The Effect of Light Intensity on Oxygen Production of *Euglena gracilis*

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Abstract

This study aims to investigate the effect of increasing light intensity on the oxygen production by *Euglena gracilis*. *E. gracilis* serves as an essential source of oxygen for salmon species to uptake, which then can be used to perform various life functions such as growth, digestion, and locomotion. Light intensity promotes vertical movement of *E. gracilis* to stay in regions where optimal photosynthesis can occur, leading to the prediction that oxygen production of *E. gracilis* will increase with increasing light exposure. To test this prediction, we exposed the *E. gracilis* to three different light intensities ($20Wm^{-2}$, $35Wm^{-2}$, $50Wm^{-2}$) and measured the oxygen concentration at the 0hr, 3hr, and 6hr mark. We also counted the number of cells at each stage in order to measure the O_2 production rate per cell. Our one-way ANOVA results showed an overall decreasing trend in the oxygen production per cell across all three different light intensities. Statistical analysis also showed no relationship between the two variables under investigation. As a result, we failed to reject the null hypothesis. This may have been caused by human errors during the course of the experiment, including gas exchange as a possible confounding variable.

Introduction

Salmonids are a keystone species in British Columbia and numerous animals, including humans, rely heavily on their existence (Otero et al., 2011). In these ecosystems, *Euglena gracilis* are known to be one of the important phytoplanktons that provide oxygen for survival of salmon. These single-celled, photosynthetic protists are found worldwide - in freshwater and soils (Wang et al., 2018). They are capable of growing in regions that has a wide range of temperatures and pH with a growth rate of approximately 1.1 d^{-1} (Wang et al., 2018). *E. gracilis* have various modes of nutrient uptake including phagotrophy and photosynthesis (Zakrys et al., 2017). In the presence of light, these species are able to photosynthesize given sufficient light intensities, and in the absence of light, they are able to uptake nutrients via phagocytosis. *E.*

gracilis are known to have very flexible cell surfaces that allow for cells to undergo metaboly, causing changes to the cell shape (Zakrys et al., 2017). Depending on the light level, *E. gracilis* have the ability to move up and down the water column via negative gravitaxis and phototaxis respectively (Vogel et al., 1993). This movement within the water column allows for these species to stay in areas of optimal light conditions favourable for growth and survival.

Oxygen concentration in freshwater is an extremely important abiotic variable in terms of salmon life processes as salmonids are considered to be hypoxia-sensitive species (Barnes et al., 2011 & Olsvik et al., 2013). Without adequate oxygen concentration, the growth of salmonids can be negatively influenced as it could lead to a decrease in appetite; thus, not being able to support the processes involved in growth due to the lack of energy (Talloni-Alvarez et al., 2019 & Del Rio et al., 2019). In addition, prolonged exposure to hypoxia could result in stress responses that affect metabolism, osmoregulation and other bodily functions that could ultimately lead to fatalities (Kvamme et al., 2013). Insufficient delivery of oxygen could result in delayed or premature embryos and increased number of days prior to hatching, affecting the survival rate and the population of adult salmon (Del Rio et al., 2019). Similarly, the ability of salmon to swim declines with the diminishing concentrations of oxygen. This causes difficulty in growth, migration, and overall survival. During aerobic locomotion, the demand for oxygen in tissues increases by approximately five times when compared to their resting state (Zakrys et al., 2017). Thus, sufficient oxygen concentration is required to maintain the salmon population in British Columbia.

Light is considered to be one of the most important external abiotic factors that affect cell response (Vogel et al., 1993). *E. gracilis* was used as a model organism to observe whether or

not an increase in light intensity would affect the oxygen production of these species as they are widely used in laboratory setting as a photosynthetic model species (Zakrys et al., 2017 & Ludwig et al., 1951). Vertical migration of *E. gracilis* mitigates photo-inhibitory light levels to sustain optimal conditions for photosynthesis (Kingston, 1999 & Ogawa et al., 2016). As light intensity increases, the number of *E. gracilis* at the surface of water increases; thus, the photosynthetic rate increases. We predict that given this fact, the production of oxygen will increase, creating a favourable environment for more photosynthetic plankton. Based on previous literature on the effects of light on oxygen concentration, we hypothesize that there will be a significant difference in the oxygen level as light intensity increases. Our null hypothesis for this experiment was that light intensity will not affect net oxygen concentration whereas our alternative hypothesis was that light intensity will affect the net oxygen concentration of *E. gracilis*. We predict that the increasing light intensity will increase the net oxygen production of *E. gracilis.*

Methods

To prepare our experiment, we set up a 150 Watt lamp over a container filled with water, and maintained a temperature of 25°C using a thermometer and ice. As shown in Figure 1, we repeated this step to create 3 stations, one for the control (20 Wm^{-2}), treatment 1 (35 Wm^{-2}) and treatment 2 (50 Wm^{-2}). The appropriate light intensities were set up by placing the lamps at different heights, and measured using a light meter. We labelled four plastic vials (3 for replicates and 1 for negative control) for the 0 hr mark. For the control and each treatment, eight

plastic vials were labelled to indicate the 3 hr and 6 hr mark. Once all the vials were labelled, we prepared our *E. gracilis* solution.

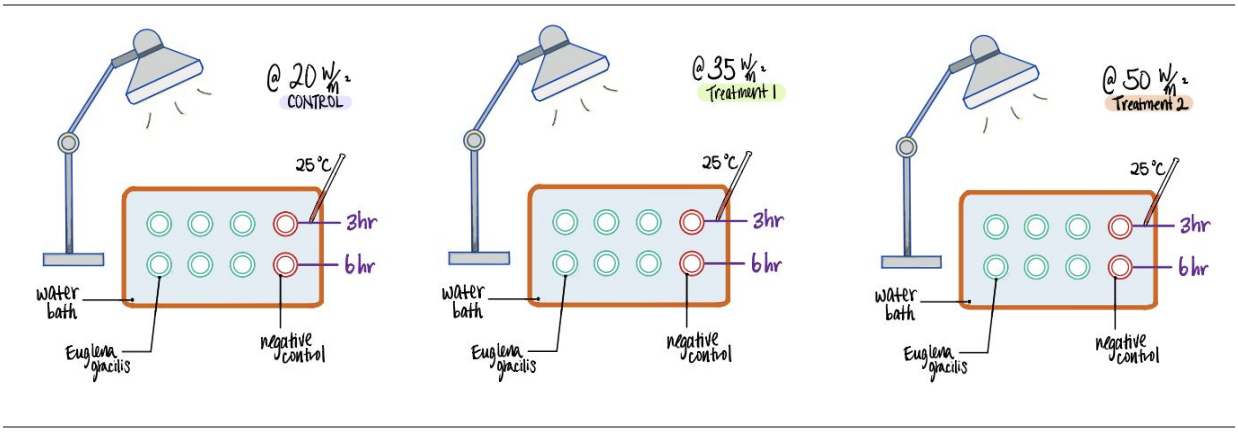

Figure 1. Station set up of the Control group, Treatment 1 and Treatment 2.

We were provided with 700 mL of *E. gracilis* mixture and 700 mL of fresh media formulated by UTEX Culture Collection of Algae at The University of Texas at Austin. To create our solution, we mixed 420 mL of the new media and 105 mL of the *E. gracilis* mixture into a 600 mL beaker by pouring 400 mL of the new media, then pipetting the remaining 20 mL into the beaker. Similarly, we poured 100 mL of the *E. gracilis* mixture, then pipetted 5 mL into the solution. The purpose of this mixture was to provide *E. gracilis* with ample nutrients from the fresh media in order to produce oxygen. To prepare our negative control, we poured another 200 mL of the new media into a separate 250 mL beaker. Once all the solutions were prepared, we divided the 525 mL of the new *E. gracilis* solution into the individual vials (Figure 2).

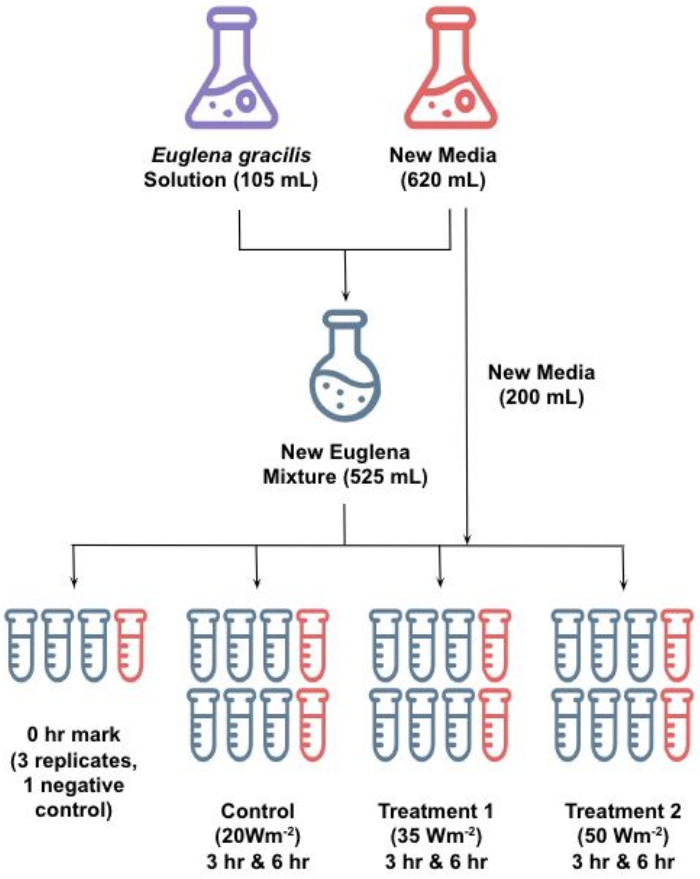

Figure 2. Vial distribution of the *E. gracilis* solution and negative control.

At the 0 hr mark, we measured the oxygen concentration of the four vials immediately after they were filled (1 with negative control and 3 with the new *E. gracilis* solution) using an oxygen meter. Once the measurements were taken, we counted the number of cells in the vials containing *E. gracilis* using a microscope and a haemocytometer. At the 3 hr mark, steps from the 0 hr mark were repeated. As soon as the vials were taken out of the water bath, the oxygen concentration was measured, followed by cell counting. At the 6 hr mark, we repeated the steps from the 3 hr mark.

The number of *E. gracilis* cells in each vial were counted as soon as the oxygen concentration was measured. To do so, we set up a Zeiss Axiostar compound microscope using

the 10X objective. Using a micropipette, we added 10 μL of the prefer fixative to 100 μL of the *E. gracilis* mixture in a separate tube. After thoroughly mixing the two solutions, we placed 20 μL of this sample onto the haemocytometer using a micropipette. From one vial of the *E. gracilis* mixture, we created 3 samples (prefer + *E. gracilis* cells) to find the average number of cells. This step was repeated for the 3 hr and 6 hr mark. With the oxygen concentration and number of cells, we were able to calculate the oxygen production per cell.

We conducted a one-way ANOVA statistical analysis to determine if the change in O_2 concentrations produced at different light intensities were statistically significant. Our data was plotted onto a bar graph in Excel to compare the changes in O_2 concentrations produced per cell between the control group and the two treatment groups.

Results

Our statistical analysis using one-way ANOVA reveals the p-value of 0.95 between the three groups, indicating that the results are insignificant across varying light intensities tested. Based on this very large p-value, we fail to reject the null hypothesis that there is no relationship between light intensity and the oxygen producing ability of *E. gracilis*.

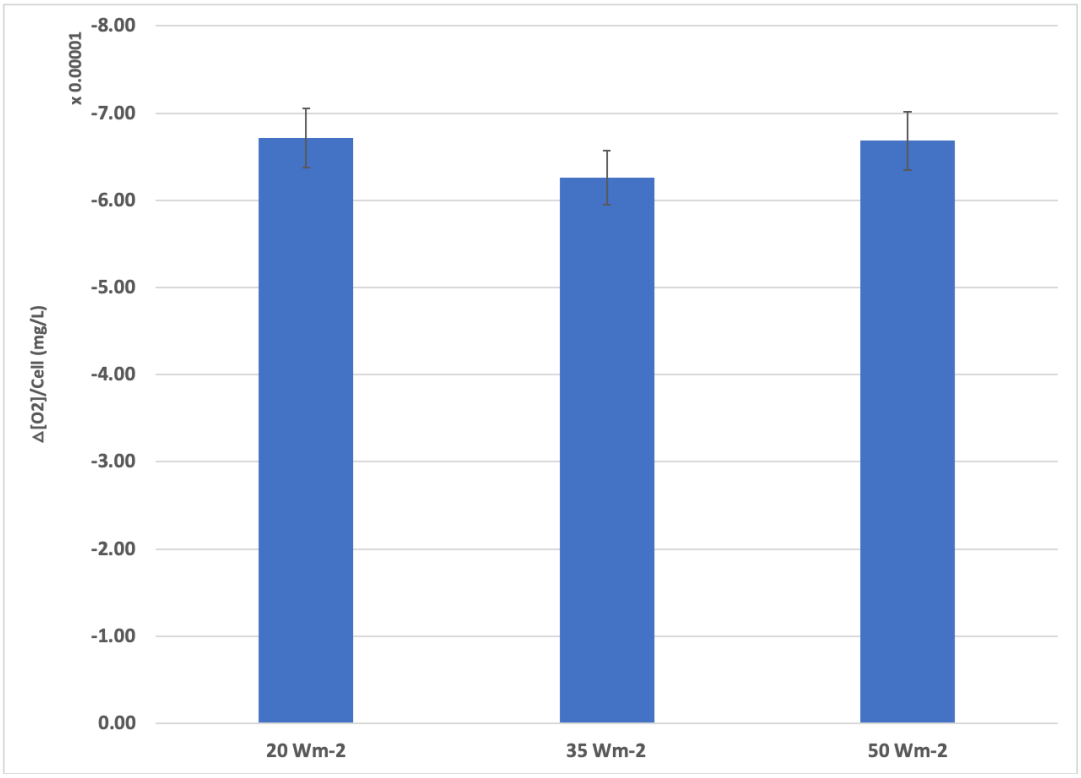

Figure 3. Change in average O_2 production of *E. gracilis* per cell in three different treatments. Control (20 Wm^{-2}), Treatment 1 (35 Wm^{-2}) and Treatment 2 (50 Wm^{-2}). Error bars showing 95% confidence intervals. One-way ANOVA test performed,
F = 0.05336211, p-value = 0.95, n=3 per treatment and df = 8 with 0.05 as significance.

Discussion

Our results fail to reject the null hypothesis, as oxygen production of *E. gracilis* did not increase with increasing light intensities. From the one-way ANOVA test, we found that the changes in oxygen production were not statistically significant. We speculate that our results may have been affected by a series of human errors, as well as gas exchange and growth media type as potential confounding variables.

Although changes in light intensity affects the vertical mobility of *E. gracilis* for optimal photosynthesis, the absence of a relationship between the light intensity and oxygen production,

despite the large p-value, may be explained by factors that contradict our prediction. From literature it has been found that the light intensity at the highest *E. gracilis* growth rate is 100 μmol m^{-2} s^{-1} (20 Wm^{-2}) and that the growth rate starts to decrease from 150 μmol m^{-2} s^{-1} (35 Wm^{-2}) (Kitaya et al., 2005). We suspect that decreasing growth rate could be accountable for the observed decrease in oxygen production over time in the two treatment groups since they were exposed to intensities exceeding 35 Wm^{-2}. As growth rate decreases, the net oxygen production would also decrease from less photosynthesis occurring at the same time. Also, high irradiances cause photoinhibition in microalgae (Grima et al. 1996). The exact values considered as high irradiance for *E. gracilis* has not yet been defined, therefore the light intensities we tested (35Wm^{-2} and 50Wm^{-2}) may have been high enough to suppress photosynthesis. The irregular response to increasing light intensity seen for each replicate may be a result of such factors.

There are a few errors of concern that may have affected the results. Firstly, the intervals of light intensities chosen (15 Wm^{-2} between each group) may not have been drastic enough to show meaningful differences in the oxygen level produced within the 6-hour duration of the experiment. Secondly, the received light intensities by individual *E. gracilis* cell may not have been uniform depending on where in the ice baths they were placed. Of the cluster of vials placed underneath the light source, some vials had a more direct exposure to the light while some vials were placed around the periphery of the ice bath which would have increased the distance between the light source and the vial. Other possible human errors are inaccurate cell counts from counting debris or other contaminants as cells. To improve our study in the future, the sample size and the number of replicates should also be increased with longer duration of exposure to each light intensity. This experiment was conducted over the span of 6 hours,

however a previous study investigating light intensity and growth rate had 24 hour exposure time under each light intensity being tested to show significant results (Kitaya et al., 2005). Image processing programs can be used to replace manual counting of cells with a haemocytometer to ensure accuracy and reduce human errors.

We attempted to keep all variables the same for the control and two treatment groups to eliminate other factors that could affect oxygen production other than light intensity. Although we were able to maintain temperature of the three groups at 25°C by adding ice, possibility of other confounding variables still remained. One of the limitations to the accuracy of our oxygen measurements is gas exchange. The volume of the master mix was not measured during transfer into each of the vials. The different volumes of master mix would result in varying volumes of air within each vial and this could have promoted more gas exchange and led to higher oxygen concentrations in some vials containing relatively less master mix. Another confounding variable is the type of growth media used. A heterotrophic growth media, instead of an autotrophic media was used for *E. gracilis* in this study. This growth media contained organic compounds, which is used to serve as a carbon source in heterotrophic conditions and eliminate the need of light for autotrophy (Liang et al., 2009). If autotrophy had been inhibited in this media, then *E. gracilis* may not have been able to produce oxygen by photosynthesis.

The result of our study suggests that increasing light intensity does not increase the oxygen production of *E. gracilis* and thus, light intensity does not affect the amount of oxygen available for salmon species to uptake in their ecosystems. How light intensity directly affects salmon species is yet to be discovered, however, it can be concluded based on our findings that there is no relation between light intensity and salmon species' ability to access free oxygen to

support salmon viability. Oxygen concentration of less than 5mg/L would fail to support optimal fish growth, possibly leading to detrimental effects on the trophic productivity and food web (Mansour et al., 2008). Therefore, regardless of the light intensity, salmon should be able to escape hypoxia and survive as long as the oxygen concentration of their environment exceeds 5mg/L.

Conclusion

The oxygen production by photosynthetic algae, such as *E. gracilis* is a vital input in marine environments that aquatic organisms at all trophic levels, including salmon, depend on for survival (Schindler et al., 2003). Based on our statistical analysis, we failed to reject our null hypothesis as there was no increasing pattern between the oxygen producing ability of *E. gracilis* and increasing light intensity. Further investigation is required to uncover whether oxygen availability could change as a function of light intensity and its effect on salmon species in shaping the productivity of an ecosystem.

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Appendix

[O2]															
Time (hr)	Control (20 W/m^2)					Treatment 1 (35 W/m^2)					Treatment 2 (50 W/m^2)				
	Rep 1	Rep 2	Rep 3	Avg	Media	Rep 1	Rep 2	Rep 3	Avg	Media	Rep 1	Rep 2	Rep 3	Avg	Media
0 (initial)	6.9	7	6.4	6.77	7.7										
3	5.2	4.8	4.2	4.73	7.8	5.4	5.2	5.8	5.47	7.5	3.8	5.2	5	4.67	7.9
6 (final)	3.1	3.5	2.7	3.1	8	5.3	4.6	4.4	4.77	7.8	3.7	3.4	3.2	3.4	8
Number of Cells															
Time (hr)	Control (20 W/m^2)					Treatment 1 (35 W/m^2)					Treatment 2 (50 W/m^2)				
	Rep 1	Rep 2	Rep 3	Avg	Media	Rep 1	Rep 2	Rep 3	Avg	Media	Rep 1	Rep 2	Rep 3	Avg	Media
0 (initial)	1.74E+04	5.45E+04	4.57E+04	3.92E+04											
3	2.04E+04	5.17E+04	3.25E+04	3.48E+04		4.40E+04	2.37E+04	3.08E+04	3.28E+04		4.29E+04	1.43E+04	2.97E+04	2.90E+04	
6 (final)	2.42E+04	3.03E+04	3.36E+04	2.93E+04		2.64E+04	7.70E+04	2.64E+04	4.33E+04		4.24E+04	2.53E+04	2.86E+04	3.21E+04	
[O2] / Cell															
Time (hr)	Control (20 W/m^2)					Treatment 1 (35 W/m^2)					Treatment 2 (50 W/m^2)				
	Rep 1	Rep 2	Rep 3	Avg	Media	Rep 1	Rep 2	Rep 3	Avg	Media	Rep 1	Rep 2	Rep 3	Avg	Media
0 (initial)	0.000396	0.000129	0.00014	1.73E-04											
3	0.000256	9.28E-05	0.000129	1.36E-04		1.23E-04	2.20E-04	1.88E-04	1.67E-04		8.86E-05	3.64E-04	1.68E-04	1.61E-04	
6 (final)	0.000128	0.000116	8.05E-05	1.06E-04		2.01E-04	5.97E-05	1.67E-04	1.10E-04		8.74E-05	1.34E-04	1.12E-04	1.06E-04	

Table 1. Data table used for recording oxygen concentration and cell counts during experiment.

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Control (20Wm^{-2})	3	-0.0003406	-0.0001135	1.8457E-08
Treatment 1 (35Wm^{-2})	3	-0.0002378	-7.925E-05	1.239E-08
Treatment 2 (50Wm^{-2})	3	-0.0003313	-0.0001104	2.9806E-08

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2.1577E-09	2	1.0789E-09	0.05336211	0.94848142	5.14325285
Within Groups	1.2131E-07	6	2.0218E-08			
Total	1.2346E-07	8				

Table 2. Statistical analysis result performing a single factor ANOVA test using Microsoft Excel with an Alpha value of 0.05 and sample size of three replicates per group.

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