Effect of elevated temperature on *Euglena gracilis* growth rate

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Euglena gracilis, a unicellular microalgal species, play an integral role in marine ecosystems. However, climate induced temperature change is expected to decrease the abundance of these flagellated protists (Roleda et al, 2013). Here, we measured the growth rate of *E. gracilis* following incubation in high temperature environments in order to mimic potential global warming conditions. *E. gracilis* was grown for two weeks at incubation temperatures of 25ºC, 30ºC and 35ºC (n = 3 for each treatment). The average growth rate of each sample was found to be 0.00437 ± 0.00178, 0.00403 ± 0.00123, and 0.00337 ± 0.00173 %/hour respectively. A one-way ANOVA analysis resulted in an F-value of 0.634 and a corresponding p-value of 0.563. These results are statistically insignificant at $\alpha = 0.05$ and suggest that elevated temperature up to 35ºC does not affect the average growth rate of *E. gracilis*.

Introduction

Found in both fresh and saltwater environments, *Euglena gracilis* form the basis of many bioregenerative aquatic systems. Capable of fixing carbon dioxide, the presence of *E. gracilis* improves water quality and provides the necessary oxygen for higher plants and marine animals (Ebenezer et al., 2019). Like many related phytoplankton species, *E. gracilis* also constitutes a significant portion of the marine food web, particularly in relation to salmon species. Relative to salmon, *E. gracilis* serve as a primary food source for larval and juvenile salmon, as well as species preyed on by salmon in later life stages (Brown et al., 1997).

Previous work involving *E. gracilis* indicates that cell division, and subsequently population size and relative abundance is temperature dependent. Studies suggest that incubation temperatures up to 29ºC promote exponential growth (Buetow, 1962). However, with global ocean temperatures predicted to rise beyond this, microalgal populations are expected to fluctuate. This will undoubtedly have a cascading effect on dependent species, especially keystone species like salmon who are already facing a decline in population due to climate change (Ferrari et al., 2017). Changes in temperature have resulted in algal blooms occurring

outside of expected growth periods and a decrease in overall fitness and population in the Pacific Northwest (Chittenden et al., 2010; Hertz et al., 2016). As salmon depend on *E. gracilis* for food, the decrease in supply may result in salmon being unable to survive, particularly during peak migration periods (Chittenden et al., 2010)

In order to quantify the potential impact of rising temperatures on *E. gracilis* populations, we computed the growth rate of our samples under different incubation temperatures. Literature suggests that 25°C is the optimal temperature for *E. gracilis* growth (Buetow, 1962). Considering this, 25°C served as the control treatment in this study. *E. gracilis* was also cultured at 30°C as it best represents current ocean temperatures in the Pacific Northwest and 35°C to represent conditions if warming continues to rapidly increase. We hypothesized that growth rate would be highest at 25°C and significantly lower at 30°C and 35°C as these treatments fall outside of the optimal range.

Methods and Materials

Using a haemocytometer, we first determined the cell density of a cultured stock solution of *Euglena gracilis.*

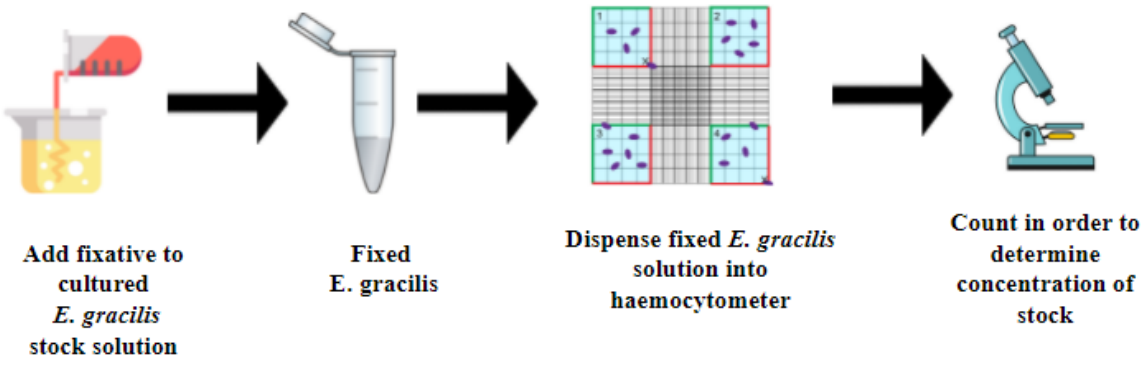

Figure 1. Schematic diagram summarizing the procedural steps taken to determine the initial stock concentration of cultured *E. gracilis.* Stock solution was diluted in a 10:2 ratio with fixative before counting.

Once the concentration of the stock solution was calculated, a working solution was prepared by mixing 9.091mL of the stock solution and 40.909mL of the growth medium, resulting in a final cell density of approximately 1×10^5 cells/mL. 5mL of the working solution was pipetted into nine individual test tubes, resulting in three replicates for each incubation temperature (25ºC, 30ºC, and 35ºC).

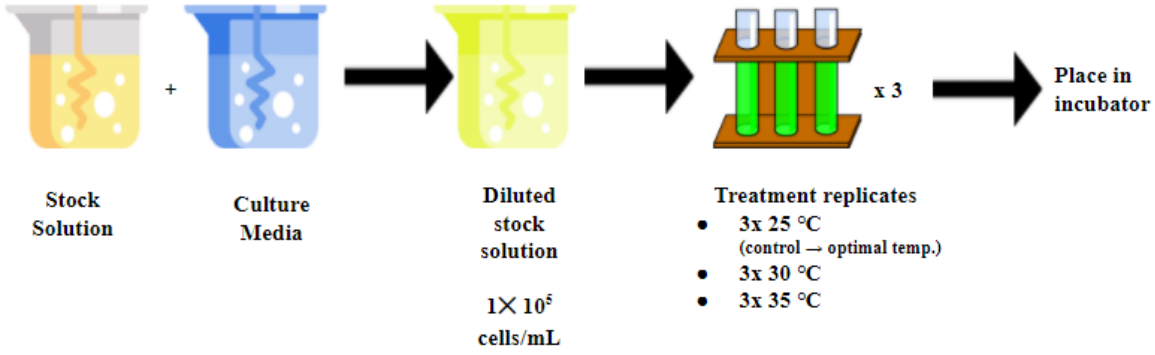

Figure 2. Schematic diagram summarizing the preparation of diluted *E. gracilis* samples for incubation at 25ºC, 30ºC and 35ºC (n=3 for each temperature treatment). Stock solution was diluted with culture media to a final concentration of 1×10^5 cells/mL.

Once removed from the incubator, the test tubes were vortexed for roughly 10 seconds in order to ensure that cells were evenly distributed throughout the sample. Each test tube was held briefly over a flame before and after sampling and a new pipette tip was used each time in order to maintain sterility. 100 µL of each sample was pipetted into an appropriately labelled PCR tubes along with 20 µL of PREFER fixative and thoroughly mixed using the pipette in order to ensure that the fixative came into contact with the entire sample and no further growth occurred. The test tubes were returned to their respective incubators and the fixed PCR samples were stored in a refrigerator at 4ºC.

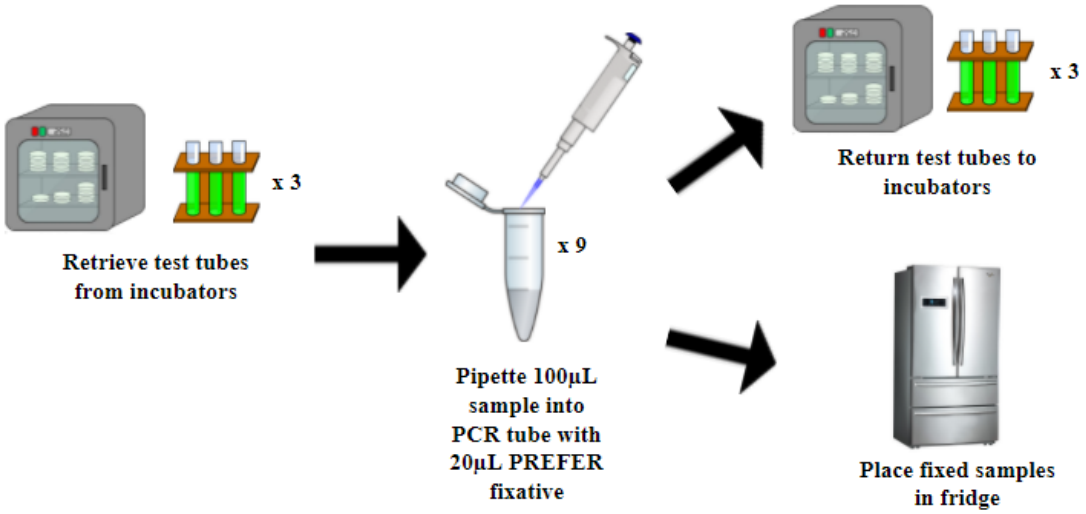

Figure 3. Schematic diagram summarizing the procedural steps taken to prepare a fixed sample of *E. gracilis*. 100µL of the *E. gracilis* solution was pipetted into a PCR tube with 20µL of PREFER fixative.

The cell densities for each sample was determined using a haemocytometer and the diluted stock calculation.

Results

Samples from different incubation temperatures appeared to differ significantly when inspected visually, While samples grown at 25ºC were bright green, samples grown at 30ºC and 35ºC were visibly paler in colour.

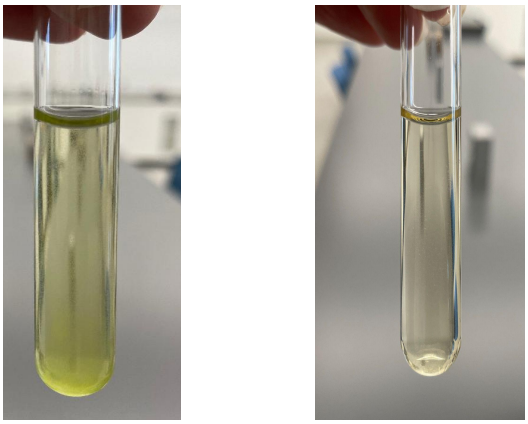

Figure 4. *Euglena gracilis* samples 25A (left) and 35A (right) grown at 25ºC and 35ºC after 168 hours of total incubation time.

The raw count data obtained from the experiment was imported into RStudio version 4.1.1. The cell density was averaged across the three replicates for each temperature to obtain a total of three cell density curves, one for each temperature treatment. This data was then graphed in order to visualize the cell population density of each temperature as the experiment progressed.

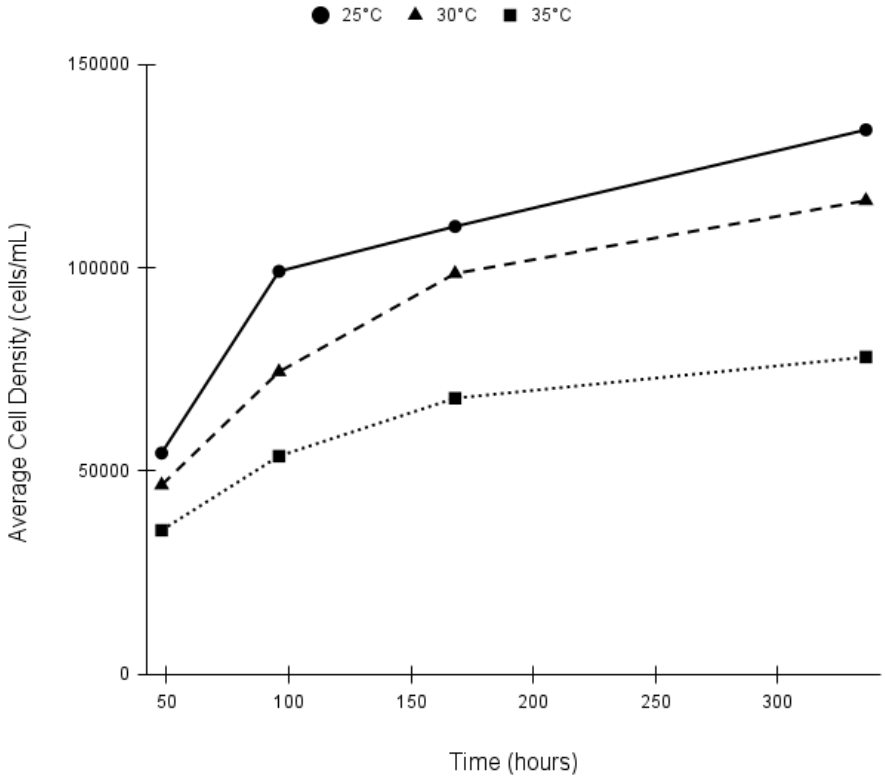

Figure 5. The average cell density in cells per milliliter of *Euglena gracilis* grown at 25ºC, 30ºC, and 35ºC over the duration of the experiment in hours.

Growth rate (*r*) for each time interval was determined using the formula $P(t) = P_0 \times e^{rt}$ with P being the population at time (t), P_0 being the initial population, r being the growth rate in %, and t being time in hours. For example, the growth rate for sample 25A at 96 hours was calculated as follows: $112667 = 57750 \times e^{r \times 96}$, resulting in r = 0.00696. These growth rates were

then averaged to obtain an overall growth rate for each replicate within each temperature, for a total of nine growth rates.The growth rates were then averaged once again to obtain a final average growth rate for each temperature. The standard deviations were calculated in a similar manner. The average growth rate was computed to be 0.00437 ± 0.00178 %/hour at 25ºC, 0.00403 ± 0.00123 %/hour at 30ºC, and 0.00337 ± 0.00173 %/hour at 35ºC. The growth rates for each temperature were graphed with their respective standard deviations.

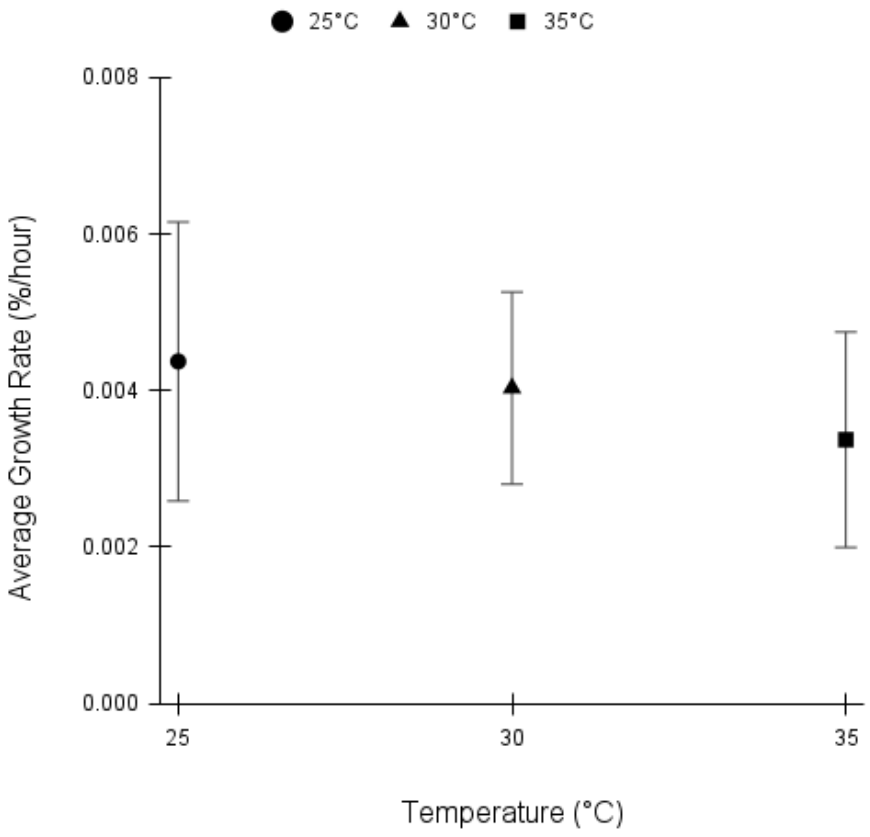

Figure 6. The average growth rate in population percent per hour of *Euglena gracilis* grown at 25ºC, 30ºC, and 35ºC with error bars.

RStudio was then used to perform a one-way ANOVA to determine if temperature had a significant effect on the growth rate. This result came back insignificant, with an F-value of 0.634 and a corresponding p-value of 0.563 at $\alpha = 0.05$.

Discussion

Trends observed in our results parallel those in the established literature and our original hypothesis. The samples grown at 25°C had the highest average cell density as well as the highest average growth rate among all temperature treatments. As the temperature increased, a decrease in the average cell density and average growth rate was observed. However, statistical analysis of our raw data suggests that elevated incubation temperature does not affect the average growth rate of *E. gracilis.* Therefore, we fail to reject our null hypothesis that temperature does not affect growth rate of *E. gracilis* and reject our alternate hypothesis that temperature affects the growth rate of *E. gracilis*. Our results appear to mimic the findings in He et al. (2021) where *E. gracilis* was found to be particularly robust and tolerant to environmental stresses. This may also be due to the growth cycle of *E. gracilis.* Based on the work of Wang et al. (2018), temperature was only found to impact the specific growth rate (exponential growth phase) of *E. gracilis* which is relatively short. As samples were fixed every other day over the course of the experiment, it is likely that insufficient samples were taken during this time and thereby failed to capture areas of significant difference. This could be remedied in future experiments by taking more samples in the initial stages of experimentation.

The discrepancy may also be due to systematic error. For example, it was difficult to guarantee that *E. gracilis* was evenly distributed when preparing the fixed samples or when pipetting onto the haemocytometer for counting. While three replicates were used in order to

prevent this, it is possible that the fixed and counted samples were not truly representative of cell density measurements, and thus overall growth rate.

Conclusion

In conclusion, our results indicate that elevated temperatures up to 35^{o}C does not play a significant role in the growth rate of *Euglena gracilis*. However, considering that *E. gracilis* plays a significant role in supporting keystone species in the marine ecosystem, it is still crucial to implement climate change initiatives that control ocean temperatures and support microalgal growth. Areas for further study include investigating growth rate over longer periods of time as opposed to the week-long period tested here as well as in situ to imitate the natural environment.

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References

1. Brown, M. R., Jeffrey, S. W., Volkman, J. K., & Dunstan, G. A. (1997). Nutritional properties of microalgae for mariculture. Aquaculture, 151(1), 315-331. https://doi.org/10.1016/S0044-8486(96)01501-3
2. Buetow, D.E. (1962). Differential effects of temperature on the growth of *Euglena gracilis*. *Experimental Cell Research*, 27(1), 137-142. https://doi.org/10.1016/0014-4827(62)90051-4
3. Chittenden, C. M., Jensen, J. L. A., Ewart, D., Anderson, S., Balfry, S., Downey, E., Eaves, A., Saksida, S., Smith, B., Vincent, S., Welch, D., & McKinley, R. S. (2010). Recent salmon declines: A result of lost feeding opportunities due to bad timing? PloS One, 5(8), e12423-e12423. https://doi.org/10.1371/journal.pone.0012423
4. Ebenezer, T. E., Zoltner, M., Burrell, A., Nenarokova, A., Novák Vanclová, Anna M G, Prasad, B., Soukal, P., Santana-Molina, C., O'Neill, E., Nankissoor, N. N., Vadakedath, N., Daiker, V., Obado, S., Silva-Pereira, S., Jackson, A. P., Devos, D. P., Lukeš, J., Lebert, M., Vaughan, S., . . . Field, M. C. (2019). Transcriptome, proteome and draft genome of euglena gracilis. BMC Biology, 17(1), 11-11. https://doi.org/10.1186/s12915-019-0626-8
5. Ferrari, M. R., Miller, J. R., & Russell, G. L. (2007). Modeling changes in summer temperature of the Fraser river during the next century. Journal of Hydrology (Amsterdam), 342(3), 336-346. https://doi.org/10.1016/j.jhydrol.2007.06.002
6. He, J., Liu, C., Du, M., Zhou, X., Hu, Z., Lei, A., & Wang, J. (2021). Metabolic responses of a model green microalga euglena gracilis to different environmental stresses. Frontiers in Bioengineering and Biotechnology, 9, 662655-662655. https://doi.org/10.3389/fbioe.2021.662655
7. Hertz, E., Trudel, M., Tucker, S., Beacham, T. D., Parken, C., Mackas, D., & Mazumder, A. (2016). Influences of ocean conditions and feeding ecology on the survival of juvenile chinook salmon (oncorhynchus tshawytscha). Fisheries Oceanography, 25(4), 407-419. https://doi.org/10.1111/fog.12161
8. Kitaya, Y., Azuma, H., Kiyota, M. (2005). Effects of temperature, CO_2/O_2 concentrations and light intensity on cellular multiplication of microalgae, *Euglena gracilis*. *Advances in Space Research*, 35(9), 1584-1588. https://doi.org/10.1016/j.asr.2005.03.039
9. Roleda, M. Y., Slocombe, S. P., Leakey, R. J. G., Day, J. G., Bell, E. M., & Stanley, M. S. (2013). Effects of temperature and nutrient regimes on biomass and lipid production by six oleaginous microalgae in batch culture employing a two-phase cultivation strategy. Bioresource Technology, 129, 439-449. https://doi.org/10.1016/j.biortech.2012.11.043
10. Wang, Y., Seppänen-Laakso, T., Rischer, H., Wiebe, M. (2018). *Euglena gracilis* growth and cell composition under different temperature, light and trophic conditions. *PLoS ONE*, 13(4). https://doi.org/10.1371/journal.pone.0195329