The effect of temperature on DNA concentration and cell size of *Chlamydomonas reinhardtii*

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Abstract

Chlamydomonas reinhardtii is a primary producer in many freshwater ecosystems. With temperatures increasing due to global warming, the effect on their cellular processes is of concern since they form the basis of the salmon food web. The purpose of studying *C. reinhardtii* was to investigate the effects of temperature on DNA concentration and cell size. It was predicted that temperature would affect both DNA concentration and cell size of *C. reinhardtii*. Our methods consisted of two different temperature treatments, 30°C, and 39°C, consisting of 3 replicates each, over 48 hours. To conduct our research, we intermittently extracted the cultures to measure cell growth, perform cell counts and isolate the DNA. This was done using both a hemocytometer and compound microscope in addition to multiple fixatives. The results generated from a two-way ANOVA produced p-values of <0.0001, 0.0556, and 0.0037 for DNA concentration corresponding to time, temperature, and interaction respectively. Consequently, we were able to determine that temperature had no significant effect on DNA concentration ($p>0.05$). Conversely, the p-value for time and interaction was less than 0.05 ($p<0.05$) inferring that there was a significance. Additionally, for cell size, the p-values obtained were 0.0137, 0.0330, and 0.4050 for time, temperature, and interaction respectively. Thus, no significance was found for interaction ($p>0.05$), however, for time and temperature, there was an observed significance ($p<0.05$). However, despite ANOVA results for cell size, Sidak's test was unable to identify where the significance resulted from. In conclusion, the temperature was found to affect C. *reinhardtii* cell size, but DNA concentration was relatively unaffected in comparison with the two temperature treatments.

Introduction

Chlamydomonas reinhardtii is a unicellular green alga, with a diameter of 10 μm (0.01 mm), that is largely found in freshwaters like streams and rivers. Being a photoautotrophic organism, *C. reinhardtii* serves as a primary producer in freshwater ecosystems and forms the base of the aquatic food chain (Norambuena et. al., 2015). *C. reinhardtii* is a useful model organism due to its small size, exponential growth, and unicellular nature (Zhang et. al., 2021). Concerning salmon, *C. reinhardtii* is part of the planktonic portion of the salmon food web,

therefore is a major food source for these fish to consume (Norambuena et. al., 2015). The presence of small amounts of algae such as *C. reinhardtii* in fish diets has been demonstrated to improve growth performance and feed utilization efficiency (Norambuena et. al., 2015). With the growing concern of climate change, and changing temperatures of local B.C. freshwaters, the impact on aquatic life is important to study and understand.

Temperature is an important factor to photosynthetic algal cells like *C. reinhardtii* since it can affect cell processes like growth and division by controlling photosynthetic rate (Vítová et. al., 2011). Three distinct temperature ranges affect an organism's cell physiology: optimal growth temperature, below and above optimal where growth and cell division still occur but at a lower rate, and temperatures that are too high or too low where cell division and growth are affected (Zachleder et. al., 2019). Previous studies have proven that increasing temperatures result in an increase in growth rate and shortened cell cycle duration. Further increases above optimum, however, result in a decline in growth rate and prolonged cell cycle (Vítová et. al., 2011). For *C. reinhardtii,* the optimal temperature is around 30ºC and the lowest effective temperature where cell division is inhibited is 39ºC (Zachleder et. al., 2019). Cells that undergo growth in supraoptimal temperatures have been shown to result in cell cycle arrest where growth continues, due to cell division being more sensitive to temperature changes (Zachleder et. al., 2019).

In this experiment, we investigated the effects of temperature on the changes in DNA concentration and cell size growth of *C. reinhardtii*. The objective of this study was to observe the difference in the impact that supraoptimal temperature versus optimal temperature has on

Chlamydomonas cell cultures grown in the laboratory. We chose to examine average DNA concentration per cell, and cell growth, over 48 hours, between cells grown in a controlled temperature of 30ºC and a supraoptimal temperature of 39ºC. The aim of this study will further provide insight into the effects of temperature on *C. reinhardtii,* in particular the impacts of increasing temperatures on fundamental cellular processes.

Our hypotheses for the two variables are as follows:

For DNA concentration, we hypothesize that both temperature and time will affect the DNA concentration of *C. reinhardtii* cells.

For cell size, we hypothesize that both temperature and time will affect the size of *C. reinhardtii* cells.

Following previous research by Zachleder et. al. (2019), we predict that DNA concentration per cell will increase with time until cell division occurs in the 30ºC, and remain relatively the same in cells at 39ºC, as cell division does not occur. In terms of cell size, we predict that at 30ºC, the cell size will increase with time until the cells divide, while at 39ºC, the cell size will increase with time due to cell cycle arrest inhibiting cell division.

Methods

We received a culture of *Chlamydomonas reinhardtii* which was evenly transferred into bioreaction tubes for centrifuging. The tubes were centrifuged to separate the media from the cells, which allowed us to discard the original media using a micropipette. The addition of a special high salt media was then added: a doubled concentration of Ca2+ ions and a 10-fold

increase of Mg2+ ions, creating our stock solution (Zachleder et. al., 2019). 5000 µL of the stock solution was separated into 6 test tubes. We used three replicates for each temperature treatment to determine the optimal cell cycle rate of *C. reinhardtii* and sterile techniques were always used when extracting the organisms for cell count and DNA isolation to avoid contamination.

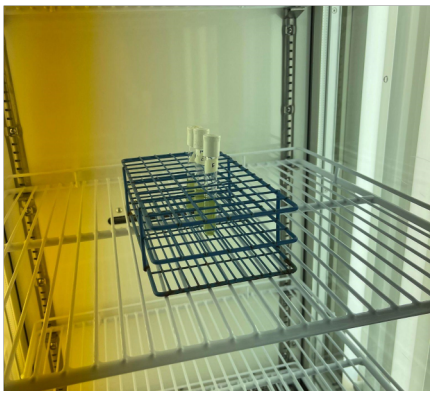
a)  b) 

Figure 1. Replicates are placed in a test tube rack containing *C. reinhardtii* in special high salt media. 3 replicates (labelled A, B, C) placed in the incubator a) 30ºC and 3 replicates (labelled D, E, F) placed in the incubator b) 39ºC.

Cell Fixation & Cell Counting

Over three days, 100µL of each replicate was extracted every 24 hours (including 0hrs) and fixated with 10µL of IKI to count the cells under a hemocytometer. Before counting, the counting tubes that contained the extracted culture were vortexed to disperse the concentration of *C. reinhardtii* mainly at the bottom. Under the hemocytometer, *C. reinhardtii* cells were counted to at least 150 cells or if under 150, until the full grid (blue square) was counted at magnification 10x under a compound microscope. Following the count, calculations corresponding to the dilution factor were made.

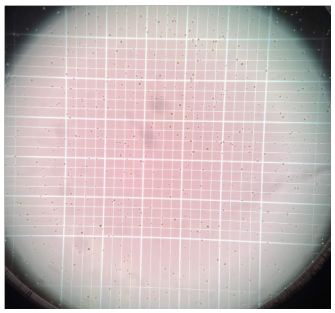


Figure 2. Haemocytometer grid viewing *C. reinhardtii* under a compound microscope at 10X magnification. Image of replicate F (39°C) taken on day 3 at 48hr. Counting was done at 0hr, 24hr, and 48hr.

Cell size:

In addition to extracting for cell count, 100µL of each replicate was also extracted every two hours (0, 2, 4, 22, 24, 26, 28, 46, 48) to determine the size of growth of *C. reinhardtii* cells into Eppendorf tubes. Similar to cell counting, tubes were likewise vortexed, and 10 measurements were recorded for each replicate for all six replicates at magnification 100x under a compound microscope.

DNA isolation

To determine the amount of DNA present, DNA isolation was done to see the regulation of the cell cycle over 48 hours. Into Eppendorf tubes, cultures from each replicate were extracted and fixed with 300µL cell lysis solution with proteinase K. Subsequently, the Eppendorf tubes were placed in an incubator at 65°C for a total of 15 minutes while being vortexed at 5-minute intervals before being placed on ice for an additional 5 minutes. Following the cooling of the tubes, 150µL of protein precipitate reagent was added, centrifuged and the supernatant removed before the addition of 500 ul of cold isopropanol was also added to the pellet to precipitate DNA from the solution before it was centrifuged once more. Isopropanol was likewise discarded, and

ethanol was used to rinse off the salts that may still be present before the pellet was able to dry for one hour.

After this duration, 995µL of distilled H_2O and 5µL of the DNA solution were placed in a cuvette for spectrophotometric analysis to measure the absorbance at wavelengths 260 nm and 280 nm (UV).

Figure 3. DNA fixation of *C. reinhardtii* for all replicates. The addition of cell lysis solution with proteinase K, protein precipitate reagent, isopropanol, and ethanol using flame for sterile technique.

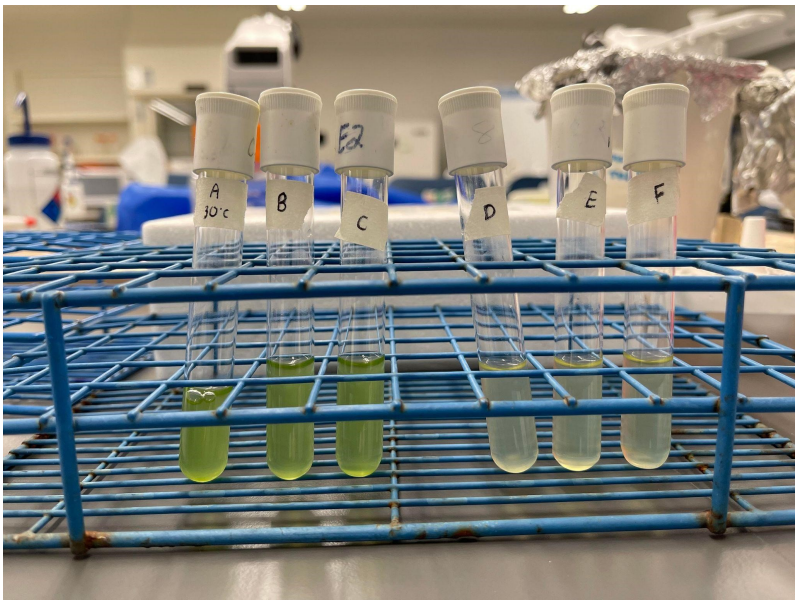


Figure 4. DNA concentration of *C. reinhardtii* after 48 hours. Replicates labelled A, B, C placed in incubator 30ºC, and replicates labelled D, E, F placed in incubator 39ºC. DNA concentration at 30ºC is more concentrated than at 39ºC

Results

To determine the effect of time, temperature, and interaction on DNA concentration per cell, a two-way ANOVA test was conducted. The p values for time, temperature, and interaction were determined to be <0.0001, 0.0556, and 0.0037 respectively. This indicates that time and interaction had a significant effect on DNA concentration per cell as the p-value was less than 0.05 ($p<0.05$). However, the p-value for temperature was greater than 0.05 ($p>0.05$) showing that temperature did not have a significant effect on DNA concentration per cell.

The difference between each of the time points 0, 24, 48 hours were determined to be significant through Tukey's multiple comparisons test as the p-value for 0 vs 24hrs, 0 vs 48hrs, and 24 vs 48 hrs were all less than 0.05 ($p<0.05$).

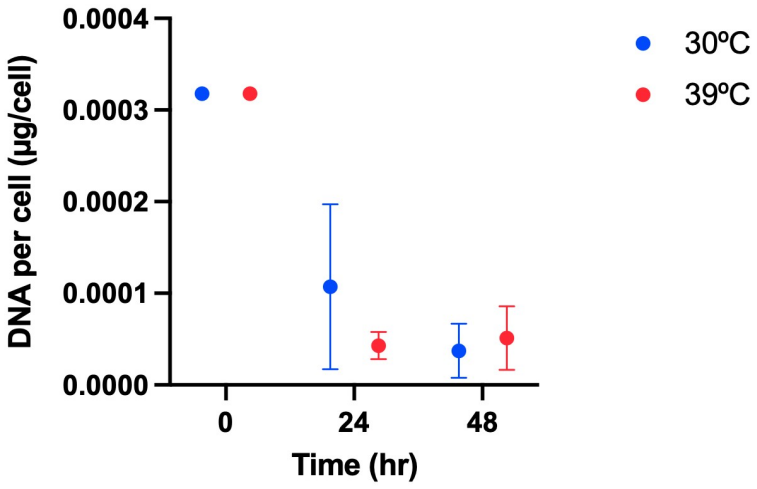


Figure 5. The DNA concentration per cell of *Chlamydomonas reinhardtii* grown at 30ºC (blue) or 30ºC (red) at 0 hr, 24hr, and 48hr of treatment. The error bars represent the 95% confidence interval.

A two-way ANOVA was also conducted to determine the effect of time, temperature, and interaction on cell size. The p-values were 0.0137, 0.0330, and 0.4050 for time, temperature, and interaction respectively. As the p-value for interaction was larger than 0.05 ($p>0.05$), the interaction did not have a significant effect on cell size. However, time and temperature both had a significant effect on cell size as their p values are less than 0.05 ($p<0.05$).

However, using Sidak's multiple comparisons test, no significant difference was identified between the temperatures or time points specifically.

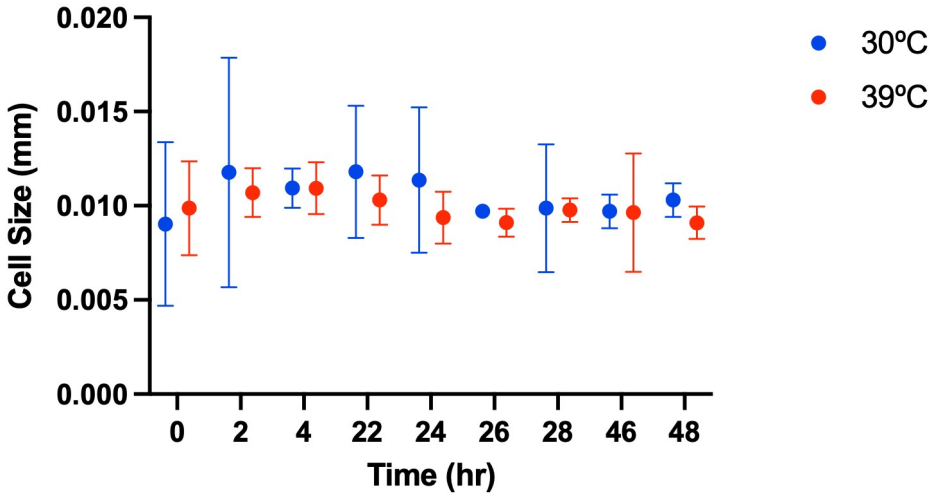


Figure 6. The course of cell size of *Chlamydomonas reinhardtii* grown at 30ºC (blue) or 30ºC (red) with error bars representing the 95% confidence interval.

Discussion

The results from our statistical analysis can confirm that a temperature change did not have a significant effect on DNA concentration but did have an effect on cell size. Whereas time had a significant effect on DNA concentration and size of *C. reinhardtii* cells. Therefore, we fail to reject our null hypothesis that temperature will not affect DNA concentration ($p > 0.05$).

Furthermore, we reject the null hypothesis that temperature and time will not have an effect on C. reinhardtii cell size ($p < 0.05$) Subsequently, significance was found using Tukey's multiple comparisons test for DNA concentration between the different time intervals (0hrs, 24hrs, and 48hrs). This indicates that DNA concentration does vary throughout the cell cycle of *C. reinhardtii*, as we had expected. On the other hand, although a significant difference in cell size as indicated in the ANOVA results between temperature and time, where the significance originated from was not identified. This was confirmed after performing Tukey's comparison test, looking within a temperature treatment, and a Sidak's comparison test, comparing between temperature treatments. To find the significance, more replicates for each treatment should be considered for future studies.

The first basis of our experiment was to determine the effects of both temperature and time on the DNA concentration of *C. reinhardtii* cells. Based upon research conducted by Zachleder et. al., (2019), the *Chlamydomonas* cell division ends after 18 hours for cells treated at 30ºC, while the cell cycle is arrested for *Chlamydomonas* cells treated at 39ºC. Thus, we expected the DNA concentration per cell to decrease at the 24-hour point for the 30ºC treatment and remain the same for the 39ºC treatment. Our results, shown in Figure 5, show that the DNA concentration per cell did indeed decrease significantly for the 30ºC treatment. However, the DNA concentration per cell also decreased significantly for the 39ºC treatment. While taking measurements for the cell size, after 22 hours we observed many burst cells for the 39ºC and some burst cells for the 30ºC treatment. Burst cells lead to a loss of DNA, which could explain

why the DNA concentration per cell decreased from 0 to 24hr for the 39ºC treatment and it may have contributed to the decrease for the 30ºC treatment as well.

As the cell cycle is arrested for cells at the 39ºC treatment (Zachleder et. al., 2019), we expected the DNA concentration per cell would stay the same from 24 to 48 hours. While the difference was much less, Tukey's comparison test still showed there was a significant difference between the 24 and 48-hour marks ($p<0.05$). Following the timeline for the cell cycle at 30ºC, we would expect the second cell division to be completed at 36 hours and the third cell division to be completed at 54 hours. Thus, at 48 hours we would expect the DNA concentration per cell to increase from the 24-hour mark as the cell prepares for cell division. However, the DNA concentration per cell decreased significantly. These results for both our 30ºC and our 39ºC treatments can also be attributed to the burst cells observed after the 22-hour mark, which resulted in a loss of DNA.

The second basis of our experiment was to determine the effects both temperature and time will have on the cell size of *C. reinhardtii.* During the cell cycle of *C. reinhardtii*, an increase in cell size is a prerequisite before cell division (Zachleder et. al., 2019). Cells are expected to grow to a critical size, usually double, until undergoing cell division and giving rise to new daughter cells, which are of normal size (Zachleder et. al., 2019). Based upon previous research results from Zachleder et. al (2019), we expected that cells grown at both temperatures (30ºC and 39ºC) will similarly increase in size until control cells undergo division, and cells in 39ºC will continue growing. Our results that are shown in Figure 6 however, differ from what was seen in previous studies with cell size remaining relatively the same for 39ºC and slightly

increasing before cell division occurs for 30ºC, around the 22hr mark. Although the ANOVA analysis indicated significant differences in cell size found between temperature treatments and with time, expected growth patterns were not observed. *C. reinhardtii* cells grown at 30ºC did not double in size before cell division, nor did cells at 39ºC continue to grow any larger.

Sources of error that could have been encountered during our experimental procedure include human, instrumental, environmental, and procedural. A relevant human error in our study involves personal bias. When performing measurements for cell size, although multiple measurements were taken, we still made a choice in which cells, present in the sample, were recorded. Another source for human error was the counting of cells using the haemocytometer. When counting cells, discrepancies in count between group members were consistently experienced, thus multiple measurements had to be taken for a sample. A relevant instrumental error that we experienced was the inconsistency with the Spec 20 which measured the DNA content of our samples. When placing the same cuvette sample into the Spec 20, differing measurements were displayed. This could have been due to various factors like how the sample was placed and the distribution within the sample. Concerning the procedure itself, the transfer of samples in and out of the temperature treatments could have disrupted the cell cycle and growth of the *C. reinhardtii* cells. For future studies, increasing the number of replicates per treatment group will lessen the degree of variation, and increase the degree of significance. Another point of interest to study involves the effects of a larger temperature range. This includes investigating with temperatures below optimal, to view whether suboptimal temperatures have similar effects on *C. reinhardtii* cell as does supraoptimal temperatures.

Conclusion

In conclusion, we reject our hypothesis that temperature will have an effect on DNA concentration per cell. But we fail to reject our hypothesis that temperature and time will have an effect on the cell size of *C. reinhardtii*. Thus, the increase in environmental temperatures for *C. reinhardtii* is expected to have no significant effect on the change in DNA concentration of cells but affects the growth of *C. reinhardtii* cells over time.

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