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Effects of Temperature on Growth Rate of *Chlamydomonas reinhardtii*

Abstract

Chlamydomonas reinhardtii is a unicellular green algae, and as a primary producer it is an essential part of our ecosystem, affecting other consumer species such as salmon. Climate change projections show that the surface temperature of bodies of water are significantly increasing, and as the water temperature increases, this consequently affects the food chain of many species. To further explore the effects of temperature, *C. reinhardtii* was incubated at 20°C, 25°C, and 35°C and sampled over a 14 day period to determine their respective cell growth. Cell density was counted for each sampled day using a hemocytometer under a compound microscope. Results showed an increasing linear trend, with 35°C having the largest cell growth. Furthermore, we determined that the mean cell growth rate at 20°C to be 2.01 x 10^6 cells/day, 25°C to be 4.11x 10^6 cells/day, and 35°C to be 6.27 x 10^6 cells/day. Using a one-way ANOVA test, it was determined that there was a statistical difference between the means of each temperature ($p=0.0024$). Further research testing the effects of climate change using the model organism *C. reinhardtii* will provide essential insight on how to help preserve our beautiful ecosystems.

Introduction

C. reinhardtii is a freshwater unicellular green algae and is described as a model organism by researchers (Harris 2001). It has a simple life cycle, it is easy to isolate mutant types, and it provides a lot of information for cell functions and structure (Harris 2015). *C. reinhardtii* can be grown photoautotrophically using temperature and light intensity (Vítová et. al, 2011).

As projected by the Intergovernmental Panel on Climate Change, global sea surface temperature will increase by 0.6°C-2°C by the end of the 21st century (2021). Therefore, it is important to study how increased temperatures due to climate change affects the rate of algae growth. Furthermore, it is essential to research the temperature tolerance of green algae such as

C. reinhardtii, as it is the primary producer of many food chains and affects many other consumer species.

An important consumer keystone species in British Columbia is salmon, partly relying on green algae such as *C. reinhardtii* to survive (Holtgrieve and Schindler 2011). Salmon are an integral part of our ecosystem; they provide an enormous food source for many species, control insect populations, and return many nutrients and organic matter back into the ecosystem (Holtgrieve and Schindler 2011). Furthermore, there is evidence that salmon with a small amount of green algae in their diet (2.5%-10%) can positively increase growth performance (Norambuena et. al, 2015). Therefore, with water temperatures increasing in recent years, it is important to understand how cell growth of primary species such as *C. reinhardtii* is affected by this, which in-turn affects the whole food chain.

To further understand the effect of temperature on *C. reinhardtii*, we conducted an experiment testing cell growth at three different temperatures. Our null hypothesis (Ho) and alternate hypothesis (Ha) are as follows:

Ho: Changes in temperature will not have a significant effect on cell growth of *C. reinhardtii*.
Ha: Changes in temperature will have a significant effect on cell growth of *C. reinhardtii*.

The objective of this study is to acclimate *C. reinhardtii* to 20°C, 25°C and 35°C and observe the cell growth at each temperature over a two week period. Previous research conducted on the growth rate of *C. reinhardtii* has found the optimal temperature to be 27.5°C (Vítová et. al, 2011). Therefore, with this information in mind, we predict 25°C to have the greatest overall increase in cell growth with 35°C being very close to the critical growth

temperature of 39°C. We predict cell growth would have a significant increase within the first week and then plateau (Zachleder et al., 2019). Moreover, as 20°C is close to room temperature, we used this as the control, expecting the least cell growth of *C. reinhardtii*.

Methods

I. Sample preparation

Nine separate cultures of *C. reinhardtii* were used to analyze the effect of temperature on its growth rate. In an Erlenmeyer flask, a working solution of *C. reinhardtii* at a concentration of 2.2 x 10^5 cells/mL was made by diluting a stock solution of *C. reinhardtii* with growth media. The initial concentration of the stock solution was determined by using a hemocytometer. 6 mL of the prepared working culture solution was then pipetted into 9 separate test tubes and capped. Three replicates for each treatment temperature were then placed in their appropriate incubators.

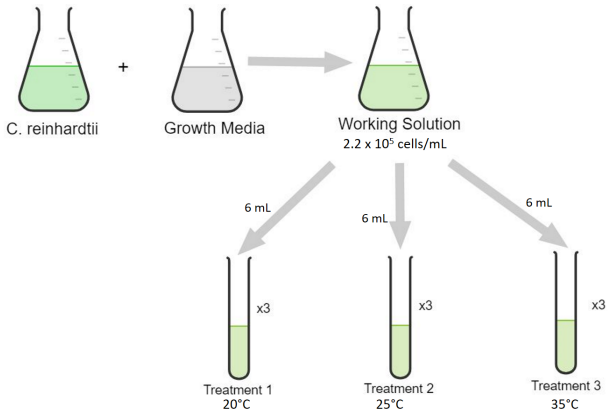

Figure 1. Schematic drawing of sample preparation of *C. reinhardtii* cultures.

II. Algae Growth

Cultures of *C. reinhardtii* were then grown for a total of 14 days starting October 26, 2021, where samples were collected every other day except for weekends. Three replicates of the

9 separate cultures of *C. reinhardtii* were grown at three treatment temperatures of 20°C, 25°C, and 35°C. The cultures were incubated over a 14-day period.

III. Cell Fixation

Samples of the 9 cultures were collected on Oct 27, Oct 29, Nov 1, Nov 3, Nov 5, and Nov 8. This was done by micro pipetting 35 µL samples of *C. reinhardtii* (total 9 cultures, 3 samples at 3 treatment temperature) incubated at each respective temperature was added to Eppendorf tubes, collecting a total of 54 samples. As *C. reinhardtii* settles to the bottom of the test tube, test tubes are mixed with a vortex before samples are collected. To inhibit further cell growth and division, 3.5 µL of iodine fixative (IKI) was added into each Eppendorf tube and thoroughly mixed with the algae. The collected samples were then placed in a fridge at 4°C until all cells were ready to count.

IV. Cell Counting

Cell counting was done on November 15 using a hemocytometer accompanied with an Axis microscope at a total magnification of 100x. Ensuring that all samples were well mixed, 12 µL of *C. reinhardtii* was placed in the hemocytometer and were counted inside one 0.2 x 0.2 mm square, the green square (Fig. 2). Cells were then counted using a hand tally and cells on the border line were not counted. The cell counts were then extrapolated to its cell density (cells/mL) by multiplying by a dilution factor of 2.5×10^5. This was then also corrected for the volume of fixative added by multiplying by a factor of 1.1 to obtain the appropriate cell density in each sample. Cell density per day (growth rates) for each temperature treatment was then determined by averaging the cell density for the three replicates at the three treatment levels. This was then graphed on Microsoft Excel. A one-way ANOVA test was then performed to determine whether

there was a difference in growth rates between each temperature treatment (20°C, 25°C, and 35°C). The statistical significance was determined at p-value < 0.05, a significant level at 95%.

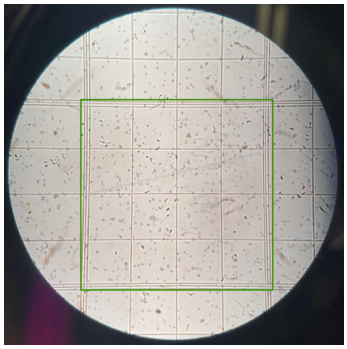

Figure 2. Cell counting done with a hemocytometer using an Axis microscope. Cells counted within the green, 0.2 x 0.2 mm square.

Results

The growth rates of *C. reinhardtii* for all samples for each separate treatment temperatures were extrapolated by graphing its cell density (cells/mL) per day. After determining the linear equation of each line, a positive linear relationship was then seen for all samples in each treatment temperature, thus a logarithmic transformation was not needed. A linear regression was done for all samples to find a best fit line to determine the slope for each sample, which represents the growth rate (cell density/day). The growth rates of the three samples were then plotted against its representative treatment temperatures (Fig. 3). The mean growth rates for the treatment temperatures 20°C, 25°C, and 35°C were 2.01×10^6, 4.11×10^6, and 6.27×10^6 cell density/day respectively.

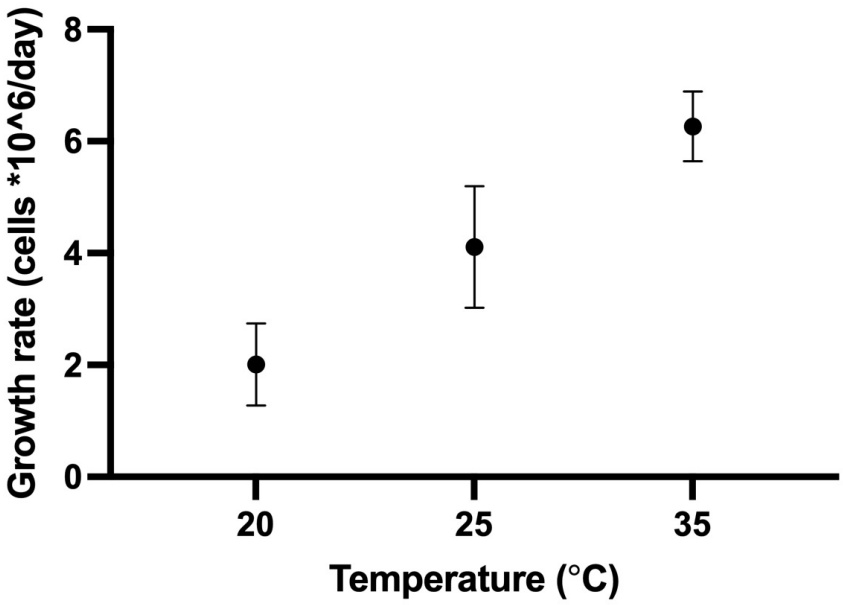

Figure 3. The average growth rate of *C. reinhardtii* (cell density/day) at 20°C (n = 3), 25°C (n = 3), and 35°C (n = 3). Error bars represent the standard deviation of the mean. (p = 0.0024).

A one-way ANOVA test revealed that there was a statistical difference between the means of the three growth rates between the independent temperature levels ($p = 0.0024$). Furthermore, a Tukey post-hoc test noted a statistically significant relationship between all treatment temperatures (20°C vs. 25°C p = 0.0499, 20 vs. 35°C p = 0.0019, 25 vs. 35°C p = 0.045).

Discussion

This study was aimed at determining how increasing ocean temperature affects the growth rate of *C. reinhardtii* and subsequent concentration in ocean environments. Cell cultures of *C. reinhardtii* were grown for 14 days and were regularly sampled and stored so that cell concentration could be counted at regular intervals. With the use of hemocytometers the amount of cells were counted, the data collected shows a significant difference in the growth rate of *C. reinhardtii* at varying temperature levels. Using a one-way ANOVA test the p value was determined to be significant (p = 0.0024). The p value collected is less than the 0.05, thus we

reject the null hypothesis that increase in temperature won't affect cell growth of *C. reinhardtii* and confirm our alternate hypothesis that changes in temperature affect cell growth of *C. reinhardtii.* A significant finding showed that the growth rate was the highest at our test temperature of 35℃ after the 14 day growth period. The results collected also showed that the second highest growth rate was at 25℃ with a rate of 4.11 x 10^6, with the lowest growth rate at the control temperature of 20℃ and a growth rate of 2.01 x 10^6. This is significant because it shows a potential benefit to the rising ocean temperatures on the rate of cell growth of *C. reinhardtii.*

Previous studies aimed at testing varying growth temperatures for *C. reinhadtii* have shown there to be a critical growth temperature where cell division is no longer possible. The supraoptimal temperature was found to be 39℃ where cell division was arrested and *C. reinhardtii* began to die (Zachleder et al., 2019). This study by Zachleder was vital in our selection of growth temperatures because it allowed us to choose a temperature, notably 35℃ where *C. reinhardtii* would still be able to grow, however still close to the growth limit threshold in order to paint an accurate picture of a wide variety of temperature. A second research study allowed our team to compare our optimal growth findings directly to another optimal growth temperature previously tested. In the paper by Lürling et al. (2012) the optimal growth temperature was determined to be 27.5°C compared to our optimal growth rate temperature of 35℃. This shows a significant difference between our two experiments that can further be tested in order to determine a more accurate growth temperature for *C. reinhardtii.*

Certain limitations of our study arise from the time constraint of a 2 week growth period and the initial concentration of cell culture. Previous research has tested over multiple weeks with varying concentration of cell cultures in order to determine a more precise growth rate at

changing temperatures. A secondary limitation that our study encountered was a small test sample. In our experiment, we had 3 temperature treatments each with 3 replicates (n=3). This sample size can lead to biases in the data because it would have provided us with a less overall accurate representation of growth rate of an entire unbound culture of *C. reinhardtii*. A larger sample size (example: n = 20) would lead to less variation when the data was collected because it would provide a more accurate mean used to calculate the significance level of varying temperature on growth rate. A third limitation/source of error in our study is that we had multiple people counting cultures on separate microscopes. This could lead to a miscounted number of cells, skewing the collected data and overall result of the study.

A surprising finding in our experiment was noticed during specimen disposal which took place 1 week after cell counting. This finding was that the test tubes in which *C. reinhardtii* was grown at 35℃ no longer had a green hue to it and a significant lack of cells compared to the other test tubes of 20℃ and 25℃. This could be due to limitation of habitat size or referring to the previously mentioned study by Zachleder et al., 2019, that 35℃ is very close to the growth threshold of 39℃ which could have led to the cells arresting the cell cycle and dying off.

In today's rapidly changing world, the climate crisis is one of the leading causes for loss of biodiversity (Pettorelli et al., 2021). Rising water temperatures is a primary abiotic factor having extraordinary effects on our ocean ecosystems and their ability to properly maintain a balance. Green algae, specifically *C. reinhardtii*, is a vital stepping stone in the nutrition dynamic of these complex ecosystems and thus, a decrease in green algae growth and reproduction will have dramatic consequences for a wide range of other aquatic organisms such as salmon. Because global temperatures are rapidly rising it is important to determine the growth rate of *C.*

reinhardtii and analyze the maximum temperature threshold in order to better understand the balance in the ocean ecosystem and prevent catastrophic loss to biodiversity.

Conclusion

As the global sea surface temperature continues to rise, it is crucial to understand the effects of increased temperatures on green algae as well as other consumer species. Our results show that the *C. reinhardtii* grew greatly in treatments with warmer temperatures, which agree with the findings from other researchers. Based on our statistical analysis, we were able to reject the null hypothesis and conclude that the *C. reinhardtii* growth rate is dependent on temperature. This suggests that climate change can in fact have negative impacts on green algae growth, thus affecting the future population of salmon. Given that there were still several limitations in our research, further studies can repeat this experiment to analyze changes in temperature over a longer period of time with the addition of other notable abiotic factors that are being affected by climate change. This research would help give a better prediction of how currently increasing temperature in our oceans will also play a role in the fundamental nutrition pyramid of our ocean ecosystems.

Acknowledgements

We would like to show our gratitude to Dr. Celeste Leander for the guidance and constructive feedback throughout the course of the experiment. We would also like to thank the University of British Columbia for providing us with the necessary materials for the experiment and granting us the opportunity to perform our study through the BIOL 342 course.

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