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

Rachel Carr, Sung Eun Kim, Delia Ma, Ace Mingchen Shi

Abstract

Chlamydomonas reinhardtii is a unicellular green alga commonly found in freshwater ecosystems. Despite this organism's wide use in research within cell biology, the optimal growth temperature is not well defined. Given the optimal temperature range of this organism provided by the literature, we hypothesized that 25°C would be the optimal temperature, resulting in the greatest growth rate (cells mL^{-1} day^{-1}), and that temperatures below and above this value (17 and 30 °C) would decrease the growth rate of this organism. We conducted an 11-day study with a total of five sampling days. *C. reinhardtii* was cultivated at three incubation temperatures of 17˚C, 25˚C and 30˚C. The number of cells was counted using a compound microscope and a haemocytometer. A one-way ANOVA test was performed, resulting in a significant p-value of 0.0033. Following, we conducted a Tukey-Kramer HSD test and found that there was a significant difference in growth rate (cells mL^{-1} day^{-1}) between treatments at 25 and 30°C and between treatments at 25 and 17°C, with p-values of 0.0031, 0.0155, respectively, lending support to our alternate hypothesis.

Introduction

C. reinhardtii is a unicellular green alga that swims with two flagella and has an 'eyespot' which senses light. This organism has been referred to as a cosmopolitan species as it can thrive in various environmental conditions (Nakada, 2010). As well, *C. reinhardtii* serves as an important model organism within the field of cell biology, and is used to study a variety of biological processes, such as flagellum structure and assembly, cell wall biogenesis and gametogenesis (Grossman, 2003). As *C. reinhardtii* is a model organism, it is important to analyze how temperature affects this species, such that an optimal temperature can be found to maximize cell density in experiments. Furthermore, recent research has highlighted the potential of algae as one of the most promising sources for biofuels (Scranton, 2015). A better understanding of the optimal

temperatures for *C. reinhardtii* strains will help pave the way for potentially maximizing biofuel yields in the lab.

The importance of this research is further emphasized by the role of C. *reinhardtii* in freshwater ecosystems and its relationship to keystone species in British Columbia, such as salmon. Salmon are hugely important to the ecological stability of British Columbia's forests. The relationship is so strong that one can look at the forest in a watershed and make predictions on how well the salmon run is doing (Hume, 2011). When salmon die, they decompose and release nitrogen, a limited resource in marine and terrestrial environments, which results in an observable increase in growth of some plant species, capable of nitrogen uptake. As green algae are at the base of the aquatic food chain, they serve a significant role in providing energy for salmon and other higher trophic level organisms. Studies have shown that the addition of small amounts of algae in fish feed has resulted in positive effects on growth performance and feed utilization efficiency (Towers, 2013). As *C. reinhardtii* and salmon are connected within the complex food system, we believe that studying the effects of temperature on the growth of *C. reinhardtii* will provide further insight into the complexity behind the fluctuations in salmon run populations. Furthermore, because of global warming caused by anthropogenic warming, the influence of temperature on this model organism, *C. reinhardtii,* is an area of particular interest. The aim of this study is to analyze the effect of various temperatures on the growth curves of the organism *C. reinhardtii*.

Previous research has demonstrated that the optimal temperature for *C. reinhardtii* is between 20-32°C (Xie et al., 2013). To obtain the predicted optimal temperature, we took the average of the range given by Xie et al. This value of the average is 26° C, however, as there was not an incubator provided at this temperature, our optimal temperature, or control for this

experiment is 25°C. To examine the effect of temperature on the growth of *C. reinhardtii*, we chose incubation temperatures of 30°C and 17°C, which are above and below the hypothesized optimal temperature.

Our null and alternate hypotheses are as follows:

H_o: Decreasing the temperature to 17°C and increasing the temperature to 30°C, compared to the organism's predicted optimal growth temperature of 25°C will not decrease the growth rate of *C. reinhardtii*.

H_a: Decreasing the temperature to 17°C and increasing the temperature to 30°C, compared to the organism's predicted optimal growth temperature of 25°C will decrease the growth rate of *C. reinhardtii*.

Methods

We conducted an 11-day study with a total of five samplings. We used three replicates for each temperature, with a total of 9 replicates. The cells were counted using a compound microscope, a haemocytometer and a hand-held counter. Sterile technique was used throughout the experiment to avoid sample contamination, which involved wiping the lab bench with ethanol and flaming the test tubes using a gas lamp before extracting samples.

Preparation

The setup of our experiment required the dilution of the provided stock solution of *C. reinhardtii* to a concentration of 2.0×10^5 cells/mL. Using a haemocytometer and a compound microscope, we found the concentration *C. reinhardtii* to be 10.5×10^5 cells/mL. To dilute the stock to the desired concentration of 2.0×10^5 cells/mL, we determined the volume of media

required for dilution using the $C_1V_1=C_2V_2$ formula. We added 19 mL of the *C. reinhardtii* stock solution and 81 mL of the provided media to a 250 mL Erlenmeyer flask. After swirling the flask to ensure a homogenous solution, we added 10 mL of the diluted culture to each of the 9 replicates. Thus, all replicates were assumed to have the same initial starting concentration of 2.0 x 10^5 cells/mL. Finally, the replicates were placed in their respective incubators at temperatures of 17˚C, 25˚C and 30˚C. Light intensity was held constant as all incubators were on the same light cycle.

Fixing

Beginning on Wednesday, November 1st to Friday, November 10th, we extracted 100 µL from each replicate tube into labelled Eppendorf tubes. Prior to extracting the samples, we ensured the replicates were well-mixed by light vortexing the test tubes, and resuspending the solution using a pipette. Then, the samples were fixed with 10 µL of IKI, mixed well with a micropipette and stored in a refrigerator for counting.

Counting

To count the cells, we placed 10 µL of the fixed solution onto the haemocytometer and counted the number of cells using an Axio compound microscope at 10X objective lens magnification and a hand-held counter. The concentration was then multiplied by 1.1 to obtain the actual concentration which was affected by the volume change due to the addition of the fixative.

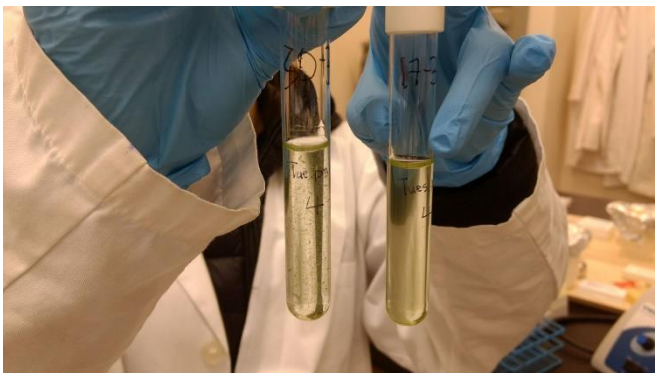

Figure 1. Picture of test tubes at 30˚C and 17˚C from left to right. Note the colonies present in the test tube at 30˚C, which are not visible in the test tube at 17 ˚C.

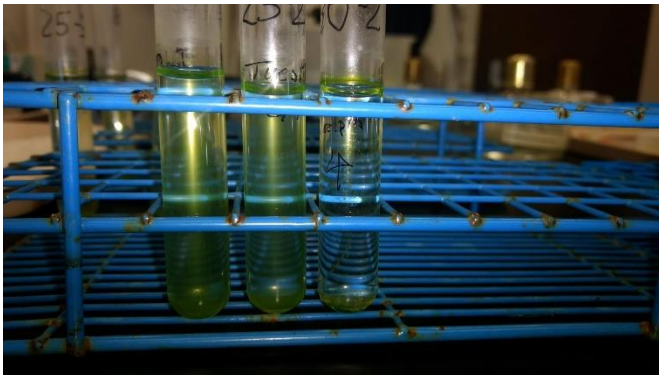

Figure 2. Picture of test tubes at 17, 25 and 30˚C from left to right. Note the film of *C. reinhardtii* at the top of the test tubes.

Data Analysis

First, to observe the trends in growth occurring for the treatments, we calculated the average cell density (cells/mL) for each treatment and plotted the data over a period of 11 days. The data is shown in Figure 3. Then, using excel, we plotted the growth curves for each replicate and added a best fit line to each graph. The slopes of the best fit lines were used as the values for the one-way ANOVA calculation, resulting in three values for each treatment, with a total of nine data points or slope values. On day 7 of the experiment, one of the replicates at 17 ˚C was spilled, which resulted in higher cell counts due to the sample being concentrated. To remove these outlier data points occurring after the spill (days 9 and 11), we obtained the slope from the data prior to the spill and used this slope for the ANOVA calculation. As well, in Figure 3, the data occurring after the spill (> 7 days) was not averaged for the growth curve at 17 ˚C. The finalized data is presented in Figure 2. Following the one-way ANOVA test, we conducted a Tukey HSD test using JMP software. The p-values from the one-way ANOVA test and the Tukey HSD test are shown in Table 1.

Results

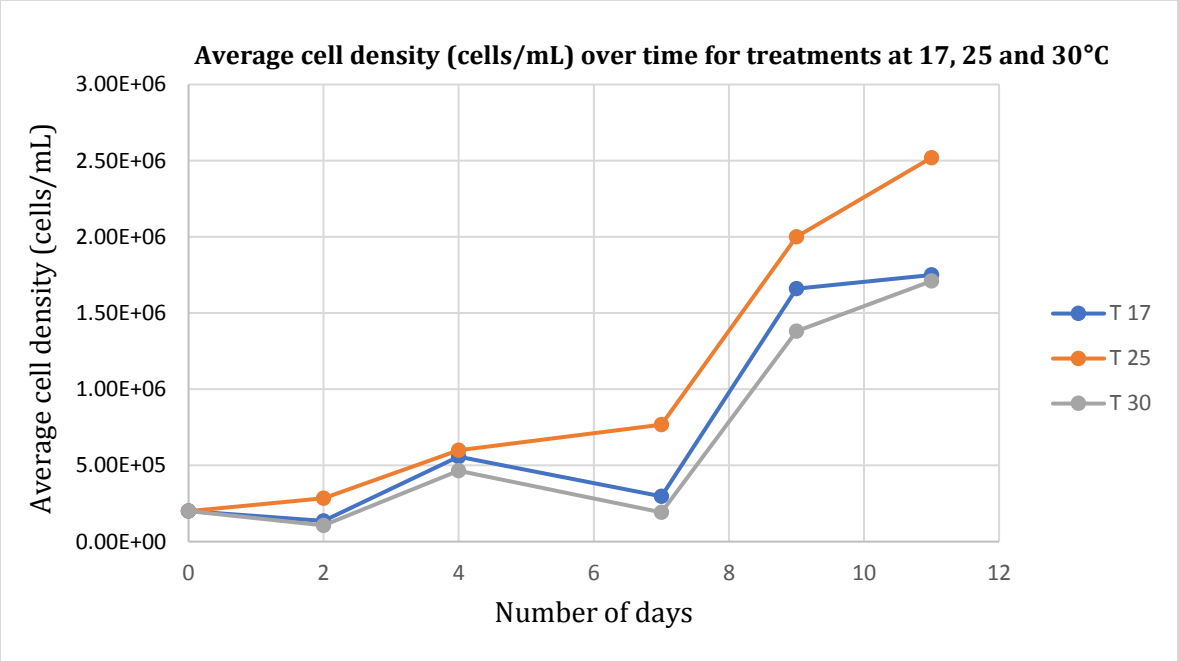

Figure 3. Average cell density (cells/mL) for the three treatments (17, 25 and 30°C) over a period of 11 days.

In Figure 3, the growth curves for the treatments at 17, 25 and 30°C are inconsistent with the typical growth curves observed for bacteria and algae which have a clear lag, exponential, stationary and decline phase (Todar, n.d.). The growth curves for the populations at 17 and 30°C have an identical growth curve pattern, whereas the populations at 25°C exhibits only positive growth over time. The increase in growth, occurring from the seventh to the ninth day of the experiment, is the steepest slope for the population at 17°C (a slope of 680,000 cells mL^{-1} day^{-1}). The second largest increase in growth occurs at 30°C, at the same time, has a slope of 593,000 cells mL^{-1} day^{-1}. The part of the growth curve from seventh to ninth day is the exponential phase and, following this rapid increase in growth, the growth curve levels off for the treatments at 17 and 30°C similar to what was observed in the stationary phase. A maximum cell density is

observed on the last day of the experiment of 1.76×10^6 cells/mL at 17°C, 2.52×10^6 cells/mL at 25°C and 1.71×10^6 cells/mL at 30°C. Thus, at 25°C, an overall maximum cell density is achieved.

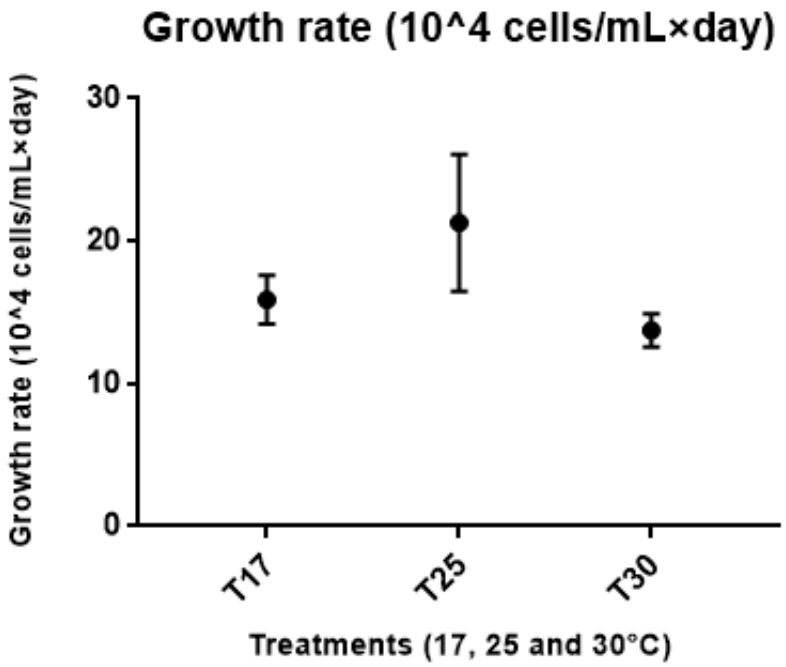

Figure 4. Growth rate (cells mL^{-1} day^{-1}) for treatments at temperatures of 17, 25 and 30°C, respectively. Error bars indicate 95% confidence intervals of the mean.

Table 1. P-value results from one-way ANOVA and Tukey-Kramer HSD analysis

Statistical test	**p-value**	
One-way ANOVA	0.0033*	
Tukey-Kramer HSD	**Treatments**	**p-value**
	T25-T30	0.0031*
	T25-T17	0.0155*
	T17-T30	0.3097

Discussion

The p-value obtained from the one-way ANOVA analysis is statistically significant, with a significance level of 0.05. The results from the Tukey-Kramer HSD test show that there is a statistically significant difference between the growth rates at 25 and 30°C and between the growth rates at 25 and 17°C. However, there is no statistically significant difference shown between the growth rates at 17 and 30°C. Thus, our data provides support for our alternate hypothesis that decreasing the temperature to 17°C and increasing the temperature to 30°C, compared to the organism's predicted optimal growth temperature of 25°C, will decrease the growth rate of *C. reinhardtii*. From the slopes calculated from the best fit line equations, the growth rates are the highest at 25°C and the lowest at 30°C. As presented in Figure 3, the highest cell density is achieved at 25°C, and the population of *C. reinhardtii* is still growing quite rapidly on day 11. The treatments at 17 and 30°C are clearly below the growth curve at 25°C and show less growth from days 9-11 in comparison.

The optimal temperature for *C. reinhardtii* varies throughout the literature. For example, Vitova et al. (2011) found the optimal temperature to be 28°C, given three temperatures of 18, 25 and 28°C, while Therien et al. (2014) found that the optimal temperature was 30°C. Thus, our results do not agree with the findings by Therien et al. (2014). It is possible that the optimal temperature for maximum growth occurs at 28°C, however, due to equipment limitations, we were not able to use an incubator at 28°C.

Errors relevant to our study include human error due to counting the cells using a haemocytometer. All replicates over the course of the experiment developed a thin film of algae at the top of the test tubes. As well, over time, all replicates except for those at 17°C became clumpy or formed colonies within the test tubes. This made counting cells difficult as it was often

challenging to resolve the number of cells in a colony using the compound microscope. Furthermore, extracting a homogenous sample was also challenging despite both lightly vortexing the replicates and resuspending the liquid using a pipette before sampling. Another error relevant to our study is the influence of bias in our experiment. When we counted the cells, we counted them as a group and shared a data table to record our values. Often, we would compare our cell counts with one another and if a large discrepancy existed between the counts, we would count the cells again to verify our results. However, this may have subconsciously induced bias into our results, as one person counting the cells may believe that the other person has obtained a more accurate value. For future experiments that involve counting cells, we will have a different data sheet for each person and we will separate group members in the lab to remove the possible influence of bias. As mentioned, half way through the experiment, we spilled one of the 17°C replicate test tubes. This concentrated the cells and the cell counts following the spill were much higher. In the analysis, we obtained the slope for the data prior to the spill to obtain a more representative value for the growth rate. By doing so, we obtained an estimate of the growth rate for this replicate, which could have decreased the accuracy of our results.

Despite considerable research on this organism, there are few studies that provide a defined optimal temperature value for *C. reinhardtii*. Further research should be conducted to define an optimal temperature for growth to maximize cell density in experiments, particularly relevant for research surrounding biofuels. As well, given its role as a major source of food for keystone species such as salmon, further research should be conducted to determine the influence of temperature on *C. reinhardtii* given future projected warming scenarios.

Conclusion

Our results provided support for the alternate hypothesis, that is, decreasing the temperature to 17°C and increasing the temperature to 30°C will decrease the growth rate of *C. reinhardtii*. A one-way ANOVA test resulted in a p-value of 0.0033, which is significant given a significance level of 0.05. Furthermore, a Tukey-Kramer HSD test resulted in a significance difference in growth rate (cells mL^{-1} day^{-1}) between treatments at 25 and 30°C and between treatments at 25 and 17°C, with p-values of 0.0031, 0.0155, respectively. Challenges included ensuring a homogenous sample and the presence of colonies when counting cells and the influence of bias. Our results differ from the findings by other researchers who have found that *C. reinhardtii* has an optimal temperature of 28-30°C (Vitova et al., 2011, Therien et al., 2014). We encourage further research regarding the relationship between temperature and the growth of *C. reinhardtii* as this organism serves as the base of the food chain for keystone species, such as salmon and has promising potential for biofuels.

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Contributions

The abstract was written by Delia Ma, methods by Sung Eun Kim and the conclusion by Ace Mingshen Shi. The analysis, results, graphs and discussion was done by Rachel Carr.

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