Effect of light wavelength on chloroplast length in cultured *Euglena gracilis*

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Abstract

The objective of our study was to determine whether exposing *Euglena gracilis* to different wavelengths of light affected their mean chloroplast length. Euglena cultures were incubated in culture tubes surrounded by coloured acetate paper, black plastic, or transparent acetate paper in order to manipulate the wavelengths of light the organisms were exposed to. In total, there were 5 different light treatments: red, green, blue, normal light, and no light. Samples were fixed and chloroplast lengths were measured using a compound microscope at 1000X magnification. A one-way ANOVA test returned an F statistic of $F_{(4, 10)} = 3.169$ and a p-value of 0.0633. We failed to reject the null hypothesis and conclude that the mean chloroplast length of Euglena cultured under different wavelengths of light does not differ. However, we did notice a trend: the no light treatment had a lower mean chloroplast length in comparison to the red, blue, green and the normal light treatments. This may indicate that the absence of light could be correlated with a decrease in chloroplast length. Understanding the ability of *E. gracilis* to change its chloroplast parameters due to changes in external environmental light conditions is important to further understand the physiological processes that underlie these organellular changes.

Introduction

Euglena gracilis is a unicellular phytoflagellate capable of conducting photosynthesis (Teerawanichpan & Qiu, 2010). Classified under phylum Eukarya, the protist *E. gracilis* has a photosynthetic eyespot which functions to detect specific wavelengths of light (James et al., 1992). In an aquatic environment, this eyespot guides Euglena towards sunlight so that the chloroplasts can utilize this light energy to produce glucose from carbon dioxide and water (Barsanti et al., 2012). With the presence of sensitive photo-receptive proteins that transduce light signals, Euglena are capable of steering themselves towards a light source (Barsanti et al., 2012).

Different ranges of wavelengths in the visible spectrum of light penetrate the water to different depths (Davies-Colley & Nagels, 2008). Due to this difference in light penetration, Euglena may cluster at specific depths depending on the wavelength that induces the greatest photosynthetic rate. The expression of chloroplast protein elongation factors are known to

increase when Euglena are exposed to specific wavelengths of light (Eberly et al., 1986). With this understanding, rather than monitoring the transcription rate of chloroplast protein factors, the present study investigates whether exposing Euglena to different light wavelength treatments affects the overall length of chloroplasts.

Chlorophyll a and b are the main photosynthetic pigments in Euglena chloroplasts and these molecules absorb two specific wavelengths of light, corresponding to blue and red in the visible spectrum (Eberly et al., 1986). If maximum light absorption occurs under blue and red light, then the length of chloroplasts in Euglena should be the greatest under wavelengths corresponding to these colors. Since different wavelengths of light are found at different depths in water, Euglena abundance would be the greatest at depths that contain the highest intensity of red and blue light. Since blue light has a shorter wavelength than red light, blue light has more energy. Since blue light has more energy, we hypothesized the mean chloroplast length to be the highest under the wavelength of light that corresponds to blue in the visible spectrum. Our null and alternative hypotheses were as follows:

H_0 = There is no difference in the mean chloroplast length between the different light treatments.
H_a = There is a difference in the mean chloroplast length between the different light treatments.

Found at the bottom of the salmon food chain, Euglena serve as an important food source for salmon. This is of significant interest because an increase in chloroplast length may result in a greater primary productivity of the Euglena. As phytoplankton such as Euglena are a primary food source for many salmon species, a greater productivity of Euglena may directly benefit salmon populations through a bottom-up trophic cascade.

Methods and Materials

Refer to Figure 1 for a schematic of the major procedural steps.

Culture Tube Preparation

In total, there were 5 different light treatments: red (R), green (G), blue (B), normal light (L), and no light (D). The normal light treatment was the positive control, while the no light treatment was the negative control. For each treatment, 3 culture tubes were prepared. The no light treatment tubes were wrapped in a sheet of black plastic. For the red, green, and blue light treatments, sheets of coloured acetate paper that filtered light at peak wavelengths of 680 nm, 500/740 nm, and 410/740 nm respectively were used to cover the culture tubes. For the normal light treatment, the culture tubes were surrounded by transparent acetate paper and wrapped in 10 layers of cheesecloth in order to reduce the light intensity to a similar level compared to the other treatments. Next, 1000 μL of growth medium and 1000 μL of vortexed Euglena culture were transferred into each of the 15 culture tubes.

Incubation

In order to minimize the discrepancy in light intensity between the treatments inside the Conviron Adaptis incubator, a VWR Traceable light meter was used to determine the optimal location for the culture tubes. This was done by covering the light meter sensor using the same materials described in the previous paragraph for each light treatment. The culture tubes were then put in separate racks and placed in the incubator such that the light intensity was within 10% of 250 lux for each treatment, except for the no light treatment which had a light intensity of approximately 0 lux. Cardboard boxes were used to adjust culture tube height. The tubes remained in the incubator for approximately 47 hours at 20˚C. Light/dark cycles in the incubator were as follows: 8 hours light, 10 hours dark, 14 hours light, 10 hours dark, 5 hours light.

Fixation

To fix the cells, 10 μL of 3% glutaraldehyde was added to 15 counting tubes. From each of the 15 culture tubes, 100 μL of Euglena culture was transferred into the 15 different counting

tubes and resuspended using a micropipette. A slide was prepared using a sample from the L1 counting tube. This slide was examined using a Zeiss Axio compound microscope at a magnification of 1000X to verify if the cells had been immobilized. Upon verification that the fixative had been effective, the counting tubes were stored in a refrigerator at 4 °C until further analysis could be conducted.

Measurement and Data Analysis

After approximately 22.5 hours, the counting tubes were removed from the refrigerator. Slides were prepared using 25 μL samples from each counting tube. The ocular micrometers on 2 Zeiss Axiostar compound microscopes were calibrated for measurement at 1000X magnification (refer to Fig. 2). For each slide, 3 Euglena cells were sampled and the lengths of 5 chloroplasts per cell were recorded. For all 5 treatments, 3 different individuals each measured chloroplast lengths using one slide from each treatment. Length was defined as the longest end-to-end distance of the chloroplast being measured. The average chloroplast length was determined for each replicate in each of the 5 treatments. A one-way analysis of variance (ANOVA) test was conducted to determine if the mean chloroplast length was significantly different between the 5 different light treatments.

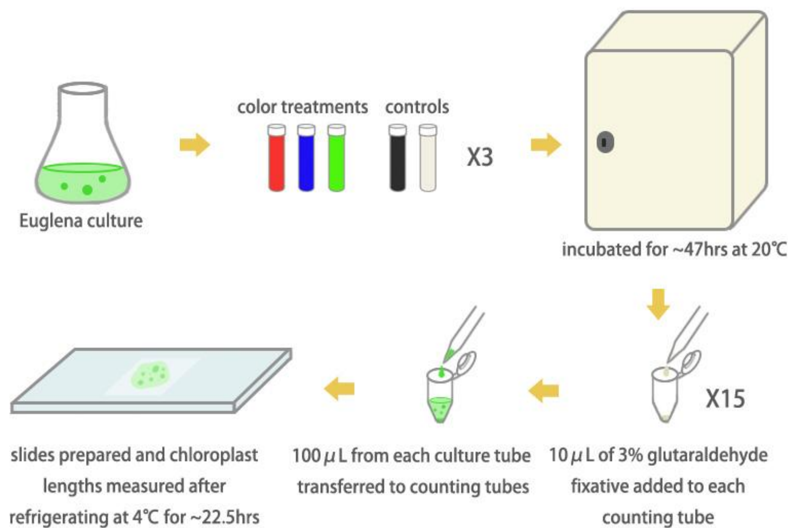

Figure 1. Overview of the major procedural steps performed.

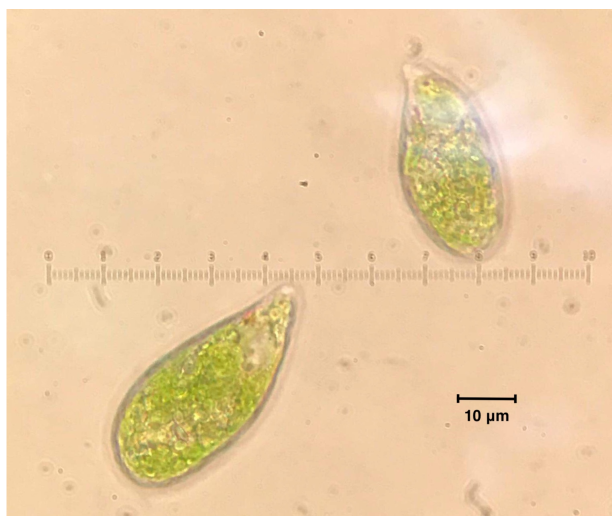

Figure 2. Euglena cells from the red treatment viewed through a Zeiss Axiostar compound microscope at 1000X magnification.

Results

For each light treatment, there were 3 replicates (n = 3) and 5 pseudo-replicates. The ANOVA test returned an F statistic of $F_{(4, 10)} = 3.169$ and a p-value of 0.0633. Based on these results, we fail to reject the null hypothesis, which states there is no difference in the mean chloroplast length between different light treatments. Figure 3 shows the average chloroplast length and 95% confidence intervals for no light, red, green, blue & normal light treatments to be M= 1.711µm, 95% CI [0.0773, 3.345], M= 3.156µm, 95% CI [0.7141, 5.597], M= 2.511µm, 95% CI [1.979, 3.043], M =2.978µm, 95% CI [2.595, 3.360] & M= 2.911µm, 95% CI [2.094, 3.728] respectively. Figure 3 depicts a noticeable trend in the means of the different treatment groups: the no light treatment group seems to have an average value that is roughly half of all the other treatment averages.

The observed Euglena showed variation in chloroplast abundance and shape: rod-shaped or spherical. Additionally, most of the observed Euglena contained visible red eyespots. The normal and red light treatments contained relatively large amounts of Euglena, of which some presented punctured cell walls. The green light treatment had a moderate abundance of

Euglena.The blue light treatment contained a greater relative number of chloroplasts per Euglena cell and a few Euglena that had undergone lysis. The no light treatment contained Euglena with relatively smaller and less prominent chloroplasts.

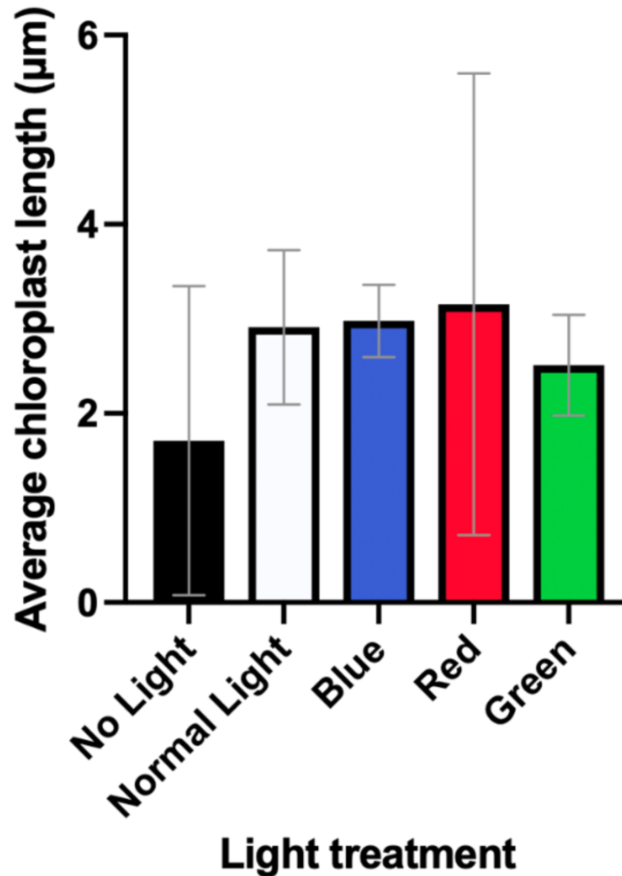

Figure 3. The average chloroplast lengths of Euglena grown in the five light treatments (n = 3) with bars representing 95% confidence intervals.

Discussion

The main purpose of this study was to determine the effect of different wavelengths of light on the chloroplast length in Euglena. Based on the results of the one-way ANOVA test, we failed to reject our null hypothesis and concluded that there was no statistically significant difference in the mean chloroplast length between the different light treatments.

The mean chloroplast length in the negative control (no light treatment) tended to be lower, almost half of the value of all other treatments (refer to Figure 3). This may be due to the

inactivation of chloroplast formation as well as the inactivation of chlorophyll synthesis in Euglena cells grown without light (Lyman et al., 1961). Secondly, we noticed a trend of a slightly lower mean chloroplast length in the green light treatment. It is possible that green chloroplasts do not utilize green light as they are pigmented green and thus reflect green light. This could have possibly led to a decreased chloroplast length, in similar fashion to the no light treatment. We expected the mean chloroplast length to be the greatest under the blue light treatment. However, the mean chloroplast length under blue light did not significantly differ from the no light, the normal light, the green light or the red light treatment.

Eberly et al. (1986) discovered that red, green, and blue lights have different effects on the synthesis of Euglena chloroplasts. Blue light (in the range of 390-490 nm) positively increased the transcription rate of chloroplast elongation factor proteins as well as chloroplast gene products while red light (600-700 nm) and green light (490-590 nm) were similarly effective, but not to the same extent (Eberly et al.,1986). This suggests that the photoresponse to red, green, and blue light is likely to be important in the development of Euglena chloroplasts. Considering the results of the previously mentioned experiment by Eberly et al. (1986), it is likely that exposure to specific wavelengths of light does indeed affect chloroplast length. However, we were not able to find statistically significant differences in our experiment.

A study by Vesteg et al. (2009) showed that light was not a factor in changing the mRNA levels that encode chloroplast proteins. If we assume higher mRNA levels in chloroplasts result in a greater concentration of chloroplast proteins and therefore a larger chloroplast size, this may be relevant to our results. Specifically, we did not find a significant difference in the mean chloroplast lengths when subjecting Euglena to different treatments of light, which is consistent with the finding by Vesteg et al. (2009).

There were two main sources of uncertainty and variation during the experiment. The first source of uncertainty was the limited sample size. In our experiment, we only had 3 replicates per light treatment. Within each replicate, we measured 15 chloroplasts. This limited sample size was a constraint for our experiment. When viewing each replicate under the

compound microscope, many more chloroplasts could have been measured. Due to a small sample size, our data collection was prone to error.

The second possible source of variation was the limited time given to the Euglena to effectively undergo noticeable changes in chloroplast length. We incubated our Euglena cultures for a total of 27 hours in the light. According to Cook (1961), the doubling time of Euglena is approximately 16 hours. Therefore, although the Euglena cultures may have doubled, it is possible that 27 hours of light were insufficient to allow for noticable changes in chloroplast length. Thus, it is possible that we may not have given the chloroplast elongation factors enough time to successfully act upon their target receptors.

For further research, we should obtain more data by increasing the number of replicates to minimize error. We should also increase the incubation time in order to possibly create a greater variation in the mean chloroplast length between the different light treatments.

Conclusion

In conclusion, with an F statistic of $F_{(4, 10)} = 3.169$ and a p-value of 0.0633, we fail to reject the null hypothesis and conclude that there is no significant difference in the mean chloroplast length of cultured Euglena exposed to different wavelengths of light. Our prediction of chloroplast length being highest under blue light was not observed. Furthermore, the overall effect of the tested treatments of light on the length of Euglena chloroplasts and the significance of this in connection to the salmon populations remains unknown.

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References

Barsanti, L., Evangelista, V., Passarelli, V., Frassantio, A.M. & Gualitieri, P. (2012). Fundamental questions and concepts about photoreception and the case of euglena gracilis. *Integrative Biology: Quantitative Biosciences from Nano to Macro*. 4(1), 22-36.

Cook, J. R. (1961). Euglena gracilis in synchronous division. II. Biosynthetic rates over the life cycle. *The Biological Bulletin*. *121*(2), 277-289.

Davies-Colley, R.J. & Nagels, J.W. (2008). Predicting light penetration into river waters. *Journal of Geophysical Research - Biogeosciences.* 113(G3), G03028.

Eberly, S. L., Spremulli, G. H., & Spremulli, L. L. (1986). Light induction of the *Euglena* chloroplast protein synthesis elongation factors: Relative effectiveness of different wavelength ranges. *Archives of Biochemistry and Biophysics*. *245*(2), 338-347.

James, T. W., Crescitelli, F., Loew, E. R., & McFarland, W. N. (1992). The eyespot of euglena gracilis: A microspectrophotometric study. *Vision Research. 32*(9), 1583-1591.

Lyman, H., Epstein, H. T., & Schiff, J. A. (1961). Studies of chloroplast development in Euglena I. Inactivation of green colony formation by UV light. *Biochimica et biophysica acta*. *50*(2), 301-309.

Teerawanichpan, P. & Qiu, X. (2010). Fatty acyl-coA reductase and wax synthase from *Euglena gracilis* in the biosynthesis of medium-chain wax esters. *Lipids*. 45(3), 263-273.

Vesteg, M., Vacula, R., Burey, S., Loeffelhardt, W., Drahovska, H., Martin, W. & Krajčovič, J. (2009). Expression of nucleus−encoded genes for chloroplast proteins in the flagellate *Euglena gracilis*. *Journal of eukaryotic microbiology*. *56*(2), 159-166.

Appendix

	Normal Light Treatment														
Rep-licate #	L1					L2					L3				
Chloro-plast	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e
Cell 1 (length in µm)	4	2	2	1	3	3	2	2	4	3	3	2	3	3	3
Pseudo-replicate average	2.4					2.8					2.8				
Chloro-plast	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e
Cell 2 (length in µm)	2	4	5	2	4	3	5	4	4	2	3	4	3	3	4
Pseudo-replicate average	3.4					3.6					3.4				
Chloro-plast	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e
Cell 3 (length in µm)	2	1	2	2	2	4	3	3	3	1	4	3	3	3	3
Pseudo-replicate average	1.8					2.8					3.2				
Treat-ment average	2.911														

	No Light Treatment														
Rep-licate #	D1					D2					D3				
Chloro-plast	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e
Cell 1 (length in μm)	1	2	1	1	1	3	2	3	3	4	2	1	1	1	1
Pseudo-replicate average	1.2					3					1.2				
Chloro-plast	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e
Cell 2 (length in μm)	1	2	2	1	1	3	2	1	3	3	2	1	2	1	2
Pseudo-replicate average	1.4					2.4					1.6				
Chloro-plast	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e
Cell 3 (length in μm)	1	1	1	2	1	2	3	2	1	2	2	1	1	2	1
Pseudo-replicate average	1.2					2					1.4				
Treat-ment average	1.711														

	Blue Treatment														
Rep-licate #	B1					B2					B3				
Chloro-plast	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e
Cell 1 (length in μm)	4	5	4	5	2	3	2	3	2	3	3	4	2	2	1
Pseudo-replicate average	4					2.6					2.4				
Chloro-plast	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e
Cell 2 (length in μm)	2	2	2	2	2	4	3	2	3	5	2	4	4	4	3
Pseudo-replicate average	2					3.4					3.4				
Chloro-plast	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e
Cell 3 (length in μm)	3	5	2	2	4	3	2	5	2	4	2	2	3	3	3
Pseudo-replicate average	3.2					3.2					2.6				
Treat-ment average	2.978														

	Red Treatment														
Rep-licate #	R1					R2					R3				
Chloro-plast	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e
Cell 1 (length in µm)	4	6	2	5	1	1	3	3	2	2	2	2	3	4	2
Pseudo-replicate average	3.6					2.2					2.6				
Chloro-plast	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e
Cell 2 (length in µm)	2	5	3	1	4	3	3	2	4	3	3	2	3	4	4
Pseudo-replicate average	3					3					3.2				
Chloro-plast	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e
Cell 3 (length in µm)	15	4	3	6	3	3	2	1	2	2	3	3	3	2	2
Pseudo-replicate average	6.2					2.0					2.6				
Treat-ment average	3.156														

	Green Treatment														
Rep-licate #	G1					G2					G3				
Chloro-plast	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e
Cell 1 (length in μm)	3	1	1	2	2	3	2	2	1	3	4	3	2	3	1
Pseudo-replicate average	1.8					2.2					2.6				
Chloro-plast	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e
Cell 2 (length in μm)	3	3	1	4	2	2	2	3	2	3	2	1	3	3	3
Pseudo-replicate average	2.6					2.4					2.4				
Chloro-plast	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e
Cell 3 (length in μm)	3	3	5	4	3	2	2	1	3	3	3	2	3	3	3
Pseudo-replicate average	3.6					2.2					2.8				
Treat-ment average	2.511														

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	3.996	4	0.9990	F (4, 10) = 3.169	P=0.0633
Residual (within columns)	3.153	10	0.3153		
Total	7.148	14			