Effects of Wavelength on the Photosynthetic Oxygen Production of *Euglina gracilis*

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Abstract

The main purpose of this study was to determine the photosynthetic oxygen production of *Euglena gracilis* when exposed to different wavelengths of visible light. Prepared *E. gracilis* was distributed accordingly into numerous vials. Prior to incubation, the prepared *E. gracilis* underwent O_2 concentration measurements using an Oxygen Probe Meter and cell counts under a compound microscope at 1000x magnification. Final O_2 concentration measurements and cell counts also took place at the end of our experiment. These vials were then placed under 3 different light treatments: red, green, and normal light (positive control). Our negative control was left away - unexposed to a specific light intensity. Each light treatment was surrounded with black plastic and covered with different colored acetate paper (red, green, and clear). Statistical analysis was performed using a one-way ANOVA test, obtaining a p-value of 0.02972 and an F-statistic value of 6.68462. Therefore, we reject the null hypothesis and conclude that the productivity of *E. gracilis* does differ between the different light treatment groups. The amount of photosynthetic oxygen production of *E. gracilis* allowed us to determine where in the environment, *E. gracilis* is producing the most oxygen - thus, benefiting other organisms (i.e. Salmon) in the food chain in a particular environment. It is crucial to understand the metabolic processes that *E. gracilis* undergoes and the role it plays in its environment.

Introduction

Euglena gracilis is a single-celled eukaryote that can live in both freshwater and saltwater (Borowitzka, 2018). It can conduct photosynthesis to create energy for itself, but it also feeds by phagocytosis, thus *E. gracilis'* is considered to be both autotrophic and heterotrophic (Yamane et al., 2001). In addition, *E. gracilis* has a red eyespot which aids in filtering light and

in phototaxis (Ogawa et al., 2016). The red eyespot, also referred to as the stigma, allows *E. gracilis* to find the most favorable environment to conduct photosynthesis (Ogawa et al., 2016).

The stigma on *E. gracilis* consists of carotenoid pigments, which are responsible for absorbing blue-green and violet wavelengths of light. However, they reflect yellow, red, and orange wavelengths of light (Krinsky and Goldsmith, 1960). Furthermore, *E. gracilis* has chloroplasts that contain large amounts of Chlorophyll A and Chlorophyll B pigments (Krinsky and Goldsmith, 1960). Chlorophyll A strongly absorbs wavelengths from 430nm (blue) to 662nm (red), while Chlorophyll B strongly absorbs wavelengths from 450nm to 640nm. Both of the chlorophylls do not absorb green light, which is why the chlorophylls appear green.

If Chlorophyll A and Chlorophyll B absorb blue and red light most efficiently but absorb green light the least, then we predict the rate of photosynthesis should be higher when *E. gracilis* is exposed to red or blue light. Additionally, microalgae absorb light at a wavelength of 660nm (red) most efficiently (Yan et al., 2016). The main objective of this study was to determine the effect of different wavelengths on the photosynthetic oxygen production in *E. gracilis*. Therefore, our null and alternative hypothesis' were as follows:

Null hypothesis: The oxygen production of *Euglena gracilis* does not differ in the red, green, and clear light.

Alternative hypothesis: The oxygen production of *Euglena gracilis* does differ between the different light treatments (red, green, and clear).

Euglena gracilis is a "promising candidate as an oxygen producer in biological life-support systems" (Richter et al., 2014). Therefore, at an optimal light intensity depth in the water column, *E. gracilis* can successfully perform photosynthesis as a source of nourishment for itself while producing oxygen in the process (Zhu, Long, & Ort 2008). The oxygen accumulation within the water helps to maintain an aerobic marine environment for various organisms (Zhu, Long, & Ort 2008). Furthermore, since *E. gracilis* is a primary producer, it can affect the marine food web according to the bottom-up cascade; juvenile marine insects prey on *E. gracilis* and salmon prey on juvenile marine insects (Maier & Simenstad 2009). Salmon are a keystone species, and they need oxygen to survive and also prey on juvenile marine insects, both of which are affected by *E. gracilis.* Therefore, *E. gracilis* have a large impact on the abundance of salmon in a marine environment (Maier & Simenstad 2009).

Methods and Materials

Refer to Figure 4 for a visual representation of the major steps used in this experiment.

Euglena Culture Preparation

The organism, *E. gracilis,* was used in this experiment. It was cultured over a span of multiple weeks in the Biological Sciences Building at the University of British Columbia. A culture of 550 mL of *E. Gracilis* and 550 mL of excess media were obtained. Given the cell culture and excess media, a 1:1 dilution of *E. Gracilis* was performed, as seen in **Figure 1**.

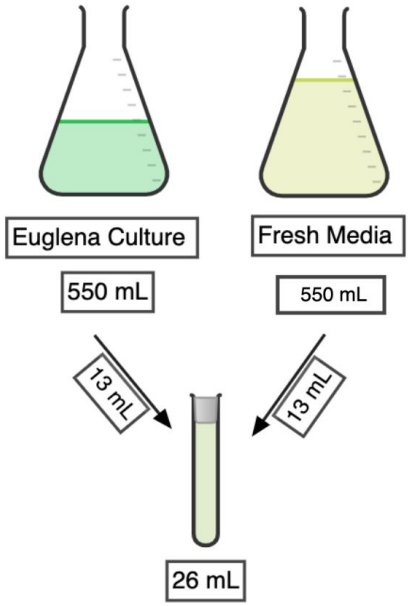

Figure 1. The Preparation of the Euglena Master Stock. An illustration that depicts the exact amount of Euglena Master Stock used for each vial within the treatments.

Proceeding the dilution, initial O_2 concentrations of all the control and treatment vials was obtained. The initial O_2 concentration was measured using an Oxygen Probe Meter, attached to a TI-84 calculator that displayed the measurement. After measuring the oxygen concentration, an initial cell count was performed. A Haemocytometer was used to count the number of cells in each vial. The cell count was performed using a compound microscope at 1000X magnification. Instructions on the proper use of a Haemocytometer were provided in the "Haemocytometer Instructions for Euglena and Tetrahymena" in the BIOL 342 laboratory. The initial and final cell count were performed in the same manner. The cell count was necessary in order to determine the oxygen production per cell.

Incubation

Once cell counts and O_2 concentration was measured, the vials were capped and placed into the bowls of the different light treatment groups. Each bowl was filled with water and ice in order to create an ice bath. The vials were placed upside down in order to prevent the formation of air bubbles in the vials. As seen in **Figure 2** the red, green, and clear light treatments were wrapped with 1 sheet of black plastic to ensure no light was escaping and no additional light was being absorbed. The red and green light treatments were then covered with coloured acetate paper that filtered light at 625/740 nm and 500/565 nm, respectively. Light intensity was measured underneath the coloured acetate paper because each colour was blocking a variable amount of light from reaching the vials. Therefore, the light intensity was kept constant underneath the coloured acetate, not above it. Since there was nothing blocking the light for the clear treatment group, the vials were receiving a higher light intensity than the green and red treatment groups. In order to control the amount of light penetrating to the vials, the clear treatment group was covered with 1 layer of cheesecloth. The light intensity of each lamp was measured using a Light Lux Meter Pro app in units of Lux, on an iPhone to ensure the amount of light received by the photosynthetic *E. gracilis* was the same. Throughout the experiment the same iPhone X was used in order to reduce variability.

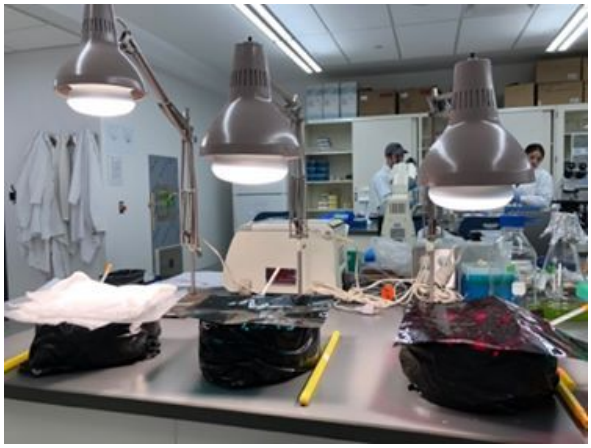

Figure 2. The layout of the light treatments during incubation. This figure depicts 3 vials (not pictured due to the coverage of acetate paper) in each light treatment, maintained in an ice bath at a temperature of 25 °C - 27 °C. In order to maintain this temperature range, ice was added during the experiment. Each of the 3 lamps provided light (1280 lx) to each treatment containing *E. gracilis.* A thermometer was placed in each light treatment in order to keep track of the temperature range and to know whether ice needed to be added. The baseline (no light treatment) was kept to the side, away from the light treatments.

Fixation

In preparation for the initial cell count, 100 µL of Euglena Master Stock and 10 µL of prefer fixative were placed into 18 eppendorf tubes with a micropipette. The solution in the Eppendorf tube was well mixed using the same micropipette. Then, 20ul of the sample in the eppendorf tube was transferred below the coverslip in a Zeiss Axio compound microscope at a magnification of 1000x to count the number of single-celled organisms. The prefer fixative used is shown in **Figure 3**, that allowed us to proceed with the cell count.

Figure 3. Prefer Fixative. The fixative used during preparation of initial cell count.

During the incubation time of 4 hours, the temperature of each light treatment was maintained by removing or adding ice to the ice bath in order to keep the temperature between 25-27°C. After the incubation time ended, the vials were removed. A final O_2 concentration measurement was performed on the 9 vials in the treatments along with a final cell count using the haemocyte.

Data Analysis

After all the data had been collected, a statistical analysis was performed. With having three treatments groups, a one-way ANOVA test was used to determine whether there were statistical differences between our treatment groups. We also interpreted our results with the Tukey-Kramer comparison test. Furthermore, the obtained p-value from the one-way ANOVA test allowed us to either reject, or fail to reject the null hypothesis.

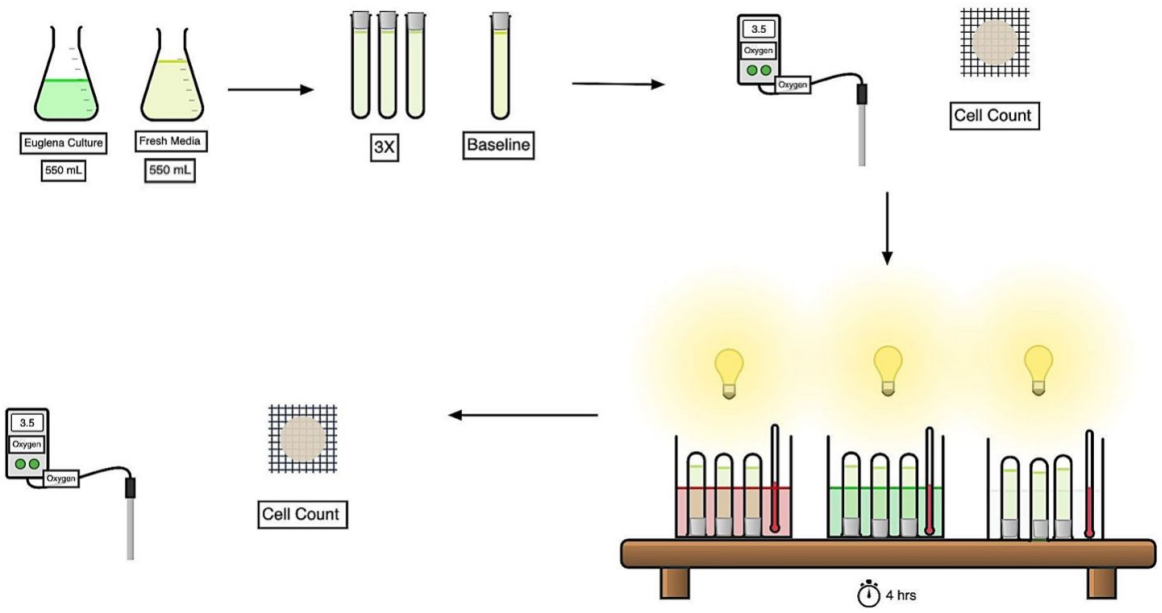

Figure 4. An overview of major procedural steps.

Results

There were 3 wavelength treatments with a total of 3 vials (n=3) per treatment. A one-way ANOVA test was used to compare the 3 treatment groups, which had an F-value of 6.685 and a p-value of 0.02972. These statistical results led to the rejection of the null hypothesis because there was a statistical difference in oxygen production depending on the wavelength of light. Figure 5 displays an observable difference in oxygen production depending on the wavelength of light because green had the highest oxygen production whereas, red had the lowest oxygen production. Figure 3 shows the difference between final and initial oxygen production means over different wavelengths of light for *E. gracilis* and 95% confidence intervals for red, green and clear light treatments to be: M=0.8333mg/L, 95% CI [0.297, 1.363], M=3.3333mg/L, 95% CI [2.32, 4.34], and M=2.2238mg/L, 95% CI [1.522,2.918] respectively.

Also, a Tukey-Kramer comparison test was performed to determine which treatments were different from each other, red vs. green, red vs. clear, and green vs. clear. The comparison test concluded only red vs. green treatments were significantly different from each other.

During the cell counts, *E. gracilis* appeared to be elongated and spindle-shaped. Also, some parts of *E. gracilis* were transparent and yellow but *E. gracilis* was mostly green.

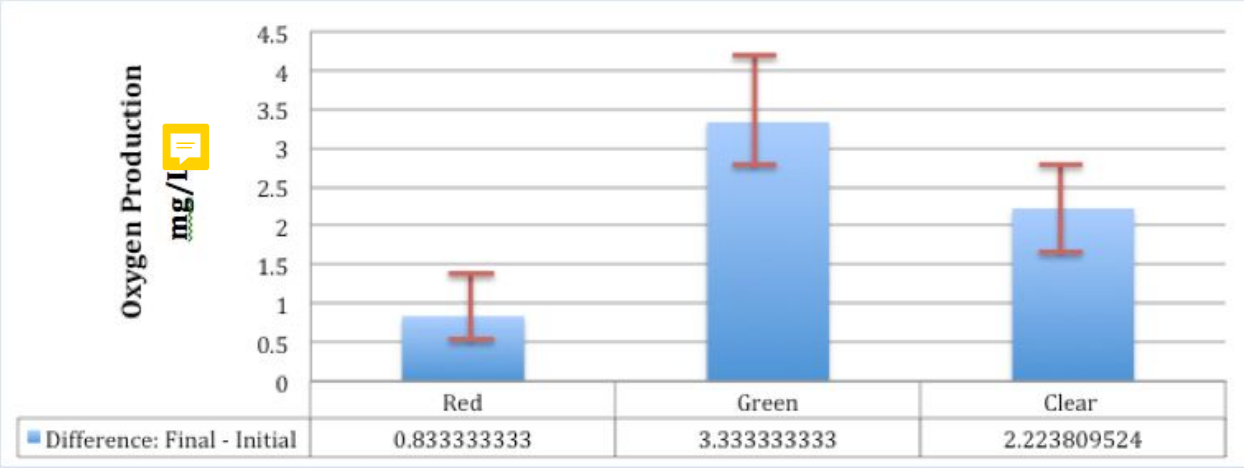

Figure 5. Bar graph of the difference between the initial and final oxygen production means over different wavelengths of light by *Euglena gracilis*, that was measured after four hours of exposure to the different wavelengths of light. Bars represent 95% confidence intervals, n=3 per treatment, p-value of 0.0297, F-value of 6.685 and degree of freedom of 2 between groups and degree of freedom of 6 within groups.

Discussion

The main objective of this study was to determine the effect of different wavelengths on the photosynthetic oxygen production in Euglena. After analyzing the data using a one-way ANOVA test, the results indicate that we reject the null hypothesis and conclude that the productivity of *E. gracilis* does differ between the different light treatment groups. With a p value of 0.02972, we can conclude that there is a statistical significance in our results. ~~This~~

means that our results are not due to chance. Also the F value was 6.685, which is significantly larger than 1, suggesting the variance between the treatment groups is greater than the variance within the treatment groups. The confidence intervals do not overlap for treatment red and green and treatment red and clear suggesting these two treatments are significantly different from one another. Whereas, the green and clear treatment do overlap suggesting that these two treatments are not significantly different from one another.

The oxygen production in the red light treatment was lower than all other treatments (refer to Figure 5). This may be due to the reflection of red light off the red acetate paper. Previous research states that microalgae absorb red wavelengths of light most efficiently (Yan et al., 2016). Our prediction was - the red light treatment group would have the highest oxygen production. If the red wavelengths of light reflected off the red acetate paper, then these wavelengths were not able to reach the *E. gracilis* cells, then the red light treatment group would have the lowest oxygen production. Since red wavelengths of light are the most important for microalgae (Yan et al., 2016), if microalgae is stripped of red wavelengths of light, then microalgae would conduct photosynthesis very slowly.

The oxygen production in the clear treatment is lower than the oxygen production in the green treatment (refer to Figure 5). We did not expect this result because green light is less effective for photosynthesis compared to red light and blue light. *E. gracilis* are both autotrophic and heterotrophic organisms, therefore in this experiment *E. gracilis* may have been growing under both autotrophic and heterotrophic conditions (Yamane, Yu-ichi, et al., 2001). After it became evident that green light exposure produced the most *E. gracilis*, we came to realize that *E. gracilis* may not only use photosynthesis as a source of energy. Having said this, a potential

alternative for the growth of *E. gracilis* may have been due to the presence of the fresh growth media used in this experiment. *E. gracilis* may have been feeding on the growth media which could have caused it to grow more.

There were three main sources of uncertainty and variation in this experiment. One problem that we ran across was maintaining a constant light intensity. Two of the three lamps were broken, and therefore kept sliding down on the stand and the arm handle would fall down throughout the experiment even after taping it. Therefore, we tried our best to have the lamps at the same level but this could have caused discrepancies between our results. This is because at some points when the arm handle would fall down, it would cause one treatment to have a higher light intensity than the other two treatments. Another source of error in our experiment was introduced when we measured the oxygen concentration. The vials with the *E. gracilis* solution were not big enough and the oxygen probe was about 1 mm above the bottom of the vial when fully submerged. For future experiments, we would wish to get a bigger vial so that we wouldn't be concerned with the oxygen probe potentially touching the bottom of the vial. Furthermore, we were required to fill the vials to the very top to prevent any air bubbles. Air bubbles would cause false results in our oxygen concentration measurements because air and water undergo a natural exchange of gases.

For future studies, we would be interested in looking at the final concentration of carbon dioxide in the vials. The final carbon dioxide concentration would be useful to know because it would signify whether photosynthesis stopped due to a lack of carbon dioxide in any of the treatment groups. Furthermore, we would check the components of the media to ensure there was no organisms that *E. gracilis* could feed on via phagocytosis. We would also increase the

incubation time in order to possibly create a greater variation in the mean oxygen production in each of the different light treatments.

Conclusion

In conclusion, our p-value of 0.02972 and F-statistic value of 6.68462 led us to reject the null hypothesis which stated, the oxygen production of *Euglena gracilis* does not differ in the different light treatment groups. Therefore, our results indicate that the oxygen production of *E. gracilis* does differ in the different light treatment groups. Our prediction of the red treatment having the highest oxygen production was not observed. Overall, more research considering *E. gracilis* and wavelength of light need to be conducted before any further conclusions are reached.

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Appendix

Appendix I. Measurements for initial and final oxygen production and cell count for each wavelength of light for *Euglena gracilis.*

Colour	**Oxygen Initial mg/L**	**Cell Count Initial**	**Oxygen Final mg/L**	**Cell Count Final**
Red 1	3.5	1.42E^5	4.0	1.60E^5
Red 2	3.5	1.18E^5	4.0	1.28E^5
Red 3	3.5	1.573E^5	5.0	1.65E^5
Green 1	3.1	9.8E^4	5.8	1.37E^5
Green 2	2.6	1.24E^5	5.3	1.05E^5
Green 3	2.6	1.517E^5	7.2	2.13E^5
Clear 1	3.9	1.739E^5	5.1	1.70E^5
Clear 2	3.0	1.184E^5	5.7	1.26E^5
Clear 3	3.5	2.18E^5	5.6	2.15E^5
Baseline	6.7	0	6.7	0

Appendix II. Data values of difference between final-initial oxygen production and cell count for each wavelength of light for *Euglena gracilis.*

Colour	**Oxygen Production Difference mg/L**	**Cell Count Difference**
Red 1	0.5	1.80E^4
Red 2	0.5	1.00E^4
Red 3	1.5	7.70E^3
Green 1	2.7	3.90E^4
Green 2	2.7	-1.90E^4
Green 3	4.6	6.13E^4
Clear 1	1.2	-3.90E^3
Clear 2	2.7	7.60E^3
Clear 3	2.1	-3.00E^5
Baseline	0	0

Appendix III. Data values of the mean for final and initial oxygen production and cell count for each wavelength of light for *Euglena gracilis.*

Colour	**Mean of Oxygen Production Initial mg/L**	**Mean of Cell Count Initial**	**Mean of Oxygen Production Final mg/L**	**Mean of Cell Count Final**
Red	3.5	1.39E^5	4.3	1.51E^5
Green	2.8	1.25E^5	6.1	1.52E^5
Clear	3.2	5.10E^5	5.5	1.70E^5

Appendix IV. Data values of the mean for the difference between final-initial oxygen production and cell count for each wavelength of light for *Euglena gracilis.*

Colour	**Mean of Oxygen Production Difference mg/L**	**Mean of Cell Count Difference**
Red	0.83	1.19E^5
Green	3.33	2.71E^5

Clear	2.22	-3.40E^5

Appendix V. Data values of the mean for the difference between final-initial oxygen production and cell count, standard deviation of oxygen production and cell count and 95% confidence intervals and ranges of oxygen production and cell count for each wavelength of light for *Euglena gracilis.*

Colour	Mean of Oxygen Production Difference	Mean of Cell Count Difference	Standard Deviation of Oxygen Production	Standard Deviation of Cell Count	95% Confidence Interval of Oxygen Production	95% Confidence Interval of Cell Count	95% Confidence Interval Range for Oxygen Production	95% Confidence Interval Range for Cell Count
Red	0.83	1.19E^5	0.471	4414.37	0.83 ± 0.533	1.19E^5 ± 4995.24	0.297 ± 1.363	1.14E^5 ± 1.24E^5
Green	3.33	2.71E^5	0.895	33845.04	3.33 ± 1.01	2.71E^5 ±38298.56	2.32 ± 4.34	2.33E^5 ± 3.09E^5
Clear	2.22	-3.40E^5	0.616	5221.96	2.22 ± 0.698	-3.40E^5 ±5909.09	1.522 ± 2.918	-3.46E^5 ± -3.34E^5

Appendix VI. Data values for F calculated and p value from performing a one-way ANOVA test.

F Calculated	**P Value**
6.685	0.02972