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

Abstract

As one of the primary sources of O_2 in many freshwater ecosystems, *Chlamydomonas reinhardtii* have well-defined genetics for photosynthesis. We have examined more specifically the effect of various light wavelengths on *C. reinhardtii* and its development of O_2 production for a more thorough perception of its photosynthetic selectivity. The method chosen used *C. reinhardtii* culture, which were subjected into 15 vials, where each 5 were separated under blue light, red light, and white light. Cell counts were measured using a haemocytometer, and average oxygen production for each treatment was then calculated. The average O_2 production was found to be very significant for white light compared to blue and red light, however the average blue and red light O_2 production values were insignificant based on the one-way ANOVA test and Tukey's multiple comparison test.

Introduction

Chlamydomonas reinhardtii is a unicellular green alga. It is structurally similar to vascular plants, but is less complex due to being unicellular (Dent, Han, & Niyogi, 2001). As a result, it is considered to be a model organism for photosynthetic research (Dent et al., 2001). *C.*

reinhardtii consists of a large cup-shaped chloroplast and an eyespot that is used to detect light (Levine & Ebersold, 1960).

C. reinhardtii is a photoautotroph that uses photosynthesis to produce energy. Photosynthesis is the process where solar energy is converted into electrochemical energy that an organism can use (Minagawa & Tokutsu, 2015). During photosynthesis, light is absorbed by the chloroplast which results in the reduction of NADP+ which generates NADPH and ATP (Minagawa & Tokutsu, 2015). During this process, oxygen is produced as a by-product when electrons are transferred from water to oxygen (Ignjatovic, 1968). As a result, *C. reinhardtii* and other algae are considered to be one of the major sources of O_2 in rivers.

C. reinhardtii has an important role in freshwater ecosystems of British Columbia (BC). One role is that it is considered to be one of the major food sources of salmon (Norambuena et al., 2015). Another, is that it is one of the major oxygen sources of the marine ecosystem (Walker, 1980), thus maintaining the amount of oxygen level in rivers and streams.

One of the keystone species of BC are salmons. This means that many different species rely on salmon as a food source that keeps the stability of BC's ecology, thus it is important to make sure salmons remain healthy. In order for salmons to stay healthy, oxygen must be maintained at an optimal level. This is because oxygen is required for a salmon to grow, develop, metabolise, swim, feed, and reproduce (Hansen et al., 2015). When oxygen is at an optimal level, it allows the salmon to stay healthy and do all of the processes stated before.

Oxygen production in *C. reinhardtii* is reliant on light. Light intensity and colour can vary, with higher intensity and more blue light during the day, and lower intensity and more red light during sunset or sunrise (Oldenhof, Zachleder, & Ende, 2006). Light colour can also differ

depending on the depth. Red light is absorbed by the ocean at around 40 m, while blue light can penetrate more than 100 m (Dickey, Kattawar, & Voss, 2011). The activation of *C. reinhardtii*'s proteins and cycles depend on different light wavelengths (red light is from 620-750 nm, blue light is from 450-495 nm, while white light is from 380-750 nm because it is made up of all of the light colours) (Mooij, Vries, Latsos, Wijffels, & Janssen, 2016). This would affect processes such as its metabolism, sexual cycle, and photosynthetic cycle (Mooij, Vries, Latsos, Wijffels, & Janssen, 2016). This makes it important to know which light wavelength *C. reinhardtii*'s photosynthetic cycle would work most efficiently in. As the cycle runs more efficiently, more oxygen is produced. For this reason, it is important to study how *C. reinhardtii* reacts under different light wavelengths.

This research was done to determine if different light wavelengths will produce different oxygen level production of *C. reinhardtii*. Our prediction is that white light would produce the most oxygen because white light contains all of the light wavelengths, thus activating all of the proteins needed for the photosynthetic cycle to run efficiently (Mooij et al., 2016). Additionally, blue light would produce the second most amount of oxygen. This is because *C. reinhardtii* is most sensitive to blue light (Oldenhof et al., 2006). Lastly, red light would produce the least amount of oxygen.

Methods

The experiment conducted consisted of 3 equal sized treatments (n = 5 per treatment) measured at the initial (t = 0 hrs) and final time (t = 6 hrs). The treatments were red light, blue light, and white light (control).

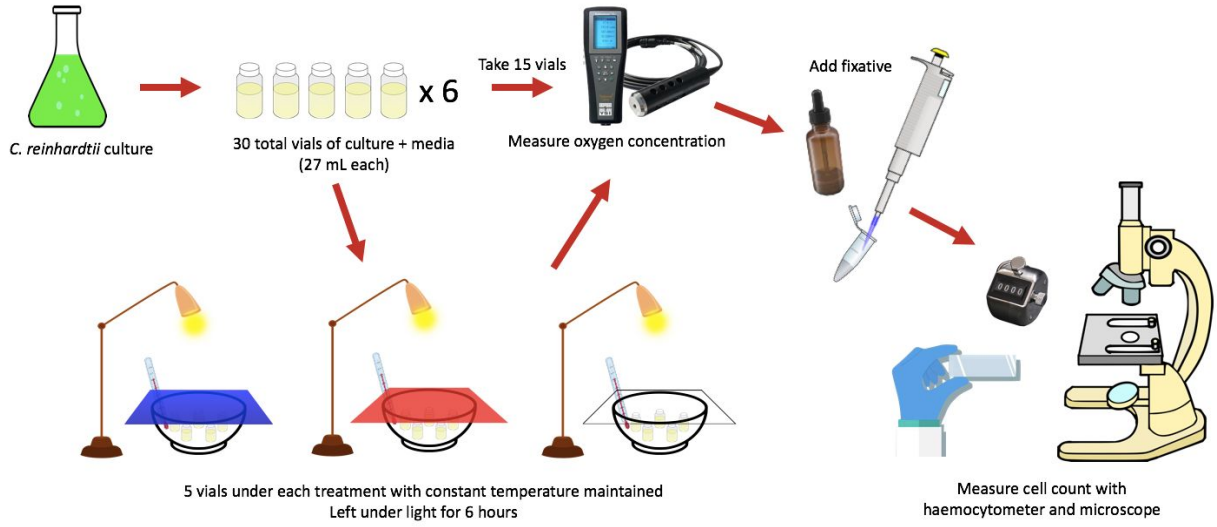

Figure 1. A schematic diagram of the complete experimental procedure.

Treatment Set Up:

The algae culture was diluted two-fold from a concentration of 5×10^5 *cells/mL* to a concentration of 2.5×10^5 *cells/mL* using standard media and separated into plastic vials. Initial measurements of cell counts and oxygen levels were performed to determine that the initial conditions were constant among all treatments. Each set of 5 vials were maintained constant throughout the experiment at 18 °C ± 2 °C by water baths. The three treatment water baths were put under white lamps as the light source, which measured a light intensity of 4400 ± 50 lux by the light meter at the distance of the water bath.

To create separate light treatments, red, blue, and clear acetate papers were used to cover the water baths of the respective treatment vials. Black plastic was used to cover the sides and bottoms of the water bath bowls to ensure that no external light source was able to reach the treatment vials.

Figure 2. The experimental set-up featuring the white light treatment using clear acetate paper. Black plastic was used to cover the sides of the bowl to only allow light to enter through the acetate paper at the top to maximize even light distribution. A thermometer, water bath, and available ice on the side was used to monitor and maintain constant temperature in the vials.

Data Collection:

For each vial, the oxygen concentration (mg/L) was measured with an oxygen meter and the cell count average was taken to calculate the oxygen concentration produced per cell. To determine the concentration of cells in each vial, 3 cell counts were taken with a sample of the culture using a haemocytometer and the average was taken to be the concentration of the vial[1]. 1 µL of IKI fixative was added for every 10 µL of cell culture.

The oxygen concentration is then calculated with the following equation:

$$Change\ in\ O_2\ Production\ per\ cell = \frac{O_2\ Concentration\ Final}{Avg\ number\ of\ cells\ final} - \frac{Avg\ O_2\ Concentration\ Initial}{Avg\ number\ of\ cells\ Initial}$$

Data Analysis:

The means and standard deviation of oxygen production per cell were calculated for each treatment group. A one-way Analysis of Variance (ANOVA) test was conducted to determine if any statistically significant difference existed between the means of each treatment group. The Tukey's Multiple Comparisons post-hoc analysis conducted was used to determine which treatment group was statistically different, given a significant ANOVA test.

[1] Appendix 1. Dilution Factor Calculation

Results

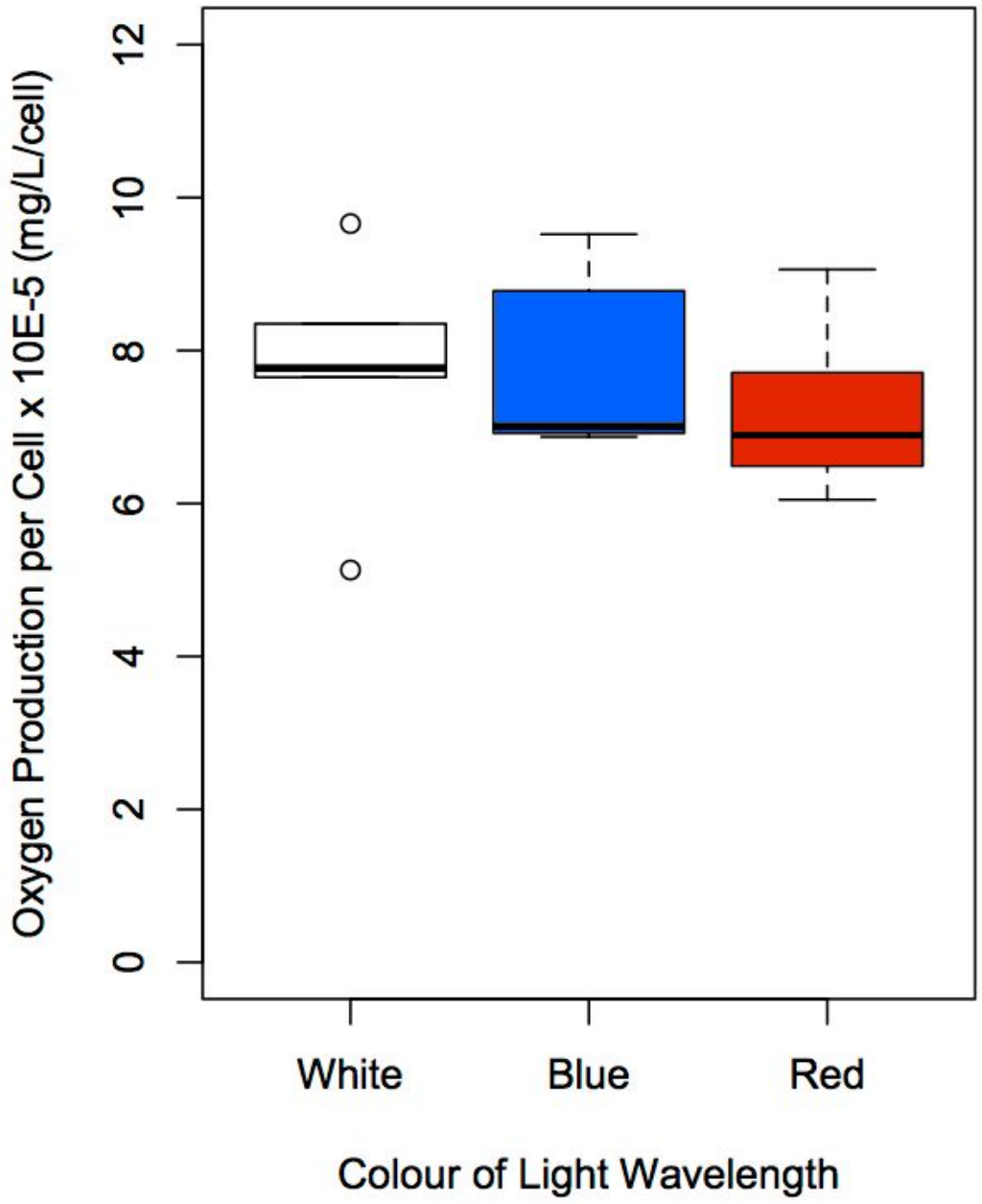

Figure 3. Side-by-side boxplots displaying the effect of light wavelength on the mean oxygen production per cell in *C. reinhardtii* prior to treatment (t = 0 hrs). The bolded black lines represent the median oxygen production at different light wavelength. The rectangles represent the interquartile ranges on the data of each treatment. The whiskers indicate variability outside the upper and lower quartiles between the maximum and minimum values, respectively. Outliers are represented by the hollow circles. A one-way ANOVA test was run and resulted in the values of: *p*-value = 0.782, F-value = 0.252, treatment df = 2, residual df = 12, n = 5.

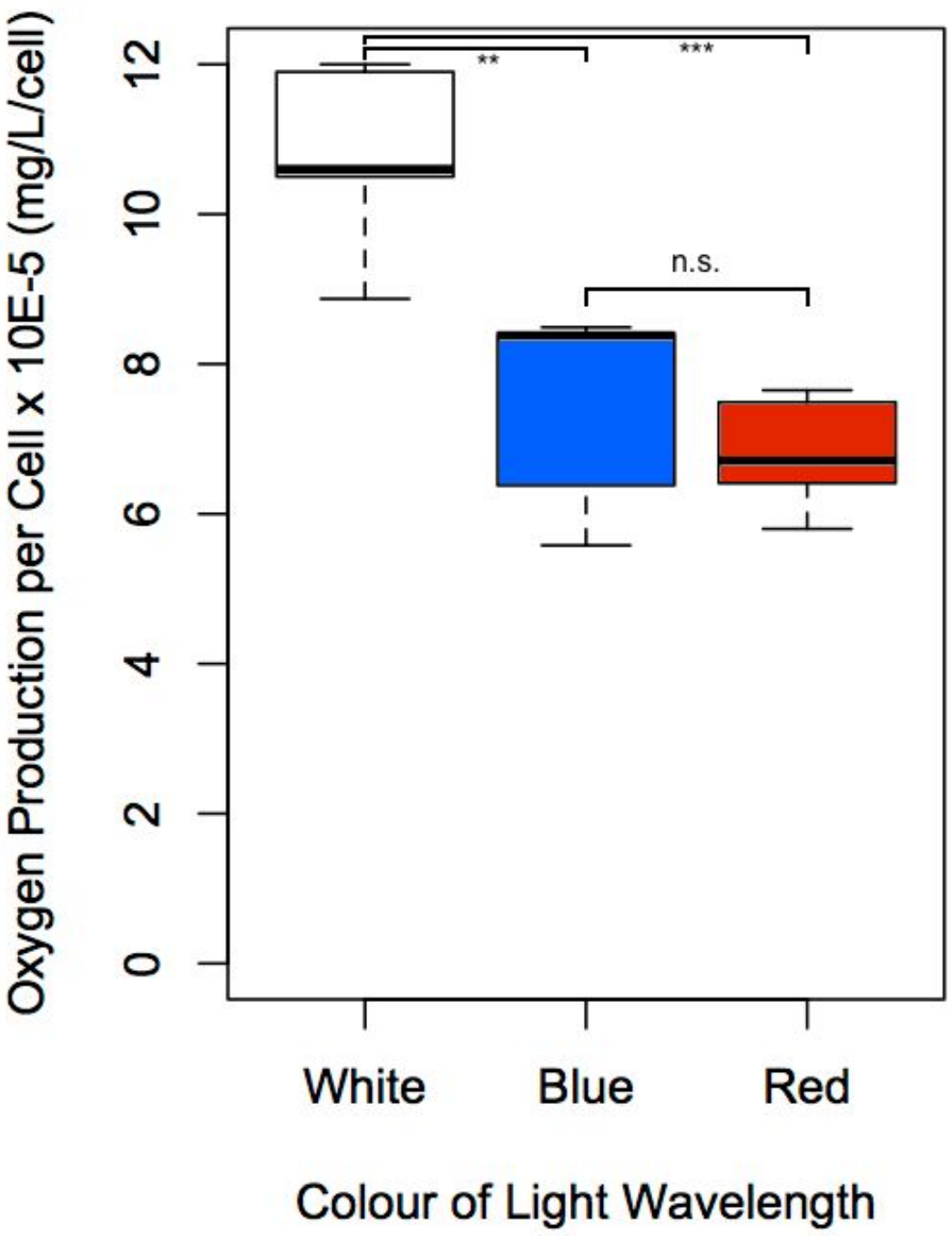

Figure 4. Side-by-side boxplots displaying the effect of light wavelength on the mean oxygen production per cell in *C. reinhardtii* after treatment (t = 6 hrs). The bolded black lines represent the median oxygen production at different light wavelength. The rectangles represent the interquartile ranges on the data of each treatment. The whiskers indicate variability outside the upper and lower quartiles between the maximum and minimum values, respectively. A one-way ANOVA test was run and resulted in the values of: *p*-value = 0.000351, F-value = 16.59, treatment df = 2, residual df = 12, n = 5.

Initial Measurements:

The oxygen production in the vials that were analyzed prior to spending time in different light wavelength treatment (where t = 0 hrs) are presented in Figure 3, which shows that the highest mean oxygen production was under the blue light treatment with a value of 7.820×10^{-5} mg/L/cell with a standard deviation of 1.243×10^{-5} mg/L/cell. The second-highest mean oxygen production was under white light which was 7.712×10^{-5} mg/L/cell with a standard deviation of 1.649×10^{-5} mg/L/cell. The lowest mean oxygen production was with the red light treatment being 7.240×10^{-5} mg/L/cell with a standard deviation of 1.187×10^{-5} mg/L/cell.

The one-way ANOVA test was performed, producing an F-value of 0.252, with 2 degrees of freedom between groups and 12 degrees of freedom within groups. The one-way ANOVA test also resulted in a *p*-value of 0.782, which is greater than α, the critical value 0.05. This indicates that there is no significant difference among the means of the three different treatments at the initial time of the experiment.

Final Measurements:

After analyzing the data that was collected after undergoing treatment, Figure 4 shows that mean oxygen production under the white light treatment was the highest with a value of 1.077×10^{-4} mg/L/cell with a standard deviation of 1.275×10^{-5} mg/L/cell. Blue light treatment resulted in the second-highest amount of oxygen as the mean oxygen production was 7.448×10^{-5} mg/L/cell and the standard deviation was 1.370×10^{-5} mg/L/cell. Lastly, mean oxygen production with the red light treatment was the least out of all the treatments, being 6.812×10^{-5}

mg/L/cell with a standard deviation of 7.678 x 10^{-6} mg/L/cell. These results are aligned with those predicted prior to conducting the experiment.

Comparing the means of all three treatments of different light wavelength with the one-way ANOVA test gives us a *p*-value of 0.000351, which is less than α, the critical value 0.05. The F-value was 16.59, with 2 degrees of freedom between groups and 12 degrees of freedom within groups.

The Tukey's multiple comparison test was also conducted to compare which of the means are significantly different from each other. Comparing the white light treatment with the red light treatment, they obtained a mean difference of 3.962 x 10^{-5} mg/L/cell, resulting in the difference between the means to be significantly different. White light treatment compared with the blue light treatment was also significantly different as they held a mean difference of 3.326 x 10^{-5} mg/L/cell. However, blue light versus red light did not have a significant difference between their means. The mean difference was 0.636 x 10^{-5} mg/L/cell. All pairings held 12 degrees of freedom with each other.

Discussion

Our experiment found that a statistically significant difference exists between the mean oxygen produced per cell between treatment groups of red, blue, and white light. Therefore, we reject the null hypothesis and conclude that light wavelength has an effect on the amount of O_2 produced by *C. reinhardtii*. This implicates that there was a change from the initial vials, which had no significant difference among the means, to the final vials that did have a significant difference among the means of the three treatments.

The Tukey's multiple comparison analysis showed that white light had a significantly higher post-treatment mean oxygen production per cell than both blue light and red light. This agrees with our prediction because white light, which is composed of both blue and red light, may be able to activate both red-specific and blue-specific proteins that allow for photosynthesis. Blue light was also found to have a higher mean oxygen production than red light, but this difference was not statistically significant. The one-way ANOVA test performed on the initial samples were insignificant, demonstrating that there are no differences in the oxygen production before the treatment. This agrees with what we expect as oxygen production should be the same for all replicates before the treatment.

These study results agree with the literature that both red light and blue light are important for photosynthesis, and therefore the production of oxygen (Mooij et al., 2016; Beel et al., 2012). Our study suggests that the photosynthetic cycle changes the amount of O_2 that is produced. Blue light also showed a higher mean than red light, which agrees with our prediction, but this statistical insignificance may be due to the small sample size or other introduced errors.

Our results demonstrate the importance that light has on the productivity of algae, which subsequently has consequences on the consumers of these organisms, such as salmon, and other factors of the environment. Since *C. reinhardtii* are primary producers, the consumers of these algae and the rest of the food chain all depend on their abundance. We find that the wavelength of light affects the amount of oxygen that is produced by *C. reinhardtii*. Since blue and red light had significantly lower oxygen production that white light, this may suggest that there are differences in oxygen production at different times of the day and at different water depths. Sunrise and sunset may have lower oxygen production rates, since there is more red light at these

times. We expect to find *C. reinhardtii* most abundant in shallow waters. This is because red light is not able to penetrate to deep waters, but they require both red light and blue light to function (Loos, Costa, & Johannessen, 2017). Having varying oxygen production rates at different times or depths may affect the feeding, development, and other physiological processes of salmon.

In order to ensure salmon survival, environmental systems should have adequate light to not only maintain metabolic and developmental processes of salmon, but also to indirectly maintain the oxygen levels and abundance of plankton and algae, which many organisms depend on. Especially in BC where salmon is so crucial as a keystone species, it may be useful to track the wavelength of light that penetrates the water in various locations to help predict algae and salmon abundance, and ultimately the health of the ecosystem.

There are various errors that may have been introduced during the experiment. The temperature fluctuated between measurements and steadily warmed up during cell counts, which may have increased oxygen production levels. Similarly, between the first and last vials measured, the cell count numbers may have increased for the later vials because they were allowed to continue to grow for a longer period of time. Errors in the lux measurement may have also caused inconsistent treatment conditions. The light intensity on top of the coloured acetate papers was consistent, but the actual light intensity was much lower under the darker red and blue acetate papers. This light intensity difference may help to explain the insignificant difference between the red and blue treatments and the significant difference between white treatment compared to the red and blue treatments.

Further studies should be conducted with other wavelengths of light, at more frequent and longer periods of study, and by measuring other response variables such as growth, cell cycle phase, and CO2 levels. Since the doubling time of *C. reinhardtii* is between 5-25 hours, a longer study may have shown different effects that were not seen in our 6 hour study (McAteer, Donnan, & Peter, 1985). This can help determine if specific light wavelengths affect other processes such as metabolism, sexual cycle, and photosynthetic cycle. These other processes will affect the abundance and oxygen production levels of *C. reinhardtii*, which in turn affect the abundance of salmon.

Conclusion

Our study rejects the null hypothesis and finds that while white light has a significant difference in O_2 production compared to red and blue light treatments. Meanwhile, the blue light and red light treatments do not have a significant difference in O_2 production even though the blue light treatment has a greater average O_2 production than the red light treatment. Despite this, our study displays the selectivity in light wavelengths in terms of *C. reinhardtii* photosynthetic properties, light-selective proteins and receptors. It also indicates that using white light will conjunctively maximize *Chlamydomonas reinhardtii* selectivity, thereby increasing O_2 production to its maximum.

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Literature Cited

Beel, B., Prager, K., Spexard, M., Sasso, S., Weiss, D., Müller, N., … Mittag, M. (2012). A flavin binding cryptochrome photoreceptor responds to both blue and red light in *Chlamydomonas reinhardtii. The Plant Cell, 24*(7), 2992–3008. doi:10.1105/tpc.112.098947

Dent, R. M., Han, M., & Niyogi, K. K. (2001). Functional genomics of plant photosynthesis in the fast lane using *Chlamydomonas reinhardtii*. *Trends in Plant Science, 6*(8), 364-371. doi:10.1016/S1360-1385(01)02018-0

Dickey, T. D., Kattawar, G. W., & Voss, K. J. (2011). Shedding new light on light in the ocean; *Physics Today, 64*(4), 44. doi:10.1063/1.3580492

Hansen, T., Olsen, R., Stien, L. H., Oppedal, F., Torgersen, T., Breck, O., . . . Fjelldal, P. G. (2015). Effect of water oxygen level on performance of diploid and triploid atlantic salmon post-smolts reared at high temperature. *Aquaculture, 435*(1), 354-360. doi:10.1016/j.aquaculture.2014.10.017

Ignjatovic, L. R. (1968). Effect of photosynthesis on oxygen saturation. *Water Pollution Control Federation, 40*(5), 151-161. doi:10.1111/j.1399-3054.1966.tb07046.x

Levine, R. P., & Ebersold, W. T. (1960). The genetics and cytology of *Chlamydomonas*. *Annual Review of Microbiology, 14*, 197-216. doi:10.1146/annurev.mi.14.100160.001213

Loos, E., Costa, M., & Johannessen, S. (2017). Underwater optical environment in the coastal waters of british columbia, canada. *Facets, 2*(2), 872-891. doi:10.1139/facets-2017-0074

McAteer, M., Donnan, L., & Peter C. L. John. (1985). The timing of division in *Chlamydomonas. The New Phytologist, 99*(1), 41-56. doi:10.1111/j.1469-8137.1985.tb03635.x

Minagawa, J., & Tokutsu, R. (2015). Dynamic regulation of photosynthesis in *Chlamydomonas reinhardtii*. *The Plant Journal, 82*(3), 413-428. doi:10.1111/tpj.12805

Mooij, T. d., Vries, G. d., Latsos, C., Wijffels, R. H., & Janssen, M. (2016). Impact of light color on photobioreactor productivity. *Algal Research, 15*, 32-42. doi:10.1016/j.algal.2016.01.015

Norambuena, F., Hermon, K., Skrzypczyk, V., Emery, J. A., Yoni, S., Beard, A., & Turchini, G. M. (2015). Algae in fish feed: Performances and fatty acid metabolism in juvenile atlantic salmon. *PLoS One, 10*(4). doi:10.1371/journal.pone.0124042

Oldenhof, H., Zachleder, V., & Ende, H. V. D. (2006). Blue- and red-light regulation of the cell cycle in *Chlamydomonas reinhardtii* (*Chlorophyta*). *European Journal of Phycology, 41*(3), 313-320. doi:10.1080/09670260600699920

Walker, J. C. G. (1980). The oxygen cycle. In O. Hutzinger (Ed.), *The natural environment and the biogeochemical cycles* (pp. 87-104). Berlin, Heidelberg: Springer Berlin Heidelberg. doi:10.1007/978-3-662-24940-6_5

Appendix

1. The dilution factor was 1×10^3 because the largest square of the haemocytometer was used to count the cells, which measured 3 mm by 3 mm. The following formula was used to determine the concentration of the cells in each vial.

$$Number\ of\ cells\ per\ mL\ =\ (Cell\ count/1\ cell)\ \times 1 \times 10^3$$