The Effect of Light Intensity on the Oxygen Production of *Chlamydomonas reinhardtii*

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ABSTRACT

The purpose of this study is to examine the photosynthetic activities of a single-celled green alga *Chlamydomonas reinhardtii (C. reinhardtii)*, under different light intensities. There were 9 replicates of *C. reinhardtii* exposed to each of the three different light intensity treatments: light (345 Lux), wrapped around cheesecloth (245 Lux), and dark (0 Lux). Controls were included for comparison purposes and contained media only. Oxygen production was measured daily from 3 replicates of each treatment for a total of 3 days. The results obtained in this experiment were analyzed using the one-way ANOVA test, which indicated an insignificant difference between the mean of the four results with a p-value of 0.1017. One of the possible explanations for the insignificant difference is that the temperature chosen in the study was not optimal for *C. reinhardtii* culture growth. Moreover, the experimental length and the short time frame for incubation resulted in less prominent and significant population differences among samples. In conclusion, we failed to reject the null hypothesis and we could not provide subsequent support for the alternative hypothesis.

INTRODUCTION

Our experiment was based on the alga *Chlamydomonas reinhardtii* commonly found in freshwater ecosystems. *C. reinhardtii* is a haploid, unicellular green alga with a diameter officially about 10 μm (About Chlamydomonas, n.d.). The organism contains two flagella which makes it motile. *C. reinhardtii* also possesses a light-sensitive eyespot. Therefore, it can grow autotrophically in light or heterotrophically in the absence of light but the presence of organic carbon (About Chlamydomonas, n.d.). *Chlamydomonas* species can also be found worldwide in soil and freshwater environments. Given that *C. reinhardtii* are classified as phytoplankton, their relative biomass is of significance with respect to the salmon biomass in its surroundings. As phytoplankton, *C. reinhardtii* form the bottom of the food chain as primary producers, therefore, any bloom in *C. reinhardtii* biomass will be seen with an overall increase higher up in the food chain, represented by salmon biomass at the top trophic levels. Additionally, as photoautotrophs,

C. reinhardtii produces oxygen which can affect the natural environment of salmon. A study by Carter et al. (2005) found that reduced oxygen levels in water can increase mortality and decrease the growth rate of salmon, therefore *Chlamydomonas reinhardtii*'s contribution of oxygen in natural environments can directly influence the health of salmon. Moreover, salmon swimming performance decreases in a low oxygen environment, and they avoid areas of low oxygen concentrations (Carter et al., 2005). Thus, salmon would typically be found in environments with a high phytoplankton population. This experiment tested how different light intensities affect the oxygen levels produced by the organism, *C. reinhardtii*. A study by Ryther (1956) investigated the effects of light intensity on photosynthetic rates of marine plants and plankton algae, the experiment results were then constructed into a general photosynthesis-light curve for marine phytoplankton. He found that photosynthesis in photoautotrophs increases as light intensity increases until saturation is reached. After this photosynthesis decreases due to photoinhibition. Furthermore, when placed under a high-light treated environment, researchers found that oxygen levels significantly increased (Roach et al., 2017). Thus, we hypothesized that at greater light intensities, *Chlamydomonas* will have higher population growth, and therefore an increase in overall oxygen production.

METHODS

Sample Preparation:

We went to the laboratory to divide the 1-lite *C. reinhardtii* into twenty-seven identical replicates. We started by using the bunsen burner to heat and disinfect the outer ring of the Erlenmeyer flask that contained the *C. reinhardtii*. We then pipetted 13.5mL of *C. reinhardtii* and 13.5mL of pure media into each 27mL glass vial to make twenty-seven replicates because this

experiment does not take cell growth into consideration, thus, for this situation, 1/2 organism verse 1/2 media appear to the most sustainable concentration.

We then divided twenty-seven replicates into three treatment groups, with one group containing nine samples. The first group was the "light treatment group," which was stored in a 25 °C incubator with a regular eight hours light and a sixteen hours dark cycle with no coverage to block light consumption of *C. reinhardtii*. The second group was the "cheesecloth treatment group." It was stored in the same incubator with regular light cycles but with cheesecloths covering the vial. The third group was the "dark treatment group" kept in the 25 °C dark incubators with no light.

The nine samples within the same treatment group were further divided into three sub-groups, and each sub-group had three replicates that would be opened on the same date, while we opened three samples from each group per day. After dividing the samples, we labelled them by their group (treatment) and sub-group (date).

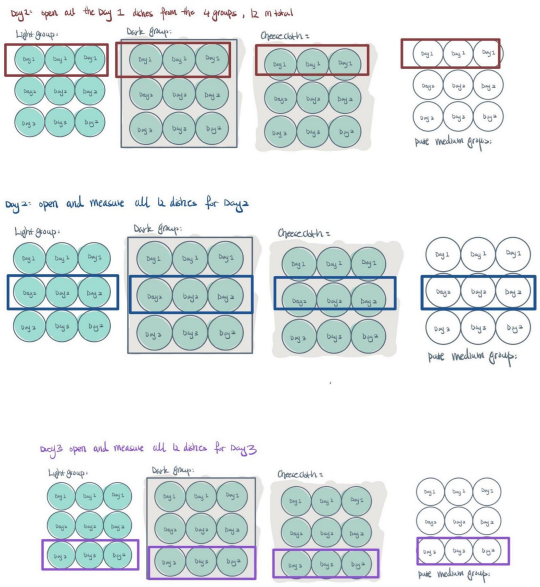

Figure 1. the planning for the experimental procedure and the measurements.

We then filled another nine glass vials with 27ml of media in each to obtain a control group that would also be kept in a regular light cycle incubator and divided them into three sub-groups opened in three days. After finishing labelling the control group, we moved all four groups, in a total of 36 containers, to the two 25 °C incubators.

When we arrived at the regular light cycle incubator, we first used the Light Meter APP to measure the light intensity in the incubator by lux to obtain the light intensity for the light treatment group. Then we covered the cell phone camera with cheesecloth to measure the light intensity of the cheesecloth treatment group. After completing the above steps, we stored the samples in the incubator and returned 24 hours later to measure oxygen production.

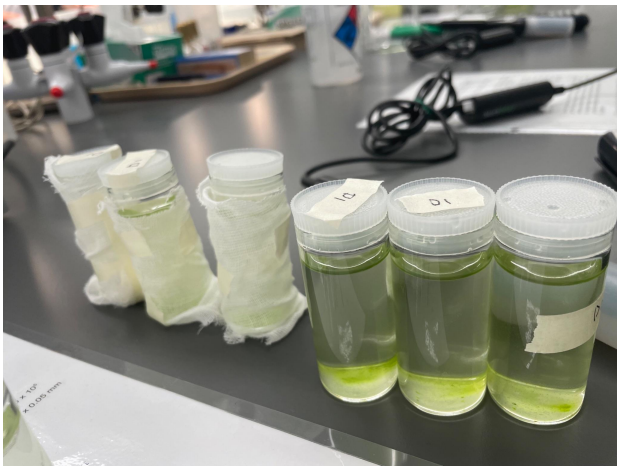

Figure 2.a

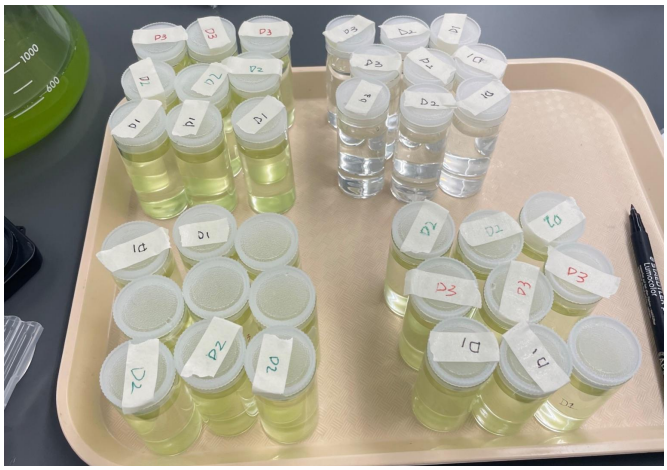

Figure 2.b

Figure 2. a) The comparison between the “cheesecloth group” (left) and “light group” (right). b) the divisions of the four groups after labeling and before moving to incubators.

Measuring Oxygen Production:

After 24 hours, one of our group members returned to the lab and took three samples labelled "Day One" from the four groups, in a total of 12 samples. We slowly swirl the vials before measurement to ensure equal distribution of organisms in the vials. Then the oxygen meter is used to measure the oxygen production of each sample and rinse the probe with

distilled water before contacting the next replicate. After recording the oxygen production in mg/L, we used a micropipette to remove 100uL of each sample into the fixative tube. 50uL of the fixative solution was then added to each tube using a micropipette, and each tube was labelled according to their treatment group, opening date, and the order of opening. All the fixative tubes were kept on a tray and maintained in a refrigerator until the next day. The above steps were repeated for three days until the oxygen production of all the samples was measured.

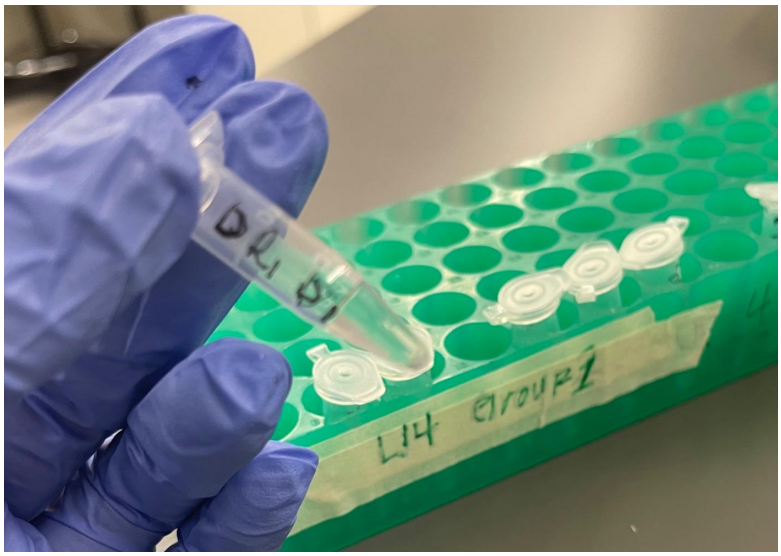

Figure 3. The labeling of fixative tubes based on the group number (D for Dark group /L for light group /C for cheesecloth group /M for media group), replicate order (R1/R2/R3), and date (D1/D2/D3)

Counting:

After three days of oxygen production measurements, we took all the fixative tubes from the refrigerator to count the number of cells per sample. From there, we could further calculate oxygen production per cell in each sample. We used a micropipette to load 20uL of the solution onto the hemocytometer and placed the hemocytometer underneath the dissection microscope. We then measure the cell amount per slide through a handheld tally counter. If the number of cells exceeded 100 on the center grid of the hemocytometer, we used the counting on the center

grid as the final result. If the number of the cells does not exceed 100 on the center grid, we move the hemocytometer to count all the cells on the entire grid.

Statistical analysis :

The data were calculated as oxygen production per replicate divided by the cell number per replicated, which we obtained the oxygen production per cell for each replicate. The significance of the data was analyzed using ANOVA test and a western box plot was generated.

RESULTS

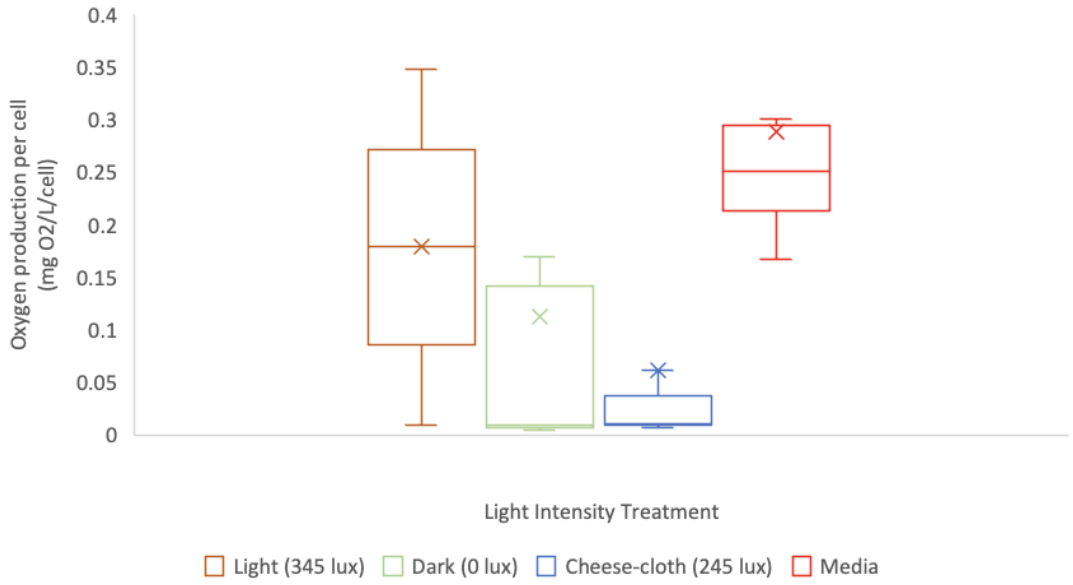

Figure 4. Oxygen production per cell of *Chlamydomonas reinhardtii* in light, dark and cheese-cloth treatment (345 Lux, 0 Lux, and 245 Lux respectively) plus media. Box plots represent 25% and 75% markers (X marker, mean); the upper and lower error bars extend to the minimum and maximum values. p=0.1017, (n=6).

Figure 4 shows an increase in mean oxygen production per cell in the light treatment group (0.179 ± 0.149 mg/L/cell) compared to the dark treatment group (0.1127 ± 0.190 mg/L/cell) and cheese-cloth treatment group (0.062 ± 0.126 mg/L/cell). Surprisingly, our control group containing only the media showed the highest oxygen production (0.289 ± 0.154

mg/L/cell). However, the difference between the mean of the four results is not significant, indicated by a p-value of 0.1017 from the one-way ANOVA test. A consistent trend of increased oxygen production with increased light intensity was not observed since the dark treatment group had a higher mean oxygen production than the cheese-cloth group. The error bars on the graph indicate that when compared to other groups, the maximum value and minimum value are farthest away from the mean in the light treatment group. On the other hand, the error bars also indicate the median value for the dark group and the cheesecloth treatment group are very close to the minimum value, shown by the lower error bar.

Qualitatively, we observed that the replicates from the dark treatment group seem to contain less *C. reinhardtii,* and they are mostly sunk at the bottom of the vials during oxygen measurement.

DISCUSSION

Given the previously stated hypothesis, a positive correlation was expected between the light intensities *C. reinhardtii* experienced and the relative oxygen production, subsequently this would also lead to an expected rise in population growth. However, as indicated within the relative p-value of 0.1017, the given results were concluded to be insignificant at a 5% significance level throughout the procedure completed. Hence, the null hypothesis is then failed to be rejected and the subsequent alternative hypothesis is rejected.

As autotrophs, *C. reinhardtii* is heavily reliant on the completion of photosynthesis to meet their energy production requirements to continuously grow, however, a few reasons can be noted for the lack of growth throughout the population as seen throughout the experiment. Abiotic factors are of significance when considering organisms living within aquatic conditions, hence, the relative temperature in which *C. reinhardtii* thrives greatly impacts their effectiveness

when undergoing photosynthesis. A study suggested by Cho et al. concluded that the level of oxygen production done by *C. reinhardtii* is altered when placed within conditions with ranging temperatures between 7ºC and 27ºC, with temperatures around 17ºC yielding the most oxygen production per cell (Cho et al, 2018). As for the experiment at hand, the samples were retained within incubators with temperatures of 25ºC, therefore, the temperature range can be deemed as suboptimal, which can explain the lack of growth noted. Moreover, as *C. reinhardtii* is predominantly found throughout North America and Japan, the ideal temperature for *C. reinhardtii* is expected to be around 12-18ºC, correlating to the results yielded by Cho et al. (Sasso et al., 2018; Cho et al., 2018). Future experiments can be conducted with different light intensities and alternative temperature ranges to better understand the growth of *C. reinhardtii*, in search of the most optimal conditions to ensure an increase in biomass of other aquatic species such as salmon.

With our results differing from that of Roach et al. (2017), some suggestions may be made on the time-frame chosen in which our samples were incubated. With the growth of the *C. reinhardtii* population expected to be doubled within a 4-hour time frame, the samples of Roach et al. were allowed to grow for a total of 5 days (Roach et al., 2017). Subsequently, our samples were incubated for two and three days, respectively, to allow for growth. By permitting a greater time frame for incubation within future experiments, larger differences in population may be noted, which would then likely yield more prominent and significant results. Additionally, both systemic and random errors are to be found within the experiment conducted. Random errors may have been implemented when micro pipetting samples of *C. reinhardtii* into each respective flask. Hence, slight differences could be noted in the amount of sample added to each flask, ultimately leading to larger uncertainties throughout all 36 samples. Another notable random

error may have been presented as samples were not pulled out of the incubators at specific times throughout the intervals of days. For example, the time of day one sample was removed may have differed from the removal time of a different sample. The lack of precision in our timing may have been impactful to the results that were yielded. Moreover, systemic errors may have risen as oxygen samples were not taken while in the incubators, hence, all samples were exposed to alternative light intensity and temperature while the oxygen probe was used to measure the relative oxygen production. Therefore, due to the fairly rapid growth rate of *C. reinhardtii*, this may have imposed alternative results from what may have been yielded if the oxygen levels were taken within the incubator itself.

Additionally, systemic errors were seen through the unusability of all samples for the first day of our experiment. As the samples were first removed for the respective incubators, media was not introduced into each sample which effectively acts to remove bacteria present within the sample while being stored. Due to this step being missed, the day one samples were all rendered unusable. Moreover, cross-contamination was also present as our control samples had a sparse presence of *C. reinhardtii* noted while undergoing the cell-counting process. Mixing of the control and *C. reinhardtii* may have been introduced when the samples were initially being prepared.

CONCLUSION

We predicted the relative light intensity to have a positive correlation between the growth in the population of *C. reinhardtii* and their overall level of oxygen production. However, with a p-value of 0.1017, no significance was noted within this hypothesis. Yet, the experiment allowed us to note the importance of other abiotic factors, such as temperature, and how it plays a vital role when considering the population growth of *C. reinhardtii*. As *C. reinhardtii* is one of the

main providers of oxygen and nutrients to the upper echelon of the food chain, without its presence, the entirety of the food chain would be vulnerable to a collapse. Therefore, learning the relative properties and the thresholds to ensure its presence throughout our ocean waters is essential moving forward.

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