# The Effects of Thermal Stress Response on Oxygen Production of Chlamydomonas *Reinhardtii*

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## Abstract

*Chlamydomonas reinhardtii* is a unicellular photosynthetic alga that is vital for ensuring the health and stability of aquatic ecosystems, specifically that of salmon populations. Temperature is well understood as an environmental factor that influences the ability of algae and plants to photosynthesize. In this study, we aimed to investigate the effect temperature plays on oxygen production in *C. reinhardtii*, as an indicator of how potential climate change could impact oxygen levels and the health of salmon populations in the future. The optimal temperature range for *C. reinhardtii* is between 20°C and 32°C. Cultures of *C. reinhardtii* were incubated at three temperature treatments of 4°C, 20°C, and 35°C for an acclimation period of 30 minutes and an exposure time of 75 minutes. Initial and final temperature and oxygen measurements were recorded, and results reflected no significant difference in oxygen levels between the controls and treatment vials at different temperatures. The statistical analysis using a One-way ANOVA provided a p-value significantly greater than 0.05, therefore we failed to reject our null hypothesis that thermal stress would have no mean effect on oxygen production levels in *C. reinhardtii*.

## Introduction

Photosynthesis is the biological process by which algae, plants, and some bacteria convert light energy into chemical energy, turning inorganic material into usable, organic energy. One of these photosynthetic organisms—which is the focus of our experiment—is *Chlamydomonas reinhardtii*, a unicellular alga that performs oxygenic photosynthesis converting light into usable energy, creating oxygen as a byproduct (Sasso et al., 2018). *C. reinhardtii* plays an active role in maintaining the health and diversity of its ecosystem, especially with regards to salmon, a keystone species in BC that contribute to their ecosystem even after death; in fact, ecosystems rely on salmon populations so much so that fluctuations in numbers have observable ramifications on their ecological community, one of which being nutrient cycling (Hilderbrand et al., 2004). In addition to being a major food source for salmon, *C. reinhardtii* significantly influences the oxygen levels of its environment. Marine organisms like salmon require oxygen to function and survive, and exposure to hypoxic environments have been found to result in catastrophic consequences for life (Carter, 2005).

This, in turn, has several impacts on the ecosystem; *C. reinhardtii* is therefore a key species in contributing towards the maintenance of ecosystem health.

Among other elements, temperature is a known environmental factor that affects the efficiency of photosynthesis in algae/plants; when temperatures fall out of optimal range, organisms experience cataclysmic changes in photosynthetic efficiency, membrane fluidity, and enzyme denaturation. Climate change presents an existential threat to ecological systems and life forms, causing warmer ocean/stream temperatures. In this experiment, we aimed to investigate how exposure to various temperatures affects the rate of oxygen production in *C. reinhardtii* as a potential indicator of what salmon populations and oxygen levels may look like in the future, in the context and consideration of C. *reinhardtii*. To investigate the effect of temperature, we exposed sample populations of C. *reinhardtii* to temperature treatments: 4°C, 20°C, and 35°C; our goal is to implement new temperature treatments to combine our data with previous experiments so that we may encapsulate a wider-ranged understanding of the effect of climate change on oxygen production. Our hypotheses are as follows:

$H_0$: thermal stress will have no mean effect on $O_2$ production levels of C. *reinhardtii.*

$H_A$: Oxygen production will be lowest at 4°C and 35°C.

$H_A$: Oxygen production will be highest at 20°C.

The optimal range of temperature for photosynthesis in *C. reinhardtii* was found to be 20°C to 32°C (Prasad et al., 2016); therefore, we predict that 4°C will be too cold and the algae will become dormant and that 35°C will be too warm and *C. reinhardtii* will experience denaturation and reduced photosynthetic activity.

## Methods

For this experiment, a 500 mL stock culture of *Chlamydomonas reinhardtii*, strain cc-1690, wild-type MLT 21g was prepared, along with 500 mL of excess standard media. In addition to this, the following materials were used: 100 mL beakers (x2), a dissolved-oxygen probe, 27 mL vials (x18), incubators (4°C, 20°C, and 35°C), and a 1000 mL beaker. Three initial $O_2$ concentration and temperature readings were taken from each of the stock cultures of *C. reinhardtii* and the standard media. In this experiment, the standard media absent of *C. reinhardtii* acted as the control. Before each measurement was taken the original stock solutions were homogenized manually by swirling the Erlenmeyer flasks in a circular motion.

Three incubators were set to temperatures of 4°C, 20°C, and 35°C and contained a light source (Fig. 1). Nine 27 mL vials were filled with a stock culture of *C. reinhardtii,* and nine 27 mL vials were filled with the standard media. Original solutions were frequently mixed to ensure equivalent concentrations among samples (Fig. 1). Each vial was filled and capped once a meniscus formed above the opening to maintain a maximum volume and prevent air bubble formation. A large beaker was placed underneath to collect spillage. Each sample was labeled according to temperature group and differentiated depending on if it was a treatment or control vial.

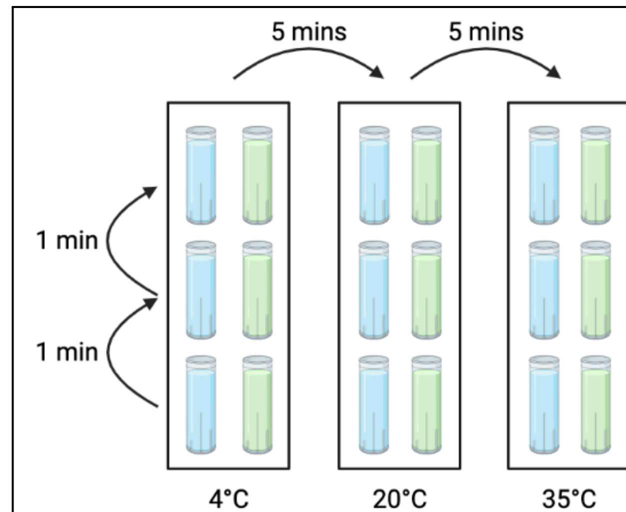


**Figure 1.** Overview of the total number of treatment (green) and control (blue) vials used. Time intervals for placing samples into incubators are shown by the arrows, 1-minute intervals between samples in the same incubator and 5 minutes between sample groups of different treatment temperatures.

Six vials were placed into each incubator, consisting of three treatment and three control vials (Fig. 2). To begin we added one treatment and one control vial into the incubator set to 4°C and staggered the other two pairs by 1-minute intervals to allow time for measurements to be recorded following incubation (Fig. 2). The next six vials were added five minutes later to the 20°C incubator in an identical manner as the previous incubator (Fig. 2). This was repeated once more for the final incubator set to 35°C. An acclimation period of 30 minutes and an exposure time of 75 minutes was applied to all samples for each treatment (Fig. 2).

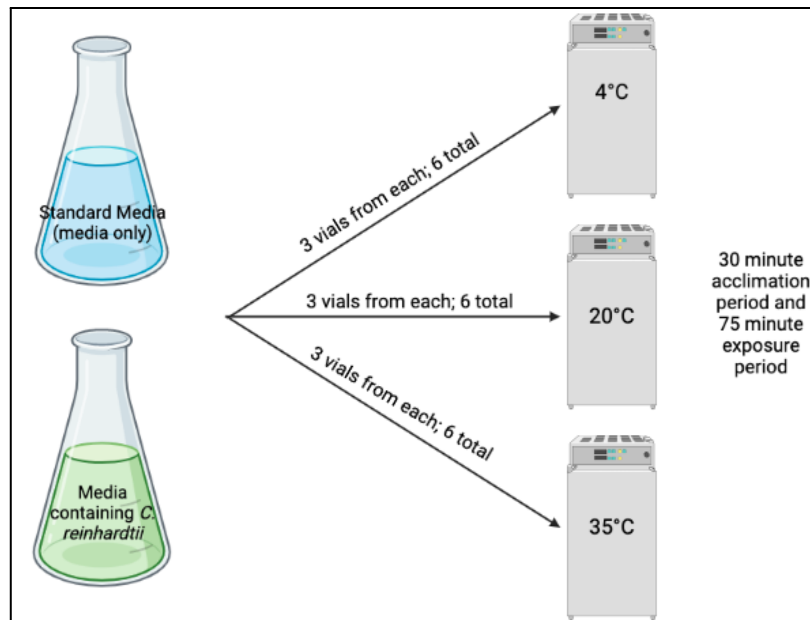


**Figure 2.** Visual demonstration of how treatment and control vials were divided among the different incubators (4°C, 20°C, and 35°C) and how long they were subjected to each temperature treatment.

Samples were removed in pairs; in the same order they were incubated initially and $O_2$ concentration and temperature readings were immediately recorded. A one-way ANOVA was utilized as the objective was to compare the mean values obtained from three different treatment types.

## Results

The oxygen levels (mg/L) calculated for each temperature treatment of 4°C, 20°C, and 35°C for the Standard Medium Test were 8.643 mg/L, 8.683 mg/L, and 11.05 mg/L, respectively. The temperature seemed to influence oxygen levels (mg / L) and not due to the different treatment groups Standard Medium Test and C. *reinhardtii*. The highest oxygen levels (mg / L) were discovered at 35°C for both treatment groups.

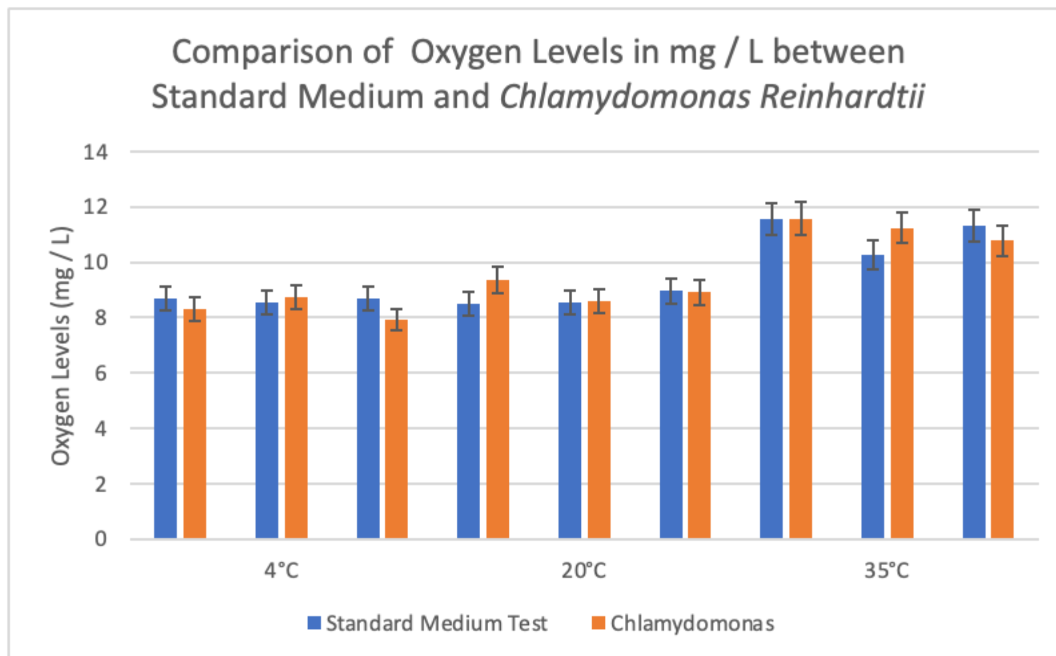


**Figure 3**. The oxygen levels of wild-type *C. reinhardtii* at temperatures 4°C, 20°C, and 35°C with units (mg / L). Bars represent 95% confidence intervals, for each temperature. Data collected in March 2023, UBC.

When comparing the oxygen levels (mg / L) at 4 °C, the error bars overlap, as shown in Figure 1 between Standard Medium Test and C. *reinhardtii*. Similarly, the error bars overlap when comparing the oxygen levels at 20°C between Standard Medium Test and C. *reinhardtii*, as well as when comparing oxygen levels at 35°C. We assume that there was no significant difference when the temperature was decreased to 4 °C or increased to 35°C from the optimal growth temperature (20°C) due to the overlap of both error bars for all scenarios.

Using one-way ANOVA, we have 3 main assumptions in our findings: a) We assume that each treatment group follows a normal distribution b) The distributions have the same variance and c) The data collected from the experiment are independent of each other. A one-way ANOVA test was performed on the results to further confirm the findings. The calculated p-value from the one-way ANOVA test was 0.2662272, 0.3689929, and 0.7597619 for oxygen levels (mg / L) between Standard Medium Test and C. *reinhardtii* at 4 °C, 20 °C

and 35 °C respectively. The data was found to be non-significant because the p-value was greater than 0.05. In conclusion, all of the 95% confidence intervals overlapped, and the one-way ANOVA test indicated that there was no statistically significant difference in measured oxygen levels (mg / L) between treatment groups at 4 °C, 20 °C, and 35 °C respectively. We fail to reject $H_0$ in this case.

**Discussion**

Based on the statistical analysis, the null hypothesis was not rejected. Little difference was found in oxygen levels between treatments and controls under any of the experimental conditions. Furthermore, no significant difference was found between the oxygen levels at different temperatures and while samples at 35°C consistently had higher $O_2$ concentrations than those at 4°C and 20°C, this was true for the 35°C controls too. Thus, the higher concentration of $O_2$ found in 35°C vials is most likely the result of other factors related to temperature, rather than increased photosynthetic activity from *C. reinhardtii* at 35°C temperature. Because *C. reinhardtii* vials did not have consistently higher $O_2$ concentrations than controls, the results do not demonstrate any correlation between temperature and oxygen production. However, because no difference between the treatment and control was observed, there is insufficient evidence to suggest that a correlation between photosynthetic activity and temperature does not exist for the given range of temperatures. To conclude that oxygen production is not correlated to temperature for these temperatures, a significant difference between oxygen production in the controls and *C. reinhardtii* vials would need to be observed, while finding no significant difference in mean oxygen production between the temperature treatments. These data show no difference in oxygen concentrations from photosynthetic organisms as compared with a control that lacks the capability to produce oxygen.

There were several limitations in this experiment that likely had a significant impact on the results, the most notable of which was the inconsistency in lighting, which would have a significant impact on photosynthetic activity. While lighting was considered in the experimental design, technical difficulties prevented the lights from remaining on for the duration of the incubation period. While all incubator lights were initially turned on, they were turned off automatically after a certain amount of time in one of the incubators. The lights in the other incubators were then purposefully turned off to maintain consistent lighting conditions across all temperature treatments. However, this would not have alleviated the suppression of photosynthetic activity caused by a lack of light; rather, it would have standardized it across treatments.

A further limitation due to the equipment was in temperature maintenance. To avoid having the light shut off automatically, the door to the refrigerator in which the 4°C samples were placed was kept slightly ajar. This likely resulted in a significant temperature increase both from the unsealed door and from heat emitted by the lightbulb that would have otherwise been turned off. As a result, the temperature of the 4°C vials was likely significantly warmer.

Finally, temperature acclimation may have impacted the photosynthetic activity of *C. reinhardtii* differently at each temperature. While all *C. reinhardtii* samples were given 30 minutes to acclimatize to their respective temperatures in addition to 75-minute temperature treatments, the 20°C samples underwent a minimal change in temperature from the temperature of the room in which they had been sitting, while the 4°C and 35°C samples had to adjust to substantial changes in temperature. This limitation means that for the 4°C and 35°C samples, oxygen production rates are likely impacted by sudden temperature changes and be different when kept at stable high or low temperatures.

The most effective approach to addressing the shortcomings of this experiment would be to repeat the experiment with better control for variables. First, to standardize the lighting conditions, external lamps with the same brightness could be used in each incubator above the samples, while the built-in lights in the incubators would remain off. While this would still introduce a confounding variable in the heat emitted by the lamps, it would prevent the issues of lights turning off unexpectedly that were encountered here, and the issue of needing to have the fridge door open for the light to remain on. In addition, doubling the duration of the acclimation period would allow the hot and cold treatments more time to adjust to the temperature, meaning the results would be less impacted by sudden changes in temperature.

## Conclusion

Following statistical evaluation, the null hypothesis could not be rejected; temperature stress has no effect on the mean oxygen production of *C. reinhardtii*. The data from this experiment reject the alternative hypotheses, oxygen content progressively increased from 4°C to 35°C.

## Acknowledgments

First and foremost, we would like to acknowledge that we have gathered on the Musqueam people's traditional, ancestral, and unceded territory at UBC. We would also like to thank our instructor, Celeste Leander, and the rest of the BIOL 342 instructional team for assisting us in our experiment when we needed help preparing our organisms and equipment, as well as teaching us the fundamental techniques and skills required to conduct research in the life sciences.

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## Appendix

| | Oxygen Levels (mg / L) | |
|---|---|---|
| | **Standard Medium Test** | **Chlamydomonas** |
| | 8.69 | 8.31 |
| **4°C** | 8.54 | 8.75 |
| | 8.7 | 7.92 |
| | 8.52 | 9.37 |
| **20°C** | 8.56 | 8.58 |
| | 8.97 | 8.92 |
| | 11.55 | 11.58 |
| **35°C** | 10.27 | 11.24 |
| | 11.33 | 10.78 |

**Table 1**. The oxygen levels of 27 mL 9 Standard Medium Test and 9 wild-type *C. reinhardtii* vials at temperatures 4° C, 20° C, and 35° C with units (mg / L) respectively.

| Anova: Single Factor | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | | |
| SUMMARY @ 4°C | | | | | | |
| *Groups* | *Count* | *Sum* | *Average* | *Variance* | | |
| Column 1 | 3 | 25.93 | 8.643333 | 0.008033 | | |
| Column 2 | 3 | 24.98 | 8.326667 | 0.172433 | | |
| | | | | | | |
| | | | | | | |
| ANOVA | | | | | | |
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Between Groups | 0.150417 | 1 | 0.150417 | 1.666975 | 0.266227 | 7.708647 |
| Within Groups | 0.360933 | 4 | 0.090233 | | | |
| | | | | | | |
| Total | 0.51135 | 5 | | | | |

**Table 2**. Calculation of One-way ANOVA between Standard Medium Test and wild-type *C. Reinhardtii* vials at temperatures 4° C respectively.

| Anova: Single Factor | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | | |
| SUMMARY @ 20°C | | | | | | |
| *Groups* | *Count* | *Sum* | *Average* | *Variance* | | |
| Column 1 | 3 | 26.05 | 8.683333 | 0.062033 | | |
| Column 2 | 3 | 26.87 | 8.956667 | 0.157033 | | |
| | | | | | | |
| | | | | | | |
| ANOVA | | | | | | |
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Between Groups | 0.112067 | 1 | 0.112067 | 1.023128 | 0.368993 | 7.708647 |
| Within Groups | 0.438133 | 4 | 0.109533 | | | |
| | | | | | | |
| Total | 0.5502 | 5 | | | | |

**Table 3**. Calculation of One-way ANOVA between Standard Medium Test and wild-type *C. Reinhardtii* vials at temperatures 20° C respectively.

| Anova: Single Factor | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | | |
| SUMMARY @ 35°C | | | | | | |
| *Groups* | *Count* | *Sum* | *Average* | *Variance* | | |
| Column 1 | 3 | 33.15 | 11.05 | 0.4684 | | |
| Column 2 | 3 | 33.6 | 11.2 | 0.1612 | | |
| | | | | | | |
| | | | | | | |
| ANOVA | | | | | | |
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Between Groups | 0.03375 | 1 | 0.03375 | 0.107211 | 0.759762 | 7.708647 |
| Within Groups | 1.2592 | 4 | 0.3148 | | | |
| | | | | | | |
| Total | 1.29295 | 5 | | | | |

**Table 4**. Calculation of One-way ANOVA between Standard Medium Test and wild-type *C. Reinhardtii* vials at temperatures 35° C respectively.

**Collection of pictures taken on the day of the experiment (above)**