The Effects of Cold Temperature Shock on *Chlamydomonas reinhardtii* Oxygen Production

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Abstract:

Chlamydomonas reinhardtii has a photosynthesis process that is multifaceted in its dependence, as it relies on temperature, carbon dioxide, water, and light exposure. This paper focuses on the temperature aspect and conducts an experiment where Chlamydomonas reacts to sudden changes in temperature and examines what kinds of impacts it has on their oxygen levels and photosynthesis. Nine samples of the stock wewere measured with an oxygen meter to find their initial oxygen levels, and then equally filled into airtight containers. The samples were divided into 3 groups, each correlating with a different temperature: 4°, 15°, and 25°C. Each group was incubated at their corresponding temperature for 60 minutes and was measured again for final oxygen levels. In addition, the cells of a sample from each group were counted and compared. The results show that the group placed at 4°C had a significant decrease in oxygen levels, the group placed at 15°C did not have any noticeable difference, and the group of samples at 25°C had quite a bit of increase in oxygen. The 25°C group had an average of 115500 cells/ml while the other two had approximately 176000 cells/ml. However, human error may have impacted the results as the Chlamydomonas had to be stirred frequently to prevent it from settling.

Introduction:

By pushing nutrients around the ocean, controlling the population of marine producers, being the source of a plethora of species, and acting as a fertilizer for forests after decomposing, salmon help the ecosystem in every stage of their life (Page & Whetung, 2020). Salmon have such a crucial role in the Pacific Northwest but are facing a decline due to overfishing and climate change. Which as a result is affecting the health of forests and marine life. In order to improve the problems that salmon are facing, it's important to take a look at biotic and abiotic factors that positively impact the salmon population to thrive. A species that should be taken into consideration is *Chlamydomonas reinhardtii*, which is a single-celled green alga that can grow independently of its environment's oxygen levels as long as it's supplied with light carbon dioxide (Graves et al., 1990). *C. reinhardtii* is a great source of food for salmon and can help the

fish with important aspects such as metabolism and feed efficiency (Norambuena et al., 2015). A study claims that *C. reinhardtii*'s cell cycle duration is a function of growth rate and not temperature, which suggests that it might not affect its rate of photosynthesis (Bisova et al., 2011). However, heat stress is an important factor in the oxygen production of several photosynthetic microorganisms. An experiment was conducted where Chlamydomonas were kept at 40 °C for variations of time, and they found that oxygen production increased for higher temperatures (Prasad et al., 2016). The purpose of this experiment is to further investigate the correlation between temperature and oxygen production of *Chlamydomonas reinhardtii*. This will be done by incubating samples of *C. reinhardtii* for some time at 4°C, 15°C, and 25°C. This sudden temperature change will cause the samples to undergo 3 different temperature shocks, and from that, the oxygen levels can measure and compare for any changes. The hypothesis going into this experiment is that the production of oxygen in *C. reinhardtii* will be affected by its exposure to a sudden change in temperature also known as heat or cold shock. In addition, it is predicted that the 4°C temperature shock will decrease the oxygen production of photosynthesis. In other words, it is expected that colder temperatures will cause oxygen production to decrease.

Methods:

Preparing the Master stock

This study used one sample of the organism *Chlamydomonas reinhardtii* divided among three conditions to determine the impact of cold shock on oxygen production. The three conditions were 4ºC, 15ºC, and 25ºC. Three replicates were used for each condition. The *Chlamydomonas reinhardtii* culture was prepared at the University of British Columbia by

Mindy Chow, following the "Chlamydomonas Maintenance" document for optimal conditions. To create the Master Stock used for each treatment, 300mL of excess media was added to 300mL of *C. reinhardtii* for a 1:1 dilution, as shown in **Figure 1**. *C. reinhardtii* was vigorously shaken before diluting.

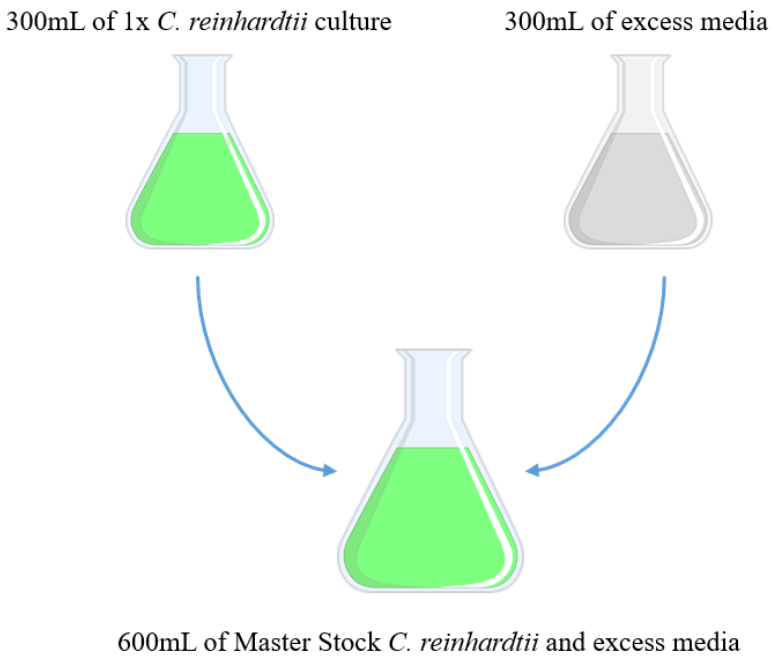

Figure 1. Creation of the 1:1 Master Stock *C. reinhardtii*. A diagram showing the volumes of *C. reinhardtii* culture and excess media required to create the Master Stock used for the experiment.

Preparing the samples

Nine 27mL vials were labeled: 1, 2, and 3 for each 4ºC, 15ºC, and 25ºC test. The Master stock was poured into one vial at a time and an oxygen meter was submerged into the vial to measure for initial O_2 concentration. After the reading was taken, the volume displaced by the oxygen meter was refilled from the Master Stock until a convex meniscus formed on the top of the vial. The vial lid was then pressed down to ensure no air bubbles were trapped inside and to

prevent gas exchange with the environment, as shown in **Figure 2**. This was repeated for all nine vials.

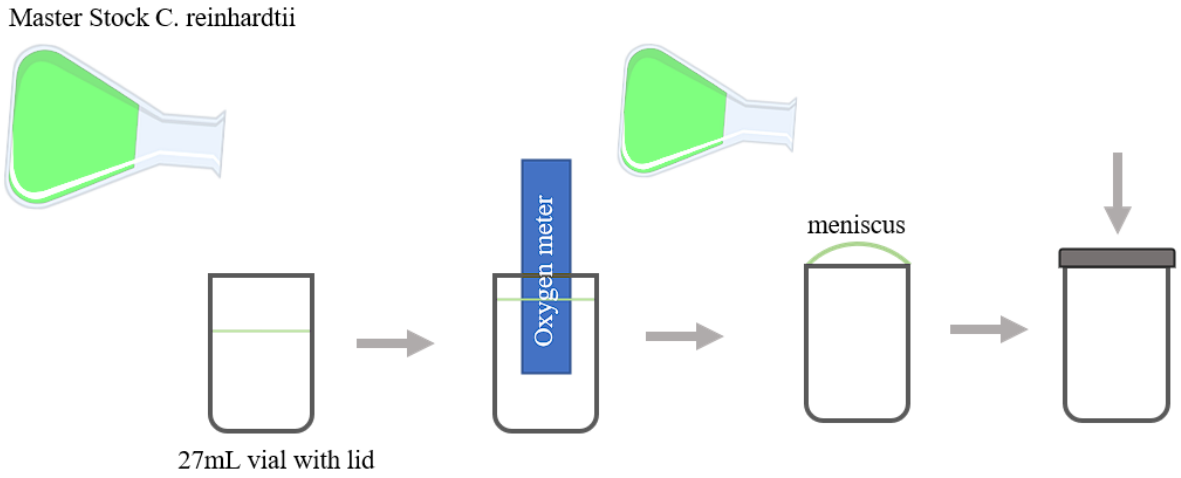

Figure 2. Preparing the samples. A diagram showing how the vials were filled with Master Stock and measured for oxygen concentration prior to treatment. Vials were filled halfway, then measured with an oxygen meter, before being filled to the top.

Cell Count

10ul of Iodine Potassium Iodide (IKI) was added to 100ul of Master Stock to fix the cells for cell counting (**Figure 3**). This solution was mixed with a pipette, which was then used to load a 0.100mm haemocytometer following the " Haemocytometer Instructions" provided in the Biology 342 lab. Cells were counted in the red, 1 mm^3 square shown in **Figure 4**. Four individual counts were performed using the same mixture, then averaged for the initial cell count.

Sample calculation

cells/mL = $\frac{number\ of\ cells\ counted}{number\ of\ squares\ counted}$ (dilution factor of the square)*(fixative dilution)*(medium dilution)

- Dilution factor = 1 x 10^4
- Fixative dilution = 1.1 (10% of solution is IKI fixative)
- Medium dilution = 2 (1:1 of *C. reinhardtii* and medium)

cells/mL = $\frac{10}{1}$ (1 x 10^4)*(1.1)*(2) = 220000 cells/mL

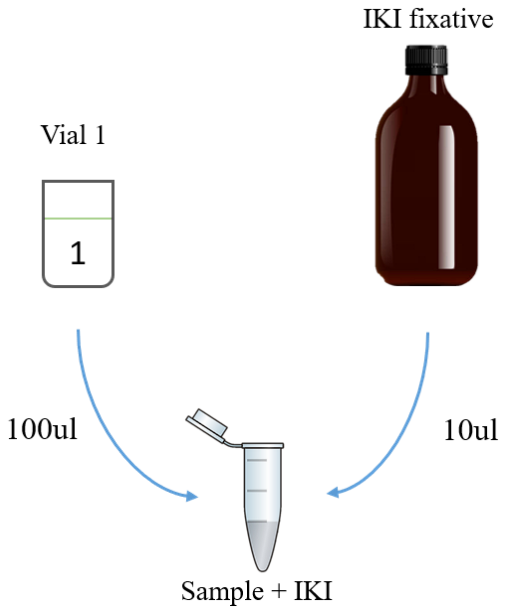

Figure 3. Preparing samples for the haemocytometer. 100ul of the sample mixed with 10ul of IKI fixative in an Eppendorf tube.

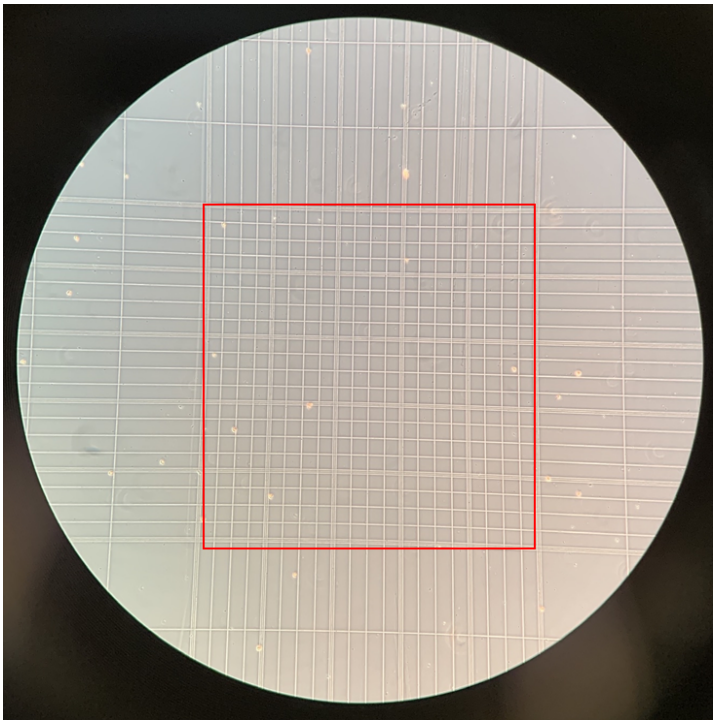

Figure 4. Haemocytometer under the microscope. An image of the haemocytometer grid used to count cells. Cells within the red, 1mm^3 square were counted and used to calculate the total cell count. Dilution factor = 1 x 10^4

Treatment set up

Vials 1, 2, and 3 of each temperature were placed together in a glass bowl as seen in **Figure 5**. One bowl was placed into its respective incubator at a time, in 10 minute intervals. A 60 minute timer was set after the incubator door was closed for each of the bowls. After 60 minutes, the first bowl was removed from the incubator and the vials were shaken before being measured for O_2 concentration. 100ul of solution was taken from vial 1 and mixed with 10ul of IKI to fix the cells for counting later. The following bowls were removed after their respective 60 minutes and the procedure above was repeated. Once all the vials had been measured for O_2 concentration, the fixed cells from each vial 1 were counted.

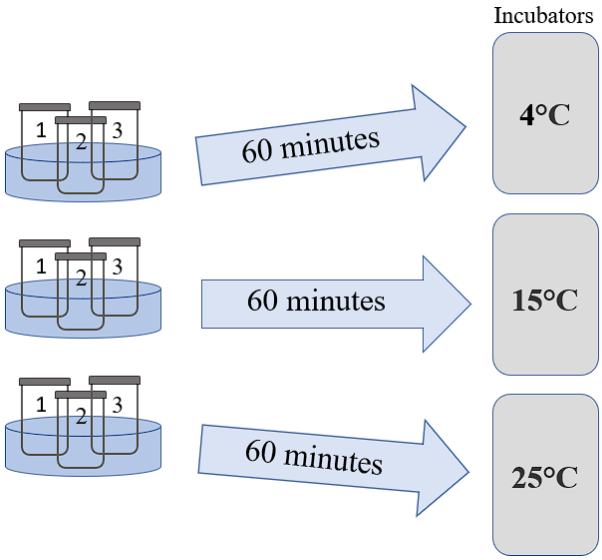

Figure 5. Treatment set-up. A diagram showing the three different temperature treatments that *C. reinhardtii* were exposed to: 4ºC, 15ºC and 25ºC. Incubation time for all vials was 60 minutes and there were three replicates per temperature (n = 3).

Statistical analysis

After obtaining the O_2 concentrations and cell count data, statistical analysis was performed. The mean O_2 concentration at each temperature was used for all analyses. A Kruskal-Wallis test was conducted to compare the means of each treatment and the p-value was used to determine whether the values were significantly different. A Mann Whitney test was also conducted and the p-value obtained from it was analyzed.

Results:

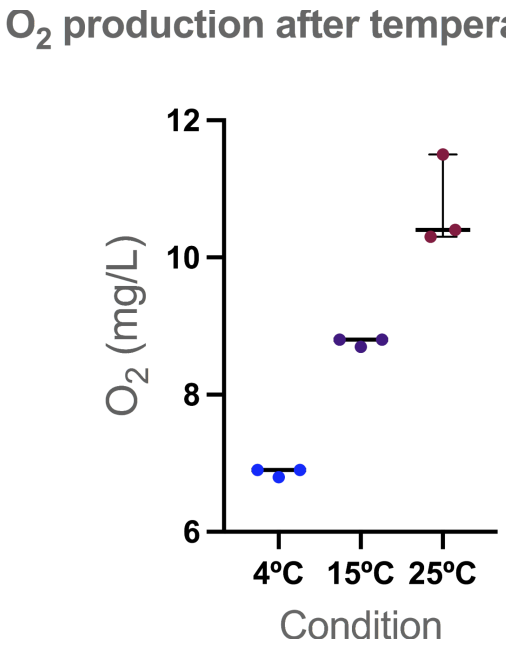

Figure 6. Oxygen concentration measurements from before and after the one hour 4ºC, 15ºC and 25ºC temperature shock are represented in blue, purple, and maroon respectively. The median values and interquartile ranges are indicated by black bars. There was a significant difference between the three treatments (p=0.0036) as calculated using a Kruskal-Wallis analysis.

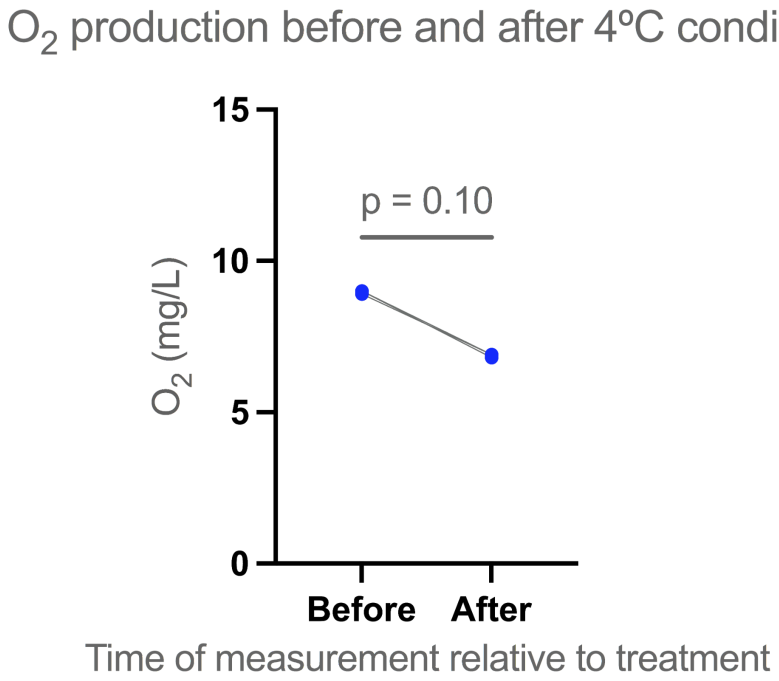

Figure 7. Measurements from before and after the one hour 4ºC temperature shock are represented in blue, the data points from each replicate are connected by a gray line. Before treatment the median oxygen concentration was 9.0 [8.9-9.0] mg/L and after treatment the median oxygen concentration was 6.9 [6.8-6.9] mg/L. The difference in oxygen concentration between the two sites was not statistically significant (p=0.10) as calculated using a Mann-Whitney analysis

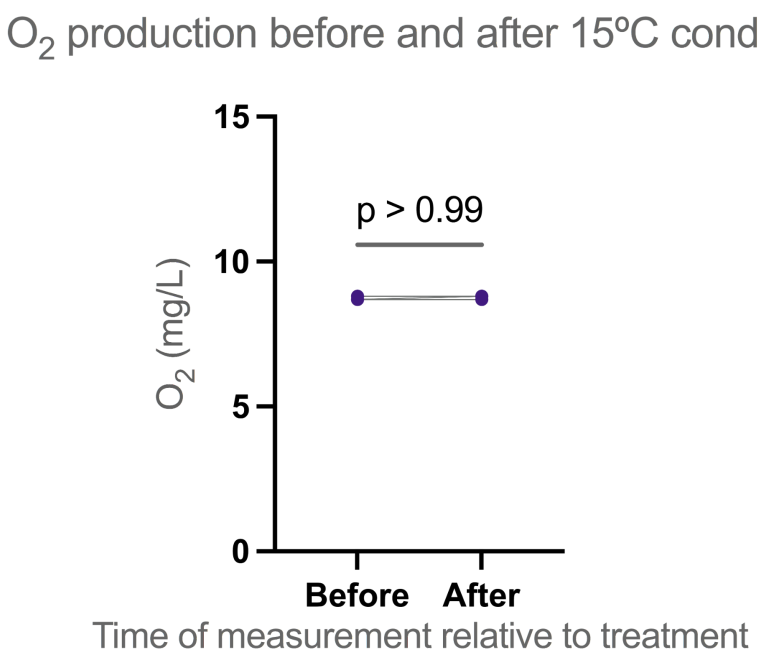

Figure 8. Measurements from before and after the one hour 15ºC temperature shock are represented in purple, the data points from each replicate are connected by a gray line. Before treatment the median oxygen concentration was 8.7 [8.7-8.8] mg/L and after treatment the median oxygen concentration was 8.8 [8.7-8.8] mg/L. The difference in oxygen concentration between the two sites was not statistically significant (p>0.99) as calculated using a Mann-Whitney analysis

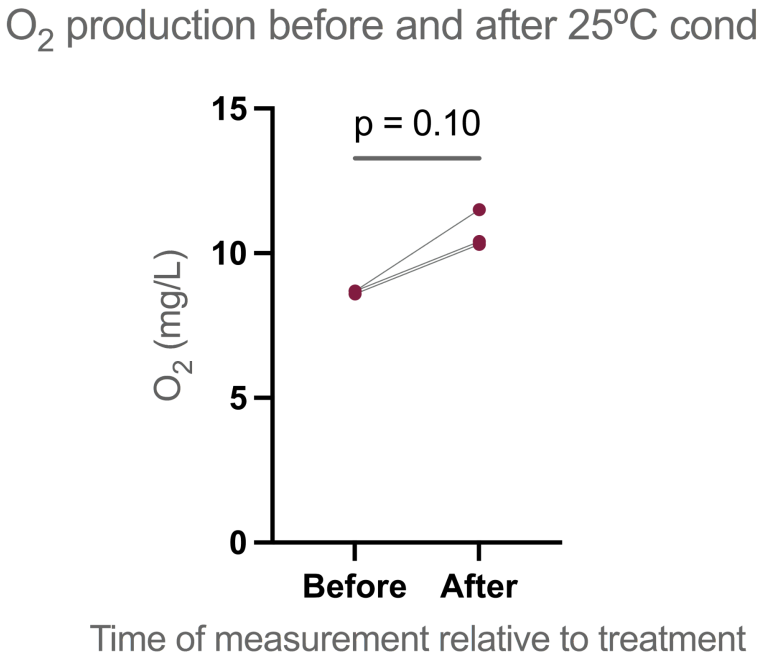

Figure 9. Measurements from before and after the one hour 25ºC temperature shock are represented in maroon, the data points from each replicate are connected by a gray line. Before treatment the median oxygen concentration was 8.7 [8.6-8.7] mg/L and after treatment the median oxygen concentration was 10.4 [10.3-10.5] mg/L. The difference in oxygen concentration between the two sites was not statistically significant (p=0.10) as calculated using a Mann-Whitney analysis

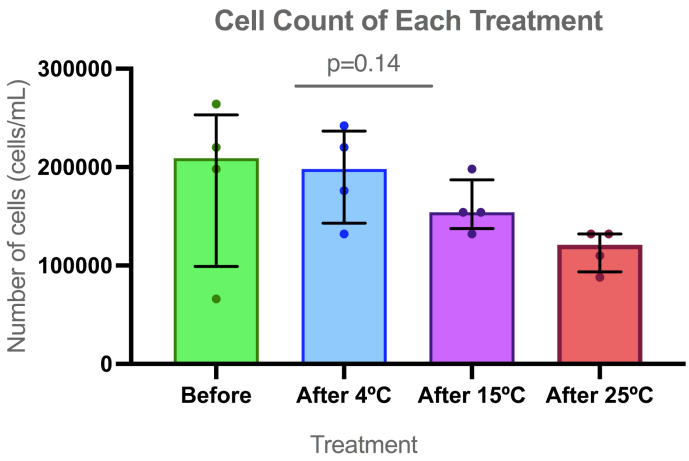

Figure 10. Cell count measurements from before any treatment and after the 4ºC, 15ºC and 25ºC temperature shock are represented in green, blue, purple, and maroon respectively. The median values and interquartile ranges are indicated by black bars. There was no significant difference between the four treatments (p=0.14) as calculated using a Kruskal-Wallis analysis.

Oxygen production was measured before and after being placed in one of the three experimental conditions. The measurements taken after the 4ºC, 15ºC and 25ºC temperature shocks are visualized in Figure 1. The Chlamydomonas samples placed in the 4ºC condition had the lowest median oxygen production level of 6.9 [6.8-6.9] mg/L, 15ºC had an intermediate median oxygen production level of 8.8 [8.7-8.8] mg/L and the 25ºC condition had the highest median oxygen production level of 10.4 [10.3-10.5] mg/L. Data were analyzed using GraphPad Prism software via Kruskal–Wallis analysis to investigate any significant difference between experimental groups. Using this analysis, it was determined that there was a significant difference between the oxygen production of the three groups (p=0.0036).

The impact of the temperature shock on oxygen production was also explored by analyzing the oxygen production of each Chlamydomonas sample before and after their respective conditions. These data are visualized in figures 7, 8, and 9. In each of the aforementioned figures the before and after treatment data for each sample are displayed with a gray line connecting the two measurements for each replicate. A Mann-Whitney analysis was performed on the data for each condition (4ºC, 15ºC and 25ºC) to determine if there is a statistically difference in the oxygen production before and after the temperature shock. Through this analysis it was found that none of the conditions produced a statistically significant difference (4ºC $p=0.10$, 15ºC $p>0.99$, and 25ºC $p=0.10$). There was however a visible trend in the data where the oxygen production of the 4ºC condition decreased, the 4ºC, 15ºC remained rather constant, and the 25ºC samples had a slight increase in oxygen production. There was no statistical difference between the cell count values depicted in figure 10. The number of cells was calculated using the following formula: number of cells counted in haemocytometer x 1.1 x 10^4 x 2.2, and the statistical analysis of these data was done using a Kruskal-Wallis analysis on GraphPad Prism software.

Discussion:

The data showed that there was in fact a difference between the measured oxygen production after the three temperature shock treatments and statistical analysis of these data reject the null hypothesis. The functionality of photosynthetic systems *C. reinhardtii* can be dependent on multiple environmental factors, such as temperature, light, and water availability (Minagawa et al. 2015). Specifically for temperature, free-living green algae like *Chlamydomonas* have been found to down-regulate photosynthetic molecules in response to

decreases in temperature (Miguez et al., 2017) as well as slowing down bodily functions and mobility (Majima et al., 1976). The hypothesis for this study was formed based on this information in addition to photosynthetic recovery being inhibited at low temperatures (Falk et al., 1990). The observed data are consistent with previous reports of decreased oxygen production at lower temperatures (Zheng, et al, 2020, Falk et al., 1990).

When comparing the oxygen production of the samples before and after treatments however, there was no statistically significant difference. Though the findings from these data were not statistically significant, they did show a downward trend in the oxygen production of the 4ºC group, no visible difference in the 15ºC group and an upward trend in the oxygen production of the 25ºC group. A larger sample size and further testing could improve the statistical power of these analyses and more investigation could determine the nature of these changes in oxygen production more definitively.

C. reinhardtii have a cell cycle of around 12 hours and can survive albeit with very little growth at 12°C and no growth at 4°C (Cross et al., 2015, Zheng et al., 2020). Because of this, the procedure allows us to assume that the number of cells per trial remains the same, making the only changing variable in the experiment oxygen produced. By averaging the haemocytometer counts, all trials except for the final count of the 25°C vial had an average of around 176000 cells/ml, with the 25°C vial holding 115500 cells/ml. This difference could be a result of technical limitations in mixing, as *C. reinhardtii* have a tendency to settle very quickly to the bottom of solution. The 25°C vials were prepared last, and the time between pipetting the first vials and the 25°C vials could have led to sedimentation of the organisms. A strong effort was made to constantly swirl the flask and solutions, but this error could have happened at any point during the preparation of the medium, the flasks, or the slides for haemocytometer counting.

Another source of error could be the oxygen measurements using the oxygen meter. Aside from the inaccuracies of the oxygen meter itself, errors in the data could have resulted from technical limitations in the experiment, such as time taken for measurements, air bubbles in the vials, as well as not mixing the solution enough when preparing them. The solubility of oxygen in water is lower than in air, making oxygen readily dissolve out of water (Liss, 1973). This is relevant to the possible errors in the experiment because the oxygen meter reading was not instant and paired along with variations in the time of opening the vials and taking a reading, oxygen could have dissolved out of the solution, altering the reading. Despite efforts to minimize the presence of air bubbles within the vials, it was not possible to completely remove them; oxygen from the solutions could have dissolved into those air bubbles and subsequently the outside air when the lids were open, altering the oxygen reading as well. As mentioned above, the concentration of different parts of the culture can vary due to sedimentation, which could have affected the preparation of the vials and culture, possibly making the number of organisms in each vial different, resulting in varying oxygen production.

Other errors such as human error or equipment errors could account for some inaccuracies in the data. The graduated cylinders, pipette, oxygen meter readings, as well as treatment times are all subject to human errors, which could explain some of the errors observed. Though human error is a possibility, the methods used across all treatment groups were consistent and as such, the findings from this study show convincing patterns which warrant further investigation. Going forward, future studies could explore whether longer temperature shock exposure would have an observable difference. It could also be beneficial to observe a more granular spectrum of temperatures to more clearly determine which temperatures cause the most harm to the organisms.

Conclusion:

The data collected in this study was in line with the stated hypothesis that colder heat shocks will result in less oxygen production in *C. reinhardtii* while warmer temperatures will increase oxygen production. Specifically, the oxygen production after treatments increased with the temperature of the shock (4ºC < 15ºC < 25ºC). These findings demonstrate the importance of temperature with regards to the wellbeing of *C. reinhardtii* providing evidence for the benefit of maintaining a constant optimal temperature in the lab and for the potential negative outcomes of changing climates to *C. reinhardtii* in the wild.

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