, and 30°C and found 13111, 58921, 75998, 32685 cells/mL/day respectively. Our one-way ANOVA analysis shows that the f-calculated value equals 20.551, is greater than the f-critical value, 3.49 at α=0.05. And the calculated p-value is less than 0.05, which indicates that there is a significant difference between the growth rates at the temperature treatments. Therefore, we rejected our null hypothesis. A Tukey-Kramer test concluded that the calculated p-values for 13°C treatment growth rate was less than the p-value of 0.05 in comparison to 20, 25, and 30°C treatments. There was no statistical difference among the 20, 25, and 30°C treatments. Therefore, 13°C is the only temperature treatment that has a growth rate that is significantly different from the other temperatures.

Introduction

Euglena gracilis are microalgae who play an important role in aquatic food production, providing food for fish, converting CO_2 to O_2 and remedying water quality (Kitaya, Azuma & Kiyota, 2005). *Euglena gracilis*, a photosynthetic protist, produces protein, unsaturated fatty acids, wax esters, and a unique β-1,3-glucan called paramylon, along with other valuable compounds (Wang et al, 2018). Of these products, Paramylon, whose production is unique to Euglena species, has gained commercial interest as important human diet (Harun et al, 2010).

As previously discussed in this course, salmons have an inconceivable importance among Canadians and people of British Columbia for its role in cultural, economic and ecosystem (Mantua et al., 2010). During spring, salmons migrate to freshwater to spawn and

lay eggs. *E. gracilis* are the primary food source for larval and juvenile salmon, and for the larvae of some crustacean and fish species in mariculture (Brown et al., 1997). They are also the primary diet of zooplankton reared as food for late-larvae and juveniles of some crustacean and fish species (Brown et al., 1997).

Previous studies have shown that temperature fluctuations due to global warming can affect the microalgae population trend in freshwater (Roleda et al., 2013). This change in trend can have a cascading effect on the lives of other species who depend on microalgal food resources. For example, consider *Chromulina ochromonoide,* as former research has found that the higher temperature in spring will cause and excess rate of reproduction in *C. ochromonoide* (Roleda et al., 2013). This excess growth leads to a time-shift in their growth pattern which then precipitates to an early decrease in their population (Roleda et al., 2013). Considering the fact that salmons migrate to fresh-waters in the spring and they partially rely on *E. gracilis* as a food source, a change in *E. gracilis* population will have impacts on salmon's life (Chittenden et al. , 2010).

In the present study, effects of temperature on of *Euglena gracilis* were investigated to determine the optimum temperature for their growth. *E. gracilis* have an optimum rate of growth at 25 degrees Celsius under 12 hours light and 12 hours dark cycle provided by cool white fluorescent lights (Li et al., 2009). Considering *E. gracilis* optimal growth rate, 25 degrees Celsius was chosen to be the control treatment in this study. As previously measured, the mean temperature of the Salish Creek was found to be 13 °C. For the sake of comparison, we have also included a 13° C treatment. In consideration of the time we have for this study and available facilities, we have included two other treatments of 20° C and 30°C which are five degrees lower and higher than the optimal growth temperature.

In this study, our null hypothesis is that temperature has no effect on the growth rate of *E. gracilis*. The alternative hypothesis would be that temperature has an effect on the growth rate of *E. gracilis*, which would require further analysis to determine which temperatures are exerting an effect. We predict that environments warmer and colder than the optimum temperature of 25° C will affect *E. gracilis* reproduction rate negatively.

Methods

Initial Preparation

An overview of the experimental methods can be seen in figure 1. The initial preparation took place on October 28th, 2019. We first determined the cell concentration of the original stock *E. gracilis* solution. Using an average of three counts with the haemocyte, the original concentration was determined to be 5.17 $x10^5$ cells/mL. We then diluted 11mL of the original concentration with growth media to a final concentration of 2.87 $x10^5$ cells/mL. 10mL of the diluted solution was put into 16 test tubes. The initial pH and temperature were 6.5 and 20°C.

Treatments

Four temperature treatments: 13, 20, 25, and 30°C had four replicates, leading to a total of 16 test tubes. The 20°C and 25°C incubators were located in room 2019 while 13°C was in room 2107 and 30°C in room 2115. These incubators had a light cycle of 12 hours. The 20°C as well as 13°C incubator model was Panasonic cooled incubator MIR-254-PA with the lumen 500 lux. The incubator for 30°C was Sanyo Versatile Environmental Test Chamber. The model for 25°C was VWR scientific while the brightness was 1153 lumens.

Counting with Hemocytes

Samples from the incubated tubes were taken every two days with the exception of the period between Oct 1st and Oct 4th as the lab was not open over the weekend. Every two days, we collected 100ul of each sample and added 10ul of fixatives to be counted into Eppendorf tubes. These samples were stored in the 4°C fridge when not being counted.

In order to count the cell density of *E. gracilis* in these samples, we visited the lab every day from Oct 30th to Nov 13th. This was done with a Zeiss microscope with 10x objective lens. On the haemocyte, 20ul of sample from the Eppendorf tubes were resuspended with a 20-200ul pipette and added to the hemocyte. Then a coverslip was placed over the hemocyte. We used Tally Counter to count over 50 cells and calculated the cell density by multiplying it with the corresponding dilution factor. In counting, we ignored cells on the right and bottom margins but included the ones on the top and left.

Methods of Graphing

To graph the growth of *E. gracilis* cell population for different temperature treatments, excel was used. For the statistical test, we used 1-way ANOVA with both f-calculated and p-values. An additional statistical test using Tukey Kramer analysis was carried out to further analyze the results. These tests were done using excel as well.

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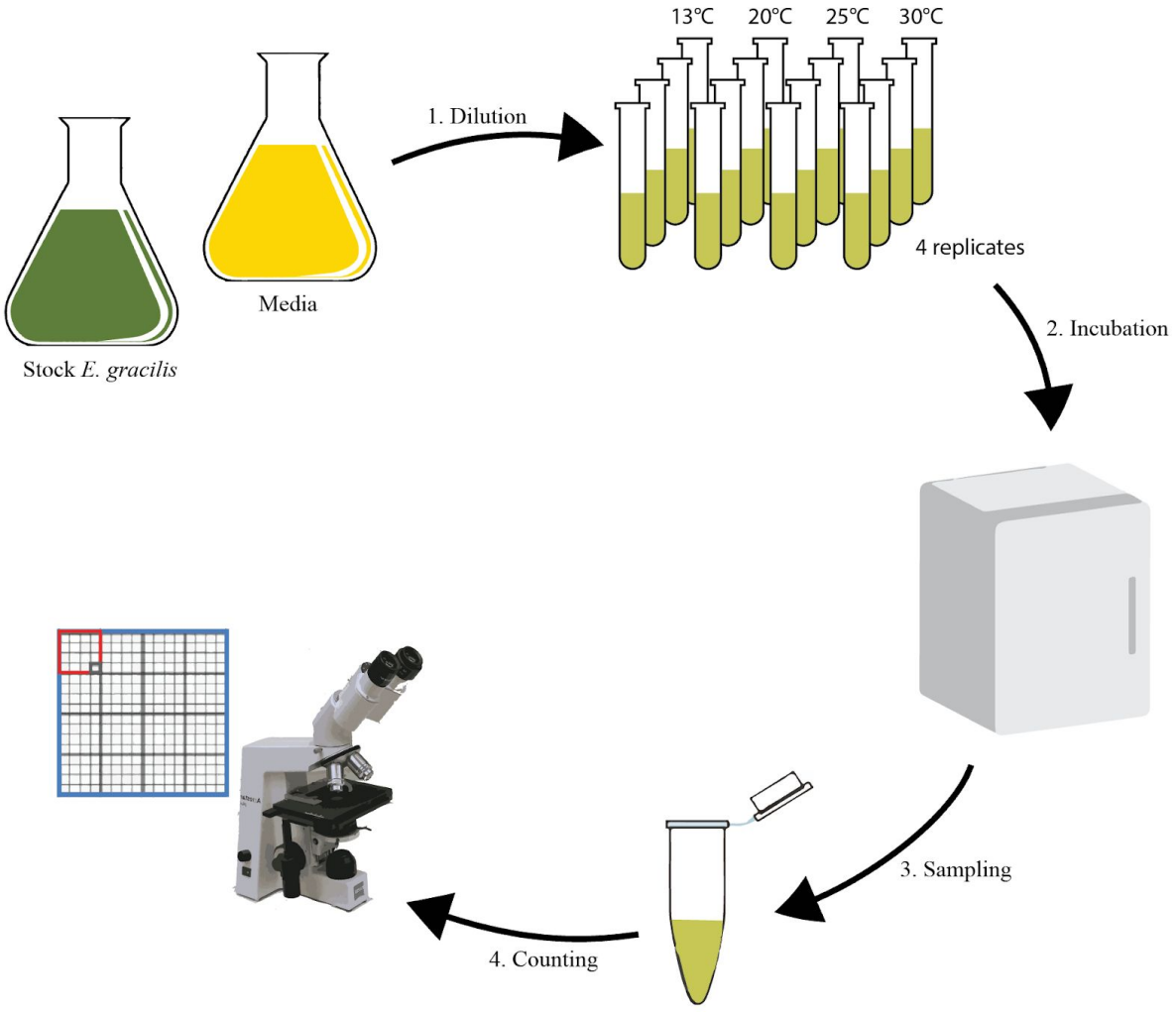

Figure 1. General overview of the experimental methods used. First, the dilution of stock *E. gracilis* and growth media was divided into 16 test tubes. These test tubes were incubated in temperature 13°C, 20°C, 25°C, and 30°C. Every two days, we would take a sample and put it into an eppendorf tube with fixative to be counted.

Results

The counted *E. gracilis* population was was adjusted for dilution through a hemocytometer. In order to plot a growth curve over 11 days, the *E. gracilis* cell density was averaged over 4 replicates for each treatment (Fig. 2). The general trend is towards increased cell density for all temperature treatments, although 13 degrees treatment had periods of decreased cell growth (Fig. 2). Of the four treatments, 25 degrees treatment had the most

exponential growth, although growth slowed past the day 7 period (Fig. 2). While we had been expecting our control, 25°C to have the most exponential growth, we predicted that all the temperature treatments would have a uniform logistic growth shape. This was not the case as evident in the 13°C and 20°C treatment (Fig. 2). 1-way ANOVA was used to compare the mean growth rates between each temperature treatment. We have omitted day 0 because *E. gracilis* has an initial lag phase during their growth.

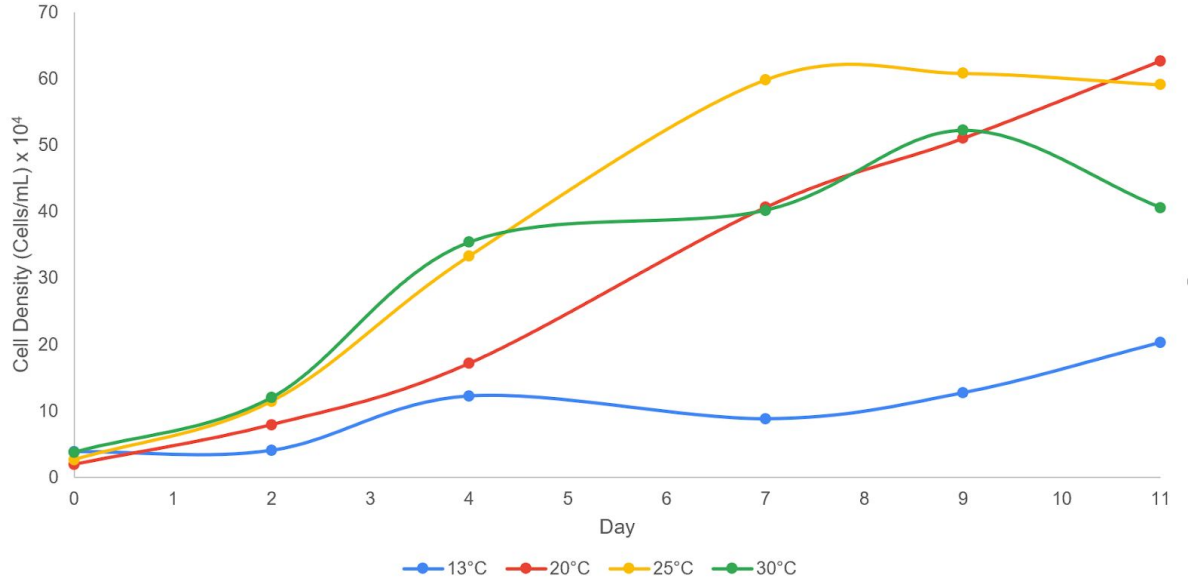

Figure 2. Growth curve of *E. gracilis* over a period of 11 days. The four temperature treatments show various degrees of growth progression.

ANOVA Test

We compared the mean growth rates at each temperature treatment. This was done by generating a scatter plot for each of the 4 replicates and determining the slope of the line of best fit as the growth rate. A scatterplot showing the average cell density among the 4 replicates is shown in figure 3. The slope obtained from each graph from the 4 replicates was analyzed with 1-way ANOVA. For the statistical test, our degree of freedom between groups was 3 as we had 4 temperature treatments, while degree of freedom within groups were 12

because we had 16 data points. The result of our statistical analysis 1-way ANOVA was that the f-calculated value was 20.551 while the f-critical value was 3.490. Here, the f-calculated is greater than the f-critical value. This result indicates that the variance between the means of the 4 treatments are significantly different. Therefore, the null hypothesis that the mean growths between each temperature are the same, can be rejected. This is also confirmed by looking at the calculated p-value of 0.00005, which is much smaller than the critical p-value of 0.05.

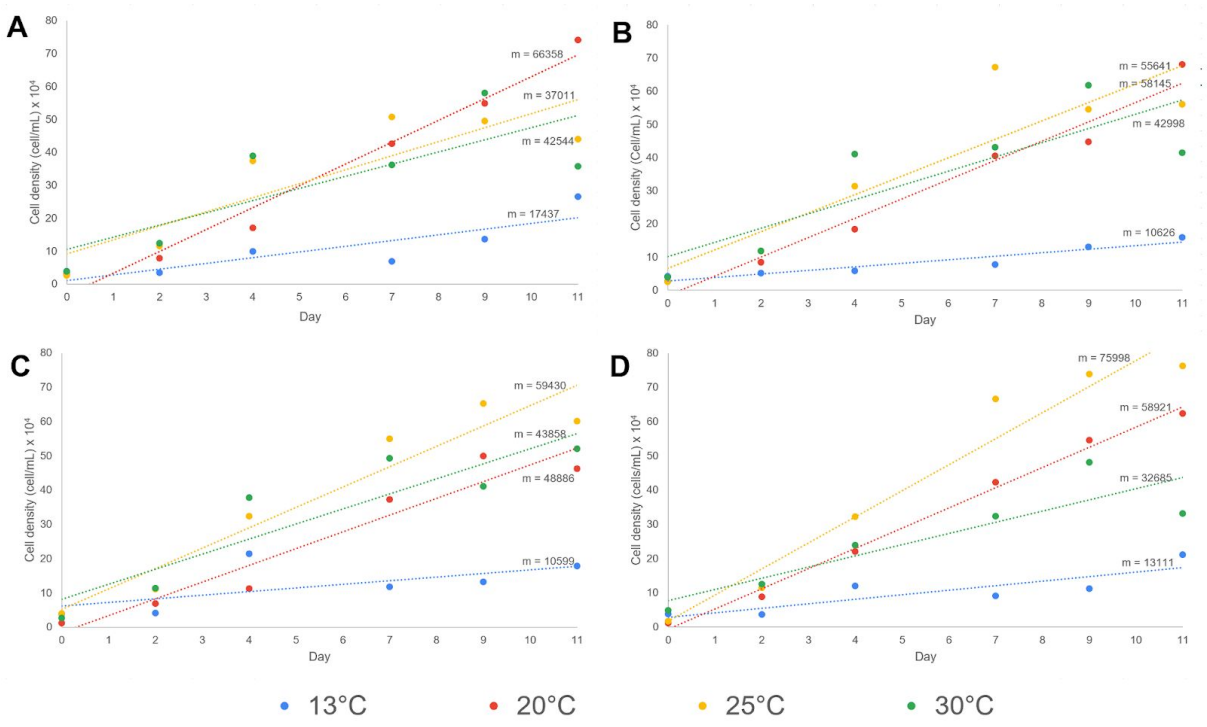

Figure 3. The cell density means of the four replicates (A-D) were plotted. A linear line of best fit was used to determine the slope (m) which is the growth rate. This growth rate was later used for one-way ANOVA.

Tukey-Kramer Test

Since the results of ANOVA indicated that one or more pairs of temperature treatments are significant, a Tukey-Kramer test was done to determine which temperature treatment differed in their mean growth rates. There were 4 treatments while the degrees of freedom was 12. The calculated p-values for 13°C treatment growth rate compared with 20,

25, and 30°C treatments was 0.001, 0.001. And 0.006 respectively. These results are less than the p-value of 0.05, indicating that the growth rate between 13°C and other temperature treatments are significantly different from each other. In contrast, 20°C and 25°C have a p-value of 0.900 which is greater than the p-value of 0.05. A similar result was observed in comparisons between 20°C and 30°C as well as 25°C and 30°C with p-values 0.083 and 0.108. Both of these have p-value greater than 0.05, which signify that the two temperature treatments do not have a significant difference between their growth rates. 13°C was the only temperature that had a growth rate that was significantly different from the other treatments. This pattern can also be seen in figure 3, where the slopes of the 20, 25, and 30°C treatments are more similar to each other than the 13°C temperature's slope. In addition, a box plot of the mean growth rates clearly illustrate how 13°C has a mean growth rate that is different from the other temperature treatments (Fig. 4).

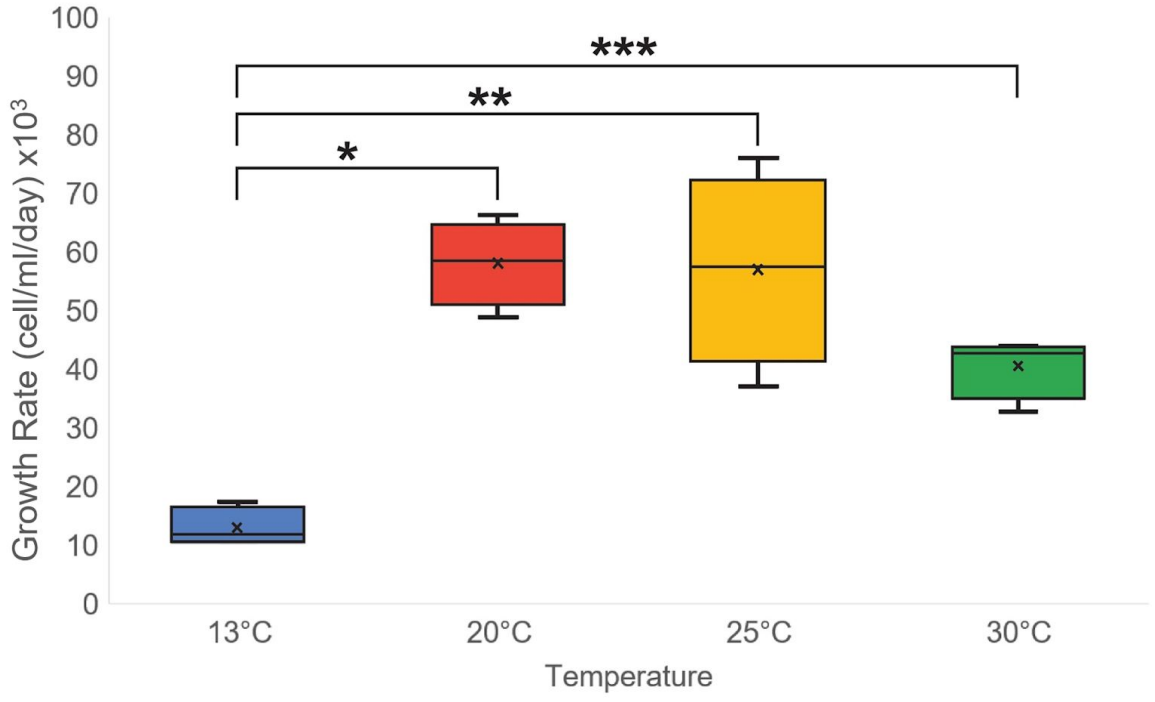

Figure 4. Box plot of growth rates for 4 temperature treatments. n=16. The upper and lower quartile are shown with whiskers. The means are visualized with an X while the median is the

horizontal line in the box. Results for one-way ANOVA showed f-calculated = 20.551 and p-value = 0.00005 with df_1= 3, df_2 =16 and n = 16. The difference between the mean growth rates are significantly different. The 13°C treatment's growth rate is significantly different from the other treatments as the calculated p-value was found to be *0.001, **0.001. And ***0.006 when compared with 20°C , 25°C and 30°C.

Discussion

Interpretation of Results

Analysis of data using 1-way ANOVA gave us the f-calculated and the p-value. The null hypothesis for ANOVA is that the mean growth rates of the temperature treatments are the same. We were able to reject the null hypothesis due the f-calculated value being greater than the f-statistic. This was also in agreement with the calculated p-value being smaller than 0.05 given by ANOVA. Therefore, since the mean growth rates of each temperature treatment are significantly different, we reject the null hypothesis that temperature does not affect growth rates. This leads us to lend support to the alternative hypothesis; temperature does have significant effect on *E. gracilis* growth. Then we sought to determine which of the temperature treatments had significant differences from each other with a Tukey-Kramer test. The results showed that the calculated p-values for 13°C treatment growth rate was less than the p-value of 0.05 in comparison to 20, 25, and 30°C treatments. However among the 20, 25, and 30°C treatments, there was no significant difference between the means of growth rates. Therefore, 13°C is the only temperature treatment that has a growth rate that is significantly different from the other temperatures. Lower temperatures have the potential to result in lower metabolic rates, resulting in lower growth rate for the 13°C treatment (Muylaert et al., 2000).

According to figure 2, our results indicate E. gracilis reaches the highest growth rates at 25°C and 30°C, since previous studies have projected optimal growth rate to range from 27 to 30°C (Wang et al., 2018). However, we anticipate that there is a lower exponential growth phase as seen in figure 3 for treatments 20°C, 25°C, and 30°C, due to an accumulation of biomass in the test tubes, leading to self-shading (Wang et al., 2018). This concept of self-shading occurs when *E. gracilis* blocks others from a light source when biomass becomes high in the given volume (Wang et al., 2018). This is possible given that we observed the the treatments: 20°C, 25°C, and 30°C, to be more pigmented in comparison to the other test tubes. Given this information, it explains why individual growth rates similar in those temperature treatments. Thus, a lower than expected individual growth rate of *E. gracilis* displayed in our results for those temperatures. On the contrary, our lowest growth rate occurred in our treatments of 13°C, which aligns with our predictions and previous literature since *E. gracilis* has the best growth rates in temperatures ranging from 27 to 30°C (Wang et al., 2018). Using these results, we can link the effects of temperature on salmon in British Columbia based off *E. gracilis* growth and biomass.

Implications on Salmon

Salmon are an integral part of the British Columbian ecosystem due to the ecological, economical, and cultural practices that depend on this keystone species (Mantua et al., 2010). One can derive that the food availability for salmon would be highly significant in maintaining salmon populations. Therefore, *E. gracilis* have an important role in the food web as mixotrophic protists since salmon feed on protists (Muylaert et al., 2000). The current freshwater temperatures in the southern British Columbia averages from 13 to 15°C (Mantua et al., 2010). Linking this to our results, since *E. gracilis* have a slow individual growth rate at 13°C, there would be low *E. gracilis* biomass in freshwater systems. This could impact

salmon populations due to lower food availability, and thus salmon populations would be expected to be lower than if there were higher *E. gracilis* biomass. Since, the optimal temperature for *E. gracilis* growth from 27 to 30°C, we attempt to extrapolate our results to the impact of higher temperature on salmon as well (Goniea et al., 2011). The issue for salmon at these higher temperatures is that salmon react lethally in temperatures over 24°C (Goniea et al., 2011). As a result, high *E. gracilis* biomass would have a negligible impact on salmon populations at 27 to 30°C because the salmon will be dead due to harsh physiological conditions of low dissolved oxygen, and higher carbon dioxide (Goniea et al., 2011).

Limitations

Due to time constraints, we were unable to complete more counts to add to our growth curve. We believe that had there been one more week of sampling, we could determine the peak *E. gracilis* cell density for each treatment group. As seen in figure 2, it appears the cell density at day five of sampling for the 20°C treatment surpasses 25°C. As aforementioned, three replicates of cell counts were done by three different people for each temperature treatment. As a result, there was a systematic variation since each individual has different accuracy and precision, leading to results such as consistently counting higher or consistently lower than the lab-partner counterparts. This was evident when reviewing our raw cell counting data.

During our study proposal, we initially wanted to have treatments at more extreme temperatures: 15, 25, 35, and 45°C. Unfortunately, we had to modify these treatment temperatures due to laboratory equipment availability, and what other students required as well. Due to this, perhaps we needed a larger temperature difference to observe results that show distinguishable growth rates between all temperatures. This may be one of the factors that caused us to have insignificant differences between 20°C, 25°C, and 30°C in our results.

In addition to time constraints, in-lab experiments can differ from in-situ experiments. This variability between different environments will generally cause in-lab experiments to have results that showcase an ideal scenario (Cauchie et al., 2000). For instance, in our study, we calculated the ideal media volume needed for E. gracilis to grow. Although we know E. gracilis is mixotrophic, the fact that organisms always had a sustenance source, is not an accurate reflection of availability of nutrients in the environment. Since in-situ conditions are not always ideal for organisms. Due to this, our results show optimized, or higher than normal cell counts than what is reflected in in-situ conditions. Furthermore, all samples were kept in incubators with an artificial light and dark cycle of twelve hours each. These light cycles were preset and out of our control, and as such, may have had different lux emitted. Since *E. gracilis* are freshwater species found in British Columbia, they experience the seasonal changes of light; so, in-situ light conditions would differ from in-lab experiments depending on seasonality. For example, experience more light in the spring and summer months, and less light in winter and fall which would affect their ability to photosynthesize. As a result, our growth curves exhibit idealized conditions for *E. gracilis* growth.

Conclusion

Euglena gracilis plays a significant role in the freshwater food web as a mixotrophic protist. Furthermore, it is the food source of salmon which is a keystone species in the British Columbian ecosystems. We found evidence that supports the hypothesis that different temperatures induce different rates of growth between 13°C and the other temperatures 20°C, 25°C, and 30°C. Our results indicate that *E. gracilis* has a higher growth rate at 20°C, 25°C and 30°C than at 13°C which conflict with the ideal temperatures for salmon growth. For future studies, we suggest further longitudinal studies of growth rates of *E. gracilis* in-situ to

imitate the natural environment. This organism sits low on the food chain, thus studying primary consumers like *E. gracilis* is bound to unveil strategies to optimize populations of reliant organisms.

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