The effect of temperature on the growth rate of *Chlamydomonas reinhardtii*

Lindsey Belsher, Brian Cheng, Claire Choi, Jenny Tang

Abstract

The green flagellate algae, *Chlamydomonas reinhardtii*, a primary producer in the aquatic ecosystem, is an important food source for salmon. Due to global warming, the change of water temperature may have strong impacts on many species' growth in the ocean. Therefore, this study investigated the effect of temperature on the growth rate of *C. reinhardtii*. It was predicted that a change in temperature would result in change in the growth rate of *C. reinhardtii*. In this experiment, *C. reinhardtii* was grown at 11°C, 20°C or 30°C. Time dependent cell densities were determined by counting cells in hemocytometers under a compound microscope over a period of 11 days. Growth rates and subsequently maximum instantaneous growth rates were determined. Results show that *C. reinhardtii* has the highest maximum growth rate at 11°C, followed by 20°C, and 30°C. Based on the result of a 1-way ANOVA test, there was no significant difference between 11°C and 20°C. However, significant differences were found between 30°C and the other two temperatures. Therefore, we concluded that temperature change would have an impact on the growth rate of *C. reinhardtii*. Further research can be focused on investigating factors other than temperature that impact the optimal growth condition for *C. reinhardtii* such as salinity and light intensity.

Introduction

Chlamydomonas reinhardtii is a single-celled green algae found in freshwater environments (Zhao *et al.*, 2007). *C. reinhardtii* has been used as a model organism to understand many aspects of cell biology including the structure and function of the basal body, chloroplast, flagellum, as well as growth rates (Zhao *et al.*, 2007). As *C. reinhardtii*. is a photosynthetic organism, its cell cycle can be related to growth rates that are affected by various abiotic factors such as temperature (Blachford *et al.*, 2016).

Along with other green algae, *C. reinhardtii* forms the base of the aquatic food chain in the local B.C. waterways. Furthermore, referring to Figure 1., through bio-magnification, *C. reinhardtii* is an important food source for wild and farmed salmon.

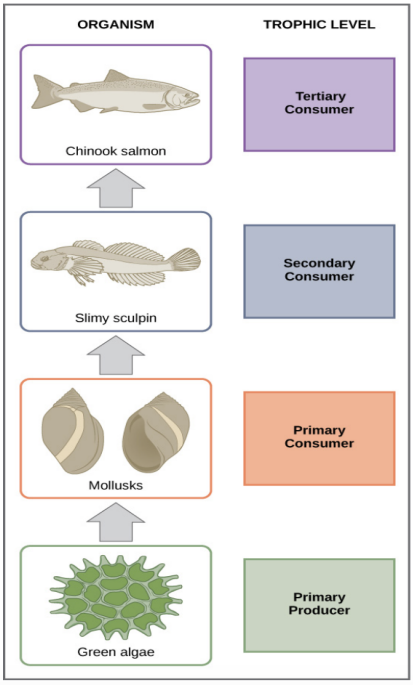


Figure 1. Green algae make up the base of the food chain as the primary producer for marine ecosystems (Image source: R. Bear and D. Rintoul, 2017).

It has been shown that including a small amount of algae in salmon's diet (2.5-10%) resulted in positive effects in growth performance for the salmon (Norambuena *et al.*, 2015). However, global warming has led to an increase in ocean temperatures in B.C. and are projected to rise by roughly 3°C by 2050 (Murdock *et al*., 2017). This change in temperature could possibly impact the aquatic life in the B.C. waterways. Therefore, it is important that we understand how a change in temperature might affect the base of the aquatic hierarchy, and resulting consumers in the upper levels of the food chain.

Under laboratory settings, we conducted a study to investigate the effect of temperature on growth rates of wild-type *C. reinhardtii*. Our null (Ho) and alternate (Ha) hypotheses were as follow:

Ho: A change in temperature will not affect instantaneous maximum growth rate of *C. reinhardtii*.

Ha: A change in temperature will affect the instantaneous maximum growth rate of *C. reinhardtii*.

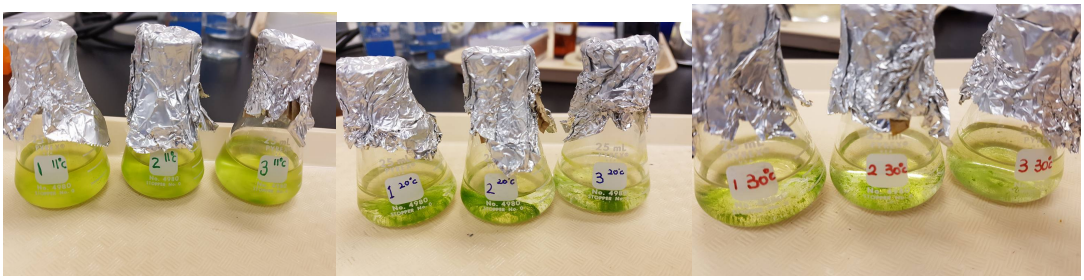
Research done by Goto and Johnson (1995) on the growth rate of *C. reinhardtii* with a temperature window of 16°C - 25°C found that cell division increases with rising temperature. The objectives of our study are to observe the growth rates of *C. reinhardtii* subjected to temperatures of 11°C, 20°C, and 30°C, and to make predictions based on these results on how freshwater temperature change in B.C. might affect algae and furthermore, salmon as a primary consumer. Based on previous research, we expect that a change in temperature will affect the growth rate of *C. reinhardtii* (Vitová et al., 2011).

Methods

A. Sample Preparation

For this project, we prepared nine separate cultures of *C. reinhardtii*. A culture solution of *C. reinhardtii* at 2 x 10^5 cells/mL was made by diluting a stock solution of *C. reinhardtii* with growth media. In nine separate Erlenmeyer flasks, we added 15 mL of culture and sealed the top with aluminum foil, before placing each flask in the appropriate incubator.

Figure 2. The samples taken on Nov 10, 2017, day 11 of the experiment. From left to right, 11°C, 20°C, and 30°C.



B. Growth Condition

Cultures of *C. reinhardtii* were grown at three different temperatures: 11°C, 20°C, and 30°C incubators. The selection of growth temperature were limited by lab availability, and those three temperatures were selected to provide a wide range of temperature for our experiment. For every temperature treatment, three replicate cultures of *C. reinhardtii* were incubated with an 8-hour light cycle over a period of 10 days.

C. Cell fixation and cell counting

After starting the 10-day incubation period, every 48 hours (with an exception on the weekend), a 100uL sample of *C. reinhardtii* was taken out from all nine cultures of *C. reinhardtii* and placed in Eppendorf tubes. Ten µL of fixative IKI was immediately added, and our fixed samples were stored in a 4°C fridge for later cell counting. We prepared our culture solution on October 30. We fixed our cells and counted on November 1, 3, 6, 8, and 10.

Cell count was determined using a hemocytometer coupled with a compound light microscope (Zeiss Axiostar) at 100x total magnification. Ten uL of previously fixed *C. reinhardtii* was placed into a hemocytometer, and the number of cells inside a 1mm x 1mm area

was determined. To account for sampling variability, we counted a minimum of 100 cells within the defined hemocytometer space, which had nine separate 1mm x 1mm quadrants, to obtain an accurate cell density. The cell density per time point at each growth temperature was then calculated by averaging the three replicates' cell counts, and calculating the cell density by dividing the average number of cells by the number of 1mm x 1mm quadrants sampled, and multiplying by the appropriate dilution factor of 2.5×10^5.

Results (Brian)

Comparing the averaged cell density plot of *C. reinhardtii* growth at different temperatures in Figure 3a, distinct regions of non-exponential growth can be observed, with special focus on the 30°C treatment cell densities having significantly different values when compared to the other two temperature treatment groups after 7 days of incubation. An analysis of maximum instantaneous growth rate (K_{max}) was then performed to determine the effect of temperature on *C. reinhardtii* growth. The instantaneous rate of change for each experimental replicate was first calculated by subtracting cell densities between neighbouring data points and dividing by the number of days incubated. Averaged K_{max} values for each temperature treatment group and their associated 95% confidence intervals are shown on Figure 3b. The K_{max} of all nine experimental replicate was then subjected to a 1-way ANOVA test to compare statistical significance, which at a 95% confidence level, there is a significant difference in K_{max} ($p = 0.021$). For both the 11°C and 30°C treatment groups, K_{max} occurred between day 4 to 7; while for the 20°C treatment group, K_{max} occurred between day 9 to 11. Furthermore, a least significant difference (LSD) test was performed on our 1-way ANOVA results for K_{max} at a 95% confidence level. Our LSD results show that K_{max} is only significantly different between the 11°C group and

30°C group, as well as the 20°C group and 30°C group (see Table 1), but not between 11C and 20C.

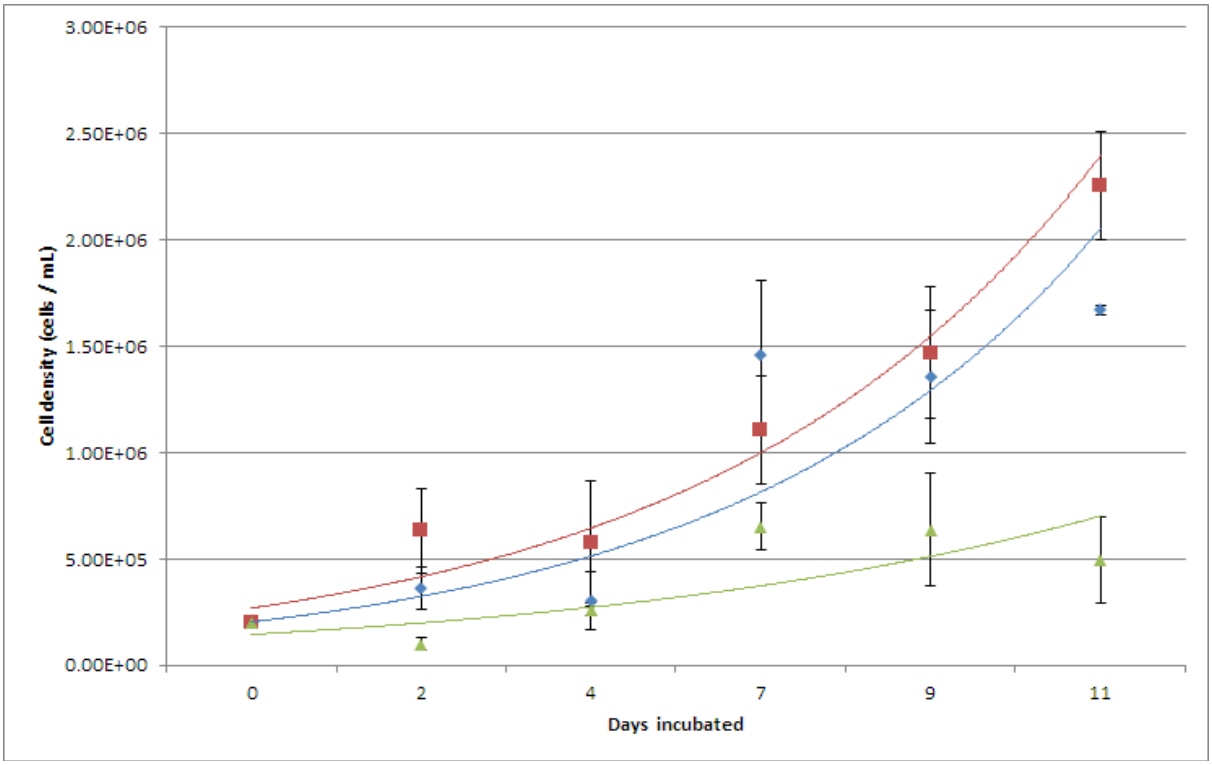


Figure 3a. Comparison of averaged *C. reinhardtii* cell densities over time between each temperature treatment, error bars indicate the 95% confidence intervals. Blue colored data points is at 11°C, red colored data points is at 20°C, and green colored data points is at 30°C. Note the distinctly lower cell densities after day 7 for the 30°C treatment group.

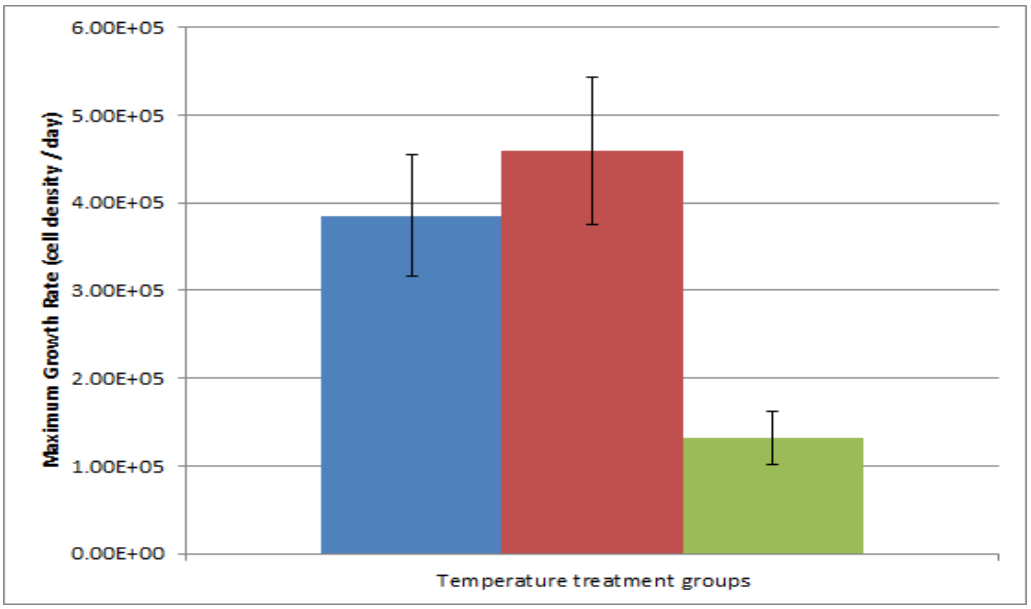


Figure 3b. Comparison of averaged K_{max} of *C. reinhardtii* between each temperature treatment, error bars indicate the calculated 95% confidence intervals. The blue colored bar is the 11°C treatment group, the red colored bar is 20°C group, and the green colored bar is the 30°C group.

Max growth rate comparison between temperature groups	Least significant difference (LSD) analysis of 1-way ANOVA results comparing maximum growth rates (α=0.05)
11◦C vs 20◦C	Not significantly different
11◦C vs 30◦C	Significantly different
20◦C vs 30◦C	Significantly different

Table 1. Least significant difference analysis of 1-way ANOVA results when comparing the maximum growth rates of *C. reinhardtii* at different temperatures.

Discussion

According to Vitová et al. (2011), the growth rate of *C. reinhardtii* increases with increasing temperature, but their findings contrast with our results because we found that 11°C

has the fastest growth rate for *C. reinhardtii.* Interestingly, our quantitative growth rate data in Figure 3b. is supported by qualitative data as seen in Figure 2, with the 30°C treatment group having the lowest *C. reinhardtii* confluency and lowest averaged K_{max} when compared to the other two growth temperatures. This may be indicative of other confounding variables that are causing slower growth at higher temperatures. Vitová et al. (2011) also found that 28°C would be the optimal temperature for the *C. reinhardtii* growth. Moreover, Xie et al. (2013) stated that the optimal temperature is between 20°C to 32°C.

Even though our results are significant, they contradict the previous studies' results. Some possibilities on the contradiction could be that Vitová et al. (2011) considered different light intensities as another factor, whereas, our experiment only considered effects of temperature on growth rate. Although Xie et al. (2013) stated that temperature as high as 39°C would provide lethal conditions for *C. reinhardtii* to grow in, their experiment only lasted 3 days. However, our experiment lasted 11 days, and Xie et al. (2013) stated that prolonged exposure to incompatible conditions could result in cell death. This may explain our reduced growth rate at 30°C. On the other hand, some studies support our result. A similar experiment was done by Blachford et al. (2016), which looked at the effect of temperature on growth rate of wild type and mutant type *C. reinhardtii.* They have also found that increasing temperature would decrease the growth rate, where their lowest temperature treatment (17°C) had the highest growth rate.

Our results from an ANOVA and LSD test lead us to reject our null hypothesis and provide support for alternate hypothesis. The results support the statement that a change in temperature will affect the instantaneous maximum growth rate of *C. reinhardtii* because our results show that growth rate is higher at lower temperature.

A possible explanation for our data being significant between some groups and not significant between others could be due to the large variation of our data points. As seen in Figure 3a, the error bars representing the standard error of cell densities overlap heavily between the 11°C and 20°C treatment group (suggesting not significant data), whereas, they do not overlap with the 30°C treatment group after day 4 in which maximum growth occurred, and we see this spacing of cell densities (suggesting significant data) (Figure 3a.).

Due to global warming, water temperatures are predicted to increase by 3°C in the next 30 years (Murdock *et al*., 2017). Our results suggest temperature does have an effect on the maximum instantaneous growth rate of *C. reinhardtii*. With *C. reinhardtii* growing the fastest at the 11°C treatment and the slowest at the 30°C treatment, we must consider how a general rise in freshwater temperature will affect the growth of *C. reinhardtii* in the local B.C. waterways and thus the salmon population, a main consumer of *C. reinhardtii*. Based on our results, we could expect to see a decrease in *C. reinhardtii* growth as water temperatures rise, thus causing a decrease in the food source for many aquatic consumers.

One of the main limitations in this study is human errors associated with measuring cell densities. Four different individuals contributed to data collection, and we were only able to have the same person count within a set of replicates. Each individual will have biases in determining cell counts, therefore, the number of cells counted for each treatment and replicate may not be consistent.

Another limitation was the possible errors from counting the cells that were clustered together. On the seventh day, the flasks of 20°C and 30°C contained large green clusters of cells. We used the vortexer to mix the cells. However, we could not achieve a truly homogeneous

mixture of *C. reinhardtii,* which contributed to our sampling bias when setting up a hemocytometer cell count. Moreover, on the second and fourth day, there was inconsistent handling of the *C. reinhardtii* cultures between individuals due to different methods of mixing. The cells would likely have sunk to the bottom of the flasks, so the 100 μL we pipetted from the flask into the Eppendorf tubes would not represent the accurate proportion of cells.

Conclusion

A 1-way ANOVA test for the maximum growth rate of *C. reinhardtii* yields no significant difference between 11°C and 20°C treatment groups but a significant difference between 11°C and 30°C, and 20°C and 30°C treatment groups. Therefore, we rejected the null hypothesis, and concluded that temperature change will affect the instantaneous maximum growth rate of *C. reinhardtii*. Further research is recommended to find the optimal growth condition for *C. reinhardtii* because not only temperature, but also salinity or light intensity could be a significant factor.

Acknowledgements

This research project was supported by the course BIOL 342 in the University of British Columbia. We would like to thank the course coordinator, Dr. Celeste Leander, for inspiring our experimental design and assisting us through experiments in the laboratory. We would also like to thank the lab technician, Mindy Chow, for providing the *C. reinhardtii* cell cultures for our experiment and checking our calculations. In this paper, all photos are provided by Claire Choi and all data plots are provided by Brian Cheng.

References

Bear, Robert, and David Rintoul. *Ecology of Ecosystems*. Rice University, 2017.

Blachford, Tianna., et al., "The effect of temperature on the growth rate of wild-type and mutant Chlamydomonas reinhardtii." *The Expedition,* vol 6, 2016.

Goto, K., and Carl H Johnson., "Is the cell division cycle gated by a circadian clock? The case of Chlamydomonas reinhardtii." The Journal of Cell Biology, vol. 129, no. 4, Jan. 1995, pp. 1061–1069.

Murdock, Trevor., et al. *Climate Projections for the Capital Region*. Capital Regional District , 2017, *Climate Projections for the Capital Region*, www.crd.bc.ca/docs/default-source/climate-action-pdf/reports/2017-07-17_climateprojectionsforthecapitalregion_final.pdf.

Norambuena, Fernando., et al. "Algae in Fish Feed: Performances and Fatty Acid Metabolism in Juvenile Atlantic Salmon." Plos One, vol. 10, no. 4, 15 Apr. 2015.

Newman, Michael., "Regression Analysis of Log-Transformed Data: Statistical Bias and its Correction." *Environmental* Toxicology and Chemistry, vol. 12, pp. 1129-1133. 1993

Vítová, Milada., et al. "Chlamydomonas reinhardtii: duration of its cell cycle and phases at growth rates affected by temperature." *Planta*, vol. 234, no. 3, 2011, pp. 599–608.

Xie, Bo., et al. "Chlamydomonas reinhardtii thermal tolerance enhancement mediated by a mutualistic interaction with vitamin B12-Producing bacteria." *The ISME Journal*, vol. 7, no. 8, 2013, pp. 1544–1555.

Zhao, T., et al. "A complex system of small RNAs in the unicellular green alga Chlamydomonas reinhardtii." Genes & Development, vol. 21, no. 10, 30 Apr. 2007, pp. 1190–1203.