Investigating the effect of Magnesium on the Oxygen Production of *Euglena Gracilis* cells.

Rasheed Abuarja, Hakha Behmanesh, Riley Cox, Juan Gomez

Abstract:

This experiment aims to shed more light on the correlation between different levels of magnesium ions and oxygen production (photosynthetic rates) of *Euglena gracilis.* This is of particular relevance with regards to salmon ecosystems, since an increased productivity of Euglena, which form the base of the freshwater salmon food web, could result in an increased oxygen level and abundance of prey, both of which are vital for salmon growth and development. There were three different magnesium levels, 0.049 mg/mL, 0.02 mg/mL and 0 mg/mL (control). Five replicates for each magnesium level were incubated for 18 hours under a light-only cycle to allow for maximum photosynthetic activity. The initial value and final values were measured and recorded for both oxygen concentration and cell count, which allowed us to find the change in oxygen production per cell for each magnesium treatment. Following this, the mean change in oxygen production per cell (n=5) was calculated per treatment, as well as a one-way ANOVA with a Tukey-Kramer test. The ANOVA showed significant differences in the means of the groups (P=0.0016), and the Tukey-Kramer test showed that the 0.049 mg/mL treatment found to be significantly different than both the 0.02 (P=0.0015) and the 0 mg/mL treatment (P= 0.0145). Whereas the 0.02 mg/mL and the 0 mg/mL were not statistically different from each other (P=0.4341). The change in oxygen production was found to be negative in all three treatments due to an inadequate light intensity which resulted in a photosynthetic rate that was less than the cellular respiration rate. Despite this, the 0.049 mg/mL experienced the smallest decrease in oxygen which could indicate that the cells experienced a higher photosynthetic rate, allowing them to better counteract the loss of oxygen from cellular respiration. Given the significant differences between the highest magnesium group and the other two groups, we were able to reject the null hypothesis in favour of our alternate hypothesis, which states that magnesium levels will affect oxygen production in *E. gracilis* cells.

Introduction:

Euglena gracilis are single-celled phytoflagellates, which are commonly found in shallow, low oxygen freshwaters, similar to environments of many salmon-spawning streams. They can exhibit both heterotrophic as well as phototrophic activity depending on the light intensity, and as such they can be considered either phytoplankton or zooplankton (Wang et al. 2018). Previous research done by Chittenden and his team of researchers has shown that phytoplankton such as Euglena are an important food source for salmon fry in the Pacific

Northwest (Chittenden et al 2010). As primary producers, *E. gracilis* carries out photosynthesis, converting an abiotic source of energy (e.g. light) into energy stored in organic compounds, which are important in freshwater systems and can sustain organisms at higher trophic levels, such as salmon.

The main background information our study relies on is the fact that chlorophyll is the pigment required for light absorption which allows for photosynthesis (Croft et al. 2017), as well as the fact that magnesium is the central atom in the chlorophyll molecule (Bohn et al. 2006). Additionally, literature has shown that the chlorophyll content can act as a proxy for photosynthetic rate due to a strong correlation between the two (Croft et al. 2017). Furthermore, previous research done by Finkle and his team of researchers (1953) has shown the varying effects of magnesium concentrations on chlorophyll abundance in the green algae, *Chlorella vulgaris*, a closely related species to Euglena. Their experiment showed that growth of this green algae in a magnesium-treated medium was accompanied by a rapid rise of chlorophyll synthesis, followed by a plateau once the magnesium concentrations were depleted. The level of magnesium initially added determined the amount of new chlorophyll synthesized (Finkle et al. 1953). His study showed this through rescued experiments where he was able to increase chlorophyll content again when magnesium was added to the cultures. The highest chlorophyll content was in treatment groups with 49 and 2.8 parts per million (ppm) of magnesium ion (Finkle et al. 1953).

These aforementioned studies show the correlation between magnesium and chlorophyll, as well as chlorophyll and photosynthetic rates, with these in mind, the main question our study

is aiming to shed light on is: what is the effect of different magnesium levels on the oxygen production by Euglena cells? Given this question, our null hypothesis states that magnesium will have no effect on the average oxygen production per cell of *Euglena gracilis* and our alternative hypothesis states that magnesium will have an effect on the average oxygen production per cell of *Euglena gracilis.* Finally, we predict that higher levels of magnesium will increase the photosynthetic rates and thus oxygen production in *Euglena gracilis* cells.

Such research is important because *E. gracilis* are found at the bottom of the salmon food web and are an important food source for salmon. An increase in photosynthesis by *E. gracilis* in the presence of increased magnesium ions can potentially increase primary productivity in the ecosystem, resulting in a bottom-up trophic cascade that could allow for a larger salmon population. Salmon ecosystems could also benefit from the increased oxygen production that result from the increased photosynthetic rates. This is because when salmon (particularly juvenile fry or alevins) are in hypoxic ranges, their development is severely stunted (Del rio et al. 2019), and thus an increase in the overall oxygen concentration of the water body could reduce the chances of such deleterious effects. This is particularly important for local freshwater systems that host large salmon populations such as Salish Creek (Patton 2003). In summary, controlled additions of magnesium could increase photosynthetic activity of *E. gracilis* cells, resulting in an increased primary productivity as well as increased oxygen production, both of which can better support and potentially increase salmon populations, having notable implications on salmon aquaculture, British Columbia's (BC) Salmon Restoration project, as well as the overall economy of BC.

Materials & Methods

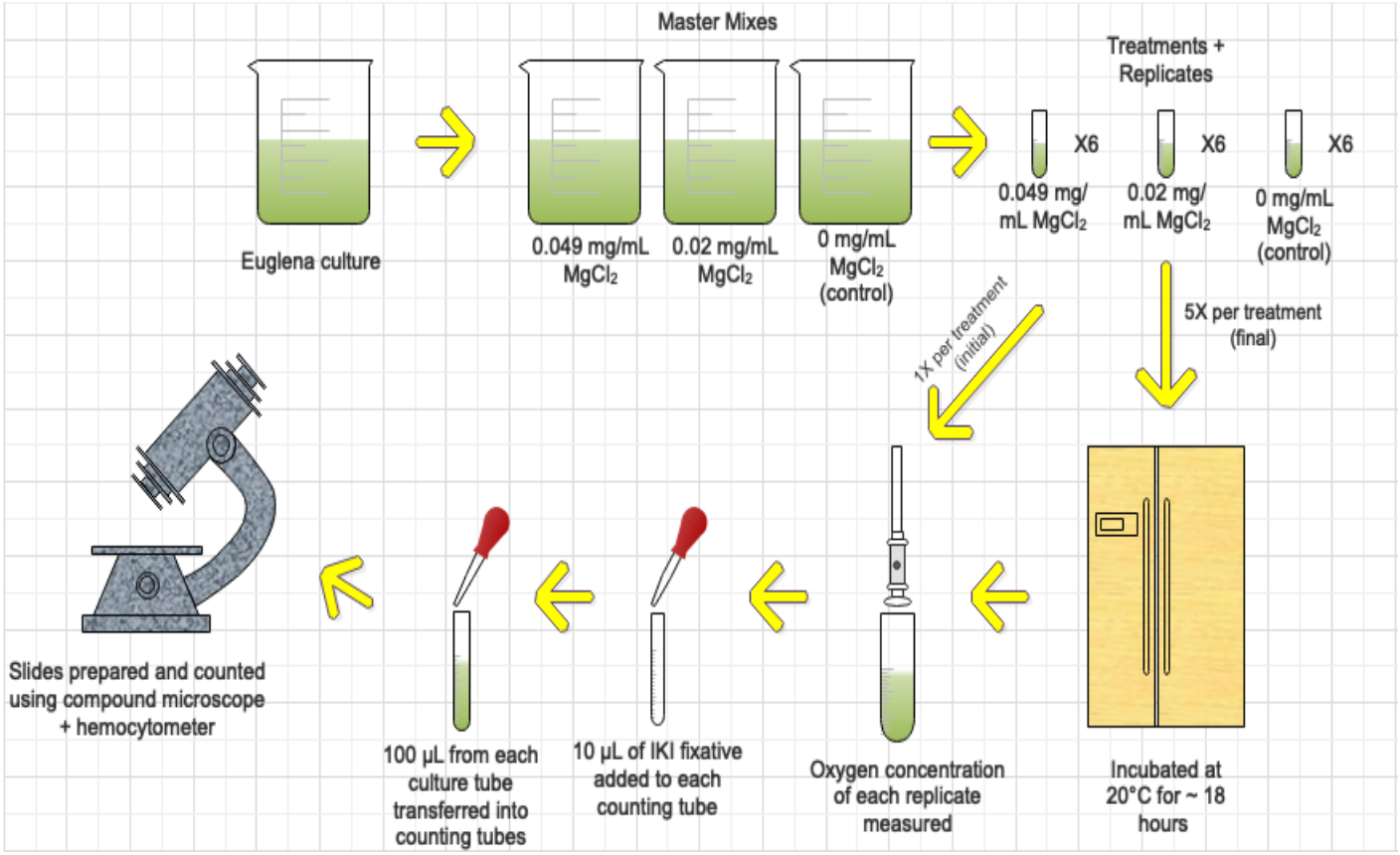

Figure 1: Overview of the major procedural steps performed, see text below for further information (created using Edrawmax software).

Culture Tube Preparation

We were provided with 500 mL of *E. gracilis,* cultured in the medium recipe from UTEX Culture Collection of Algae at the University of Texas at Austin. We were also provided 500 mL of $MgCl_2$ *E. gracilis* medium, and 500 mL of standard *E. gracilis* medium each in their own 1000 mL beakers. In our experiment, there were 3 different magnesium chloride treatments: 0.049 mg/mL, 0.02 mg/mL, and 0 mg/mL which served as our control. We created a master mix for each magnesium chloride treatment and prepared six replicates (one for the initial values, five

for the final values) for each treatment from these master mixes. Each master mix was 270 mL in total in its own 1000 mL beaker. This mastermix volume of 270 mL contains enough for 10 replicates, just as a precaution for any potential errors that would require us to recreate a replicate. Our master mixes were created using a 10 mL pipette. The control master mix was created by pipetting 10X of the following: 10 mL of *E. gracilis* culture, 17 mL of standard *E. gracilis* medium, and 0 mL of the $MgCl_2$ medium. For the highest $MgCl_2$ treatment, 0.049 mg/mL, we added 10X of: 10 mL of *E. gracilis* culture, 13.23 mL of $MgCl_2$ medium, and 3.77 mL of standard *E. gracilis* medium. For our remaining treatment with 0.02 mg/mL of $MgCl_2$, we added 10X of: 10 mL of *E. gracilis* culture, 5.4 mL of $MgCl_2$ medium, and 11.6 mL of standard *E. gracilis* medium. Using each treatment's master mix, we filled six 27 mL vials by submerging the vials into the master mix beaker and sealing them without exposure to the atmosphere to prevent air bubbles.

One vial from each treatment was then used to measure the initial oxygen concentration as well as to conduct an initial cell count. We assumed that the initial values would remain constant and thus we would only need to measure them once per treatment. As such, we used the same initial oxygen concentration and cell count for all five remaining replicates. We measured the initial oxygen concentration (mg/L) of all 3 treatments using a Vernier oxygen probe connected to a TI-84 Plus calculator. To obtain an initial cell count for each treatment, the cells first had to be immobilized, we did this by adding 10 uL of IKI fixative to 100 uL of cells from one vial of each treatment into a counting tube. The counting tube was then used to create a microscope slide with a hemocytometer. Three slides from each treatment were then prepared, examined using a Zeiss Axiostar compound microscope at a magnification of 100X, and counted

using the hemocytometer. Upon obtaining the initial cell count of the oxygen concentration for each treatment, the remaining replicates were stored in an incubator at 20°C for 18 hours until further analysis could be conducted.

Incubation

In order to ensure that our culture was photosynthesizing for the duration of our analysis period, we exposed our replicates to an 18 hours of a light-only incubation cycle at 20°C inside a Panasonic Cooled Incubator MIR-254-PA. The culture vials were then put on a tray and placed in the incubator on the top rack with light intensity at 500 lux, as measured with a VWR Traceable light meter. The replicates remained in the incubator for 18 hours and were then removed and further analyzed.

Data Analysis

After approximately 18 hours, the culture tubes were removed from the incubator. Each replicate then underwent the same procedure as the initial testing. Using the Vernier oxygen probe, each treatment's replicates final oxygen concentrations were then measured and recorded. For the final cell counts, the same methodology as described above for the initial cell count was performed.

Upon completion of all measurements, we first converted the oxygen concentration from mg/L to mg/mL then we used Equation 1 below to find the normalized Oxygen produced per cell for each replicate (milligrams of O_2/cell). Afterwards, we found the mean of the oxygen produced per cell for each treatment's replicates (n=5) and standard error of the mean for each treatment using Graphpad's descriptive statistics. $MgCl_2$

$$\frac{\text{Final Oxygen Concentration } (\frac{\text{mgO2}}{\text{mL}})}{\text{Final Cell count } (\frac{\text{cells}}{\text{mL}})} - \frac{\text{Initial Oxygen Concentration } (\frac{\text{mgO2}}{\text{mL}})}{\text{Initial Cell count } (\frac{\text{cells}}{\text{mL}})} = \text{Oxygen Produced Per Cell } (\frac{\text{mgO2}}{\text{cell}}) \quad (1)$$

In order to assess whether a difference exists between our three different $MgCl_2$ treatments, we conducted a one-way ANOVA to determine if the groups were different, and then used a Tukey Kramer post-hoc test to determine which groups were different. We used Graphpad's Prism software for descriptive statistics, plotting our graphs, as well as analyzing our statistical data. For our graph (Figure 3), we plotted the mean change in oxygen production per cell against the magnesium levels, with the error bars showing the standard error of the mean.

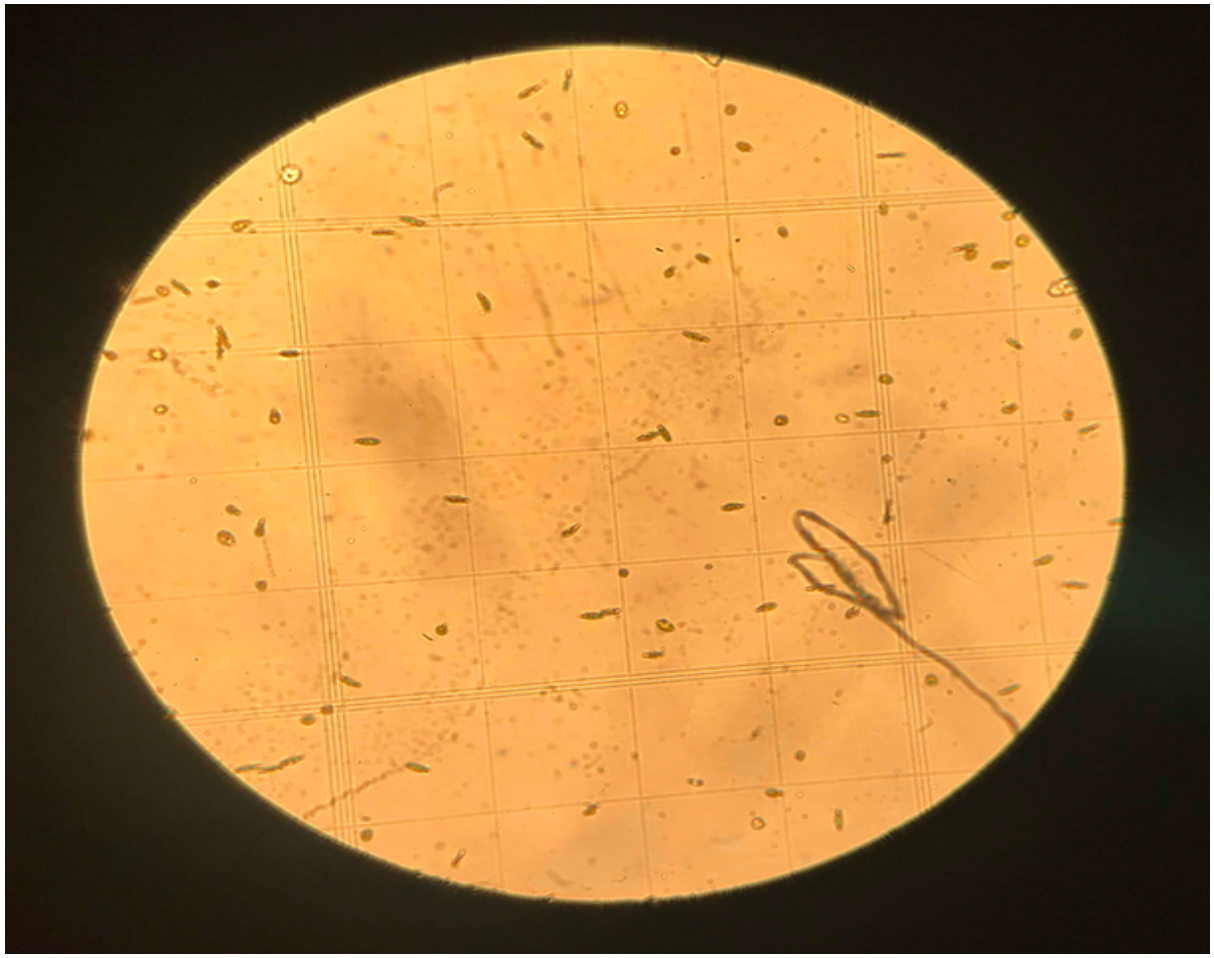

Figure 2: *E. gracilis* cells viewed through the Zeiss Axiostar compound microscope and hemocytometer at 100X magnification

Results

Recall that each magnesium treatment had five replicates (n=5) that were incubated, and one replicate that was used for the initial values and was not incubated. The values plotted in Figure 3 show the mean ± standard error of the change in oxygen production per cell. With regards to the overall trend, figure 3's results show a negative change in oxygen production for all three treatments. With the 0.049 mg/mL group showing the smallest decrease of oxygen production per cell ($-3.4 \times 10^{-8} \pm 2.2 \times 10^{-9}$ mgO_2/cell), and the 0.02 mg/mL group showing the greatest (most pronounced) decrease ($-5.2 \times 10^{-8} \pm 3.0 \times 10^{-9}$ mgO_2/cell), with the 0 mg/mL group also showing a large decrease in oxygen production ($-4.7 \times 10^{-8} \pm 2.9 \times 10^{-9}$ mgO_2/cell), one that is slightly smaller than the 0.02 mg/mL group, but larger than the 0.049 mg/mL group.

The one-way ANOVA returned a P value of 0.0016 which indicates that there is a significant difference between the groups, when compared to the alpha value of 0.05. Furthermore, the post-hoc Tukey Kramer test showed a significant difference between the 0.049 mg/mL group and the 0.02 mg/mL group (P=0.0015) and the 0 mg/mL group (P=0.0145). Whereas the 0.02 mg/mL group and the 0 mg/mL were not significantly different (P=0.4341). Given the significance of the difference between the 0.049 mg/mL group and the other two, we were able to reject the null hypothesis in favour of the alternate hypothesis which stated that magnesium levels do have an effect on the oxygen production of *E. gracilis* cells.

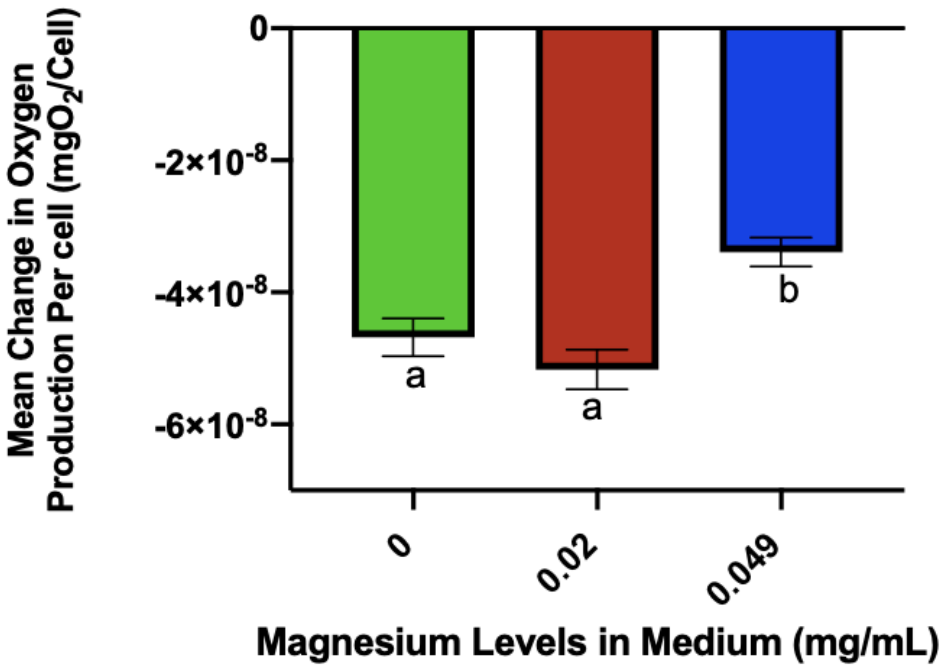

Figure 3: Bar graph showing the mean change in oxygen concentration production per cell (milligrams of oxygen/cell) for each treatment (n=5 per treatment, 3 treatments, n=15 total) versus magnesium levels in the medium (mg/mL). Error bars indicate the standard error of the mean. Different letters indicate significant differences between groups ($P<0.05$), determined through a Tukey-Kramer test (df=12 in each comparison).

Discussion:

This experiment was conducted with the goal of determining the effects of magnesium on the oxygen production of *E. gracilis* by creating treatments of three different magnesium levels (0.049 mg/mL, 0.02 mg/mL and 0 mg/mL). Our null hypothesis was that magnesium would have no effect on the oxygen production of *E. gracilis* cells, whereas our alternate hypothesis was that the magnesium addition would have an effect on oxygen production, with the prediction that oxygen production would experience an increase. Significant differences ($P<0.05$) were observed between the highest magnesium treatment group (0.049 mg/mL) and the other two (0.02 and 0

mg/mL). Since the treatment with the highest magnesium level (0.049 mg/mL) was significantly different from the other two treatment groups, were able to reject the null hypothesis in favour of the aforementioned alternate hypothesis.

However, the results do not match the prediction since they show a decrease in oxygen production, as opposed to the predicted increase. This was due to technical difficulties, we were not able to incubate the treatment vials at the literature-recommended light intensity for a maximum photosynthetic rate of *E. gracilis* at 20° Celsius, which is 8000 lux (Cramer and Myers 1952). Instead, we were only able to incubate the *E. gracilis* at 500 lux, which results in a very low photosynthetic rate (Cramer and Myers 1952). This would then mean that the oxygen being produced is less than the oxygen being consumed through the process of cellular respiration (Buetow 1963), the oxygen-consuming process of breaking down macromolecules in order to produce ATP for cellular activity.

Despite the inadequate light intensity and the resultant decrease in the change in oxygen production per cell, one could infer from the results that magnesium may have caused an increased photosynthetic activity when looking at the 0.049 mg/mL group. This can be inferred from Figure 3 since the 0.049 mg/mL treatment group was significantly different from both the 0.02 mg/mL group (P=0.0015) and the 0 mg/mL group (P = 0.0145), and had the smallest decrease in the average oxygen production per cell (-3.39×10^{-8} mgO_2/cell). Therefore, it is plausible that in the 0.049 mg/mL magnesium group, *E. gracilis* cells had a higher photosynthetic rate relative to the other groups, which allowed it to better offset the decrease in oxygen from cellular respiration. Whereas the groups with less or no magnesium (0.02 mg/mL

and 0 mg/mL) experienced a much larger decrease in oxygen production per cell (-5.17 x 10^{-8}, -4.68 x 10^{-8} mgO_2/cell, respectively). To reiterate, while the oxygen concentrations decreased due to an inadequate light intensity, the smallest decrease was seen in the *E. gracilis* treatments of the highest magnesium level of 0.049 mg/mL. This could thus indicate that the added magnesium was indeed taken up and used to synthesize new chlorophyll molecules, thus allowing for a higher oxygen production than the other groups.

An unexpected though insignificant result with the 0.02 mg/mL treatment group was seen, in that it had the greatest decrease in the mean oxygen production per cell (-5.2 x $10^{-8} \pm 3.0 \times 10^{-9}$ mgO_2/cell). There are two plausible reasonings for such a result. The first reason is the limited sample size and the fact that this treatment group had the largest confidence interval, due to a high standard deviation. To that end, one inaccurately large decrease in oxygen production per cell in one of the replicates could have greatly shifted the mean and resulted in a very low value. The second plausible reason is that a magnesium level of 0.02 mg/mL could have been insufficient for the *E. gracilis* cells to utilize and subsequently increase their photosynthetic rate. As such, the change in mean oxygen production per cell was insignificantly different from the control group of 0 mg/mL ($P>0.05$).

Aside from the technical difficulty that resulted in a low light intensity, there are three possible sources of error that can be improved on in the future. The first and foremost is that the sample size should be increased, while a sample size of five is enough to perform statistical analyses, increasing the number of replicates will give much more reliable results.

The second potential source of error is that in our methodology, there should have been five initial measurements per treatment as opposed to just one measurement that we assumed to be equal across all five replicates. This is due to the fact that despite our best efforts in mixing the *E. gracilis* culture, it is unlikely that the initial cell count was representative for all five incubated vials. Thus, by having five initial replicates instead of one, we can obtain a value that is closer to the "true" initial cell count and would result in increasingly accurate calculations.

Finally, the third source of error is the small volume of 270 mL mastermix that was in the 1000 mL beakers. This presented an issue when we were submerging our fifth and sixth vials in the beaker in order to fill them up. By the fifth vial, there was approximately only 162 mL left in a large 1000 mL beaker, which made it difficult to fully submerge the vial even if we tilted the beaker. As such, not being able to fully submerge the vials may have introduced small air bubbles, which could alter the oxygen concentrations we measured. Therefore, by filling the 1000 mL beaker with 400 mL of master mix instead of 270 mL, or by using a smaller sized beaker, this source of error would be significantly reduced and potentially eliminated.

Future research should attempt to minimize these sources of error, as well as adding another magnesium treatment group, one that is greater than 0.049 mg/mL. This would be helpful in seeing if this is significance in increased oxygen production is continuous with increased magnesium. Furthermore, one can continually increase the magnesium levels until the increase in photosynthetic activity is reduced, and instead, a toxic effect is seen. This would allow researchers to better understand the toxic range of magnesium on *E. gracilis*. The toxic range is of particular importance with regards to Salmon ecosystems. Whether one is using magnesium to

treat hypoxic waters or to increase productivity (and thus salmon prey) via a trophic bottom-up cascade, using magnesium levels that are toxic to *E. gracilis* would undoubtedly exacerbate these issues, and result in even lower oxygen concentrations or productivity. As such, despite significant results in this study, treating water bodies with magnesium should not be carried out until further analyses on a wider range of magnesium levels are conducted.

Conclusion:

In summary, the one-way ANOVA returned a statistically significant result ($P<0.05$) indicating that the groups were different, and the subsequent Tukey-Kramer test returned a significant difference ($P<0.05$) between the 0.049 mg/mL treatment group when compared with the 0.02 mg/mL, as well as when compared to the 0 mg/mL group. The 0.02 mg/mL and 0 mg/mL were not statistically different however ($P>0.05$). Contrary to our prediction, all of the treatment groups showed a decrease in the average oxygen production per cell due to an insufficient light intensity for photosynthesis. However, the highest magnesium group of 0.049 mg/mL showed the lowest decrease which suggests the cells might have been able to utilize the magnesium to synthesize new chlorophyll molecules to increase their photosynthetic rate, which in turn allowed them to better offset the decrease in oxygen from cellular respiration than the other two groups. No application using magnesium ions to support salmon populations by increasing *E. gracilis* productivity should be performed until a better understanding of Magnesium's full effects on *E. gracilis* is developed.

Acknowledgements:

We would like to thank the University of British Columbia (UBC) for giving us the opportunity to take this course (BIOL 342) which allowed us to conduct this research. As well as our instructor Jordan Hamden, and our teaching assistant Caroline Ayumi Sato for their advice

and support during the experiment. We would also like to thank the BIOL 342 laboratory technicians Chanelle Chow and Mindy Chow for providing us with the equipment required as well as any technical assistance we needed during the experiment. Last but not least, we would like to thank our peers who performed peer evaluations of this study.

References:

Buetow, D. E. (1963). Linear relationship between temperature and uptake of oxygen in *Euglena gracilis*. *Nature*, *199*(4889), 196-197.

Bohn, T., Walczyk, T., Leisibach, S., & Hurrell, R. F. (2004). Chlorophyll-bound Magnesium in Commonly Consumed Vegetables and Fruits: Relevance to Magnesium Nutrition. *Journal of food science*, *69*(9), S347-S350.

Chittenden, C. M., Jensen, J. L., Ewart, D., Anderson, S., Balfry, S., Downey, E., Eaves, A., Saskida, S., Smith, B., Vincent, S., Welch, D., McKinley, R.S. (2010). Recent salmon declines: a result of lost feeding opportunities due to bad timing?. *PLoS One*, *5*(8), e12423.

Cramer, M., & Myers, J. (1952). Growth and photosynthetic characteristics of *Euglena gracilis*. *Archives of Microbiology*, *17*(1), 384-402.

Croft, H., Chen, J. M., Luo, X., Bartlett, P., Chen, B., & Staebler, R. M. (2017). Leaf chlorophyll content as a proxy for leaf photosynthetic capacity. *Global Change Biology*, *23*(9), 3513-3524.

Del Rio, A. M., Davis, B. E., Fangue, N. A., & Todgham, A. E. (2019). Combined effects of warming and hypoxia on early life stage Chinook salmon physiology and development. *Conservation physiology*, *7*(1), coy078.

Finkle, B. J., & Appleman, D. (1953). The effect of magnesium concentration on chlorophyll and catalase development in Chlorella. *Plant physiology*, *28*(4), 652.

Patton, T. M. (2003). *Evaluation of the Salish Creek mitigation project* (Doctoral dissertation, University of British Columbia).

Wang, Y., Seppänen-Laakso, T., Rischer, H., & Wiebe, M. G. (2018). Euglena gracilis growth and cell composition under different temperature, light and trophic conditions. *PloS one*, *13*(4), e0195329.

Appendix:

Table 1: Resultant normalized values and their means per treatment

	Change in Oxygen Production per cell (Using Equation 1) (mgO_2/cell)
0.049 mg/mL Mg^{2+}	1: -3.7e-8
	2: -4e-8
	3: -3.3e-8
	4: -2.7e-8
	5: -3.3e-8
Mean 0.049 mg/mL change in Oxygen Production per cell.	-3.4e-8
0.02 mg/mL Mg^{2+}	1: -4.3e-8
	2: -4.6e-8
	3: -5.5e-8
	4: -4.9e-8
	5: -6.1e-8
Mean 0.02 mg/mL change in Oxygen Production per cell.	-5.08e-8
0 mg/mL Mg^{2+}	1: -5.3e-8
	2: -5.1e-8
	3: -5.2e-8
	4: -4.4e-8
	5: -3.8e-8
Mean 0 mg/mL change in Oxygen Production per cell.	-4.76e-8

Table 2: One-Way Anova

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	8.454e-016	2	4.227e-016	F (2, 12) = 11.48	P=0.0016
Residual (within columns)	4.420e-016	12	3.683e-017		
Total	1.287e-015	14			
Data summary					
Number of treatments (columns)	3				
Number of values (total)	15				

Table 3: Tukey-Kramer Test

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value			
0 vs. 0.02	4.900e-009	-5.340e-009 to 1.514e-008	No	ns	0.4341	A-B		
0 vs. 0.049	-1.290e-008	-2.314e-008 to -2.660e-009	Yes	*	0.0145	A-C		
0.02 vs. 0.049	-1.780e-008	-2.804e-008 to -7.560e-009	Yes	**	0.0015	B-C		
Test details	**Mean 1**	**Mean 2**	**Mean Diff.**	**SE of diff.**	**n1**	**n2**	**q**	**DF**
0 vs. 0.02	-4.680e-008	-5.170e-008	4.900e-009	3.838e-009	5	5	1.805	12
0 vs. 0.049	-4.680e-008	-3.390e-008	-1.290e-008	3.838e-009	5	5	4.753	12
0.02 vs. 0.049	-5.170e-008	-3.390e-008	-1.780e-008	3.838e-009	5	5	6.558	12