# Effect of light intensity on *Chlamydomonas reinhardtii* growth rate

Jenny Chae, SungHoo Jegal, Taylor Keraiff, Kattie Sepehri

The effect of light intensity on the growth rate of *Chlamydomonas reinhardtii* was explored. The null hypothesis stated that an increase in light intensity will either decrease or have no effect on *C. reinhardtii* growth rate, and the alternative hypothesis tested for this study was that an increase in light intensity will increase *C. reinhardtii* growth rate. Three replicates of *C. reinhardtii* solution were grown under three different light intensities: high (4660 Lux), control (2380 Lux) and low (1330 Lux). Cell growth was monitored for two weeks by taking samples from each treatment and counting the amount of cells using a haemocytometer. The obtained results for this experiment showed that compared to the control growth rate (18.6207 cells/day ± 7.2430), low light intensity had a decreased growth rate (12.8572 cells/day ± 5.7973) while high light intensity also had a relatively small decrease in growth rate (16.2760 cells/day ± 1.8620). The calculated p-value was 0.48, which indicates that no significant difference. It is suspected that the lower results in a high intensity were due to a process called non-photochemical quenching, or NPQ. In conclusion, we failed to reject the null hypothesis and could not provide support for the alternate hypothesis.

## Introduction

*Chlamydomonas reinhardtii* is a photosynthetic, eukaryotic alga that is oval shaped, usually 10 µm in diameter and utilizes two flagella for motility (Rochaix 2001). This organism is recognized as an essential model organism because of its easily storable small size, high reproduction rate and exponential growth (Griesbeck *et al*. 2006) and compared to other plant research subjects, *C. reinhardtii* a relatively small genome that allows for specific transformation and mutation (Funes *et al*. 2007). Although the primary source of its energy comes from photosynthesis, this mechanism is not vital for its survival as these green algae can grow either photoautotrophically or heterotrophically on acetate (Funes *et al.* 2007). Therefore, *C. reinhardtii* is used in many experiments that manipulate and focus on different growth rates. Each *C. reinhardtii* cell contains a single chloroplast and several mitochondria for energy

production mainly through photosynthesis, which produces complex sugars and oxygen from the reaction of carbon dioxide with water in the thylakoid membrane of the chloroplast (Rochaix 2001). The energy for this reaction is supplied by light, which is turned into chemical energy in the form of sugars that can be used as a fuel for the organism's activities such as growth and reproduction (Falk *et al*., 2006).

For this study, the objective is to further solidify that *C. reinhardtii* does, in fact, grow at a faster rate with higher light intensity due to its photosynthetic nature, provided that all other required nutrients for its growth are constant in all treatments (Bonente *et al.* 2012). Thus, our null hypothesis states that increasing light intensity will decrease or have no effect on *C. reinhardtii* growth rate, and our alternate hypothesis states that increasing light intensity will increase *C. reinhardtii* growth rate. This investigation is important because it can be used to study the effects of light intensity on the growth rate of a model organism, which uses photosynthesis as its primary source for nutrients and growth. The results of this study will not only give insight to further related studies on the effects of light on *C. reinhardtii*, but also provide a deeper knowledge of photosynthetic machinery in many other photoautotrophic organisms.

## Methods

### *Set-up*

In the lab on Day 0 of the experiment, a stock solution of CC-1690 mt+ strain *C. reinhardtii* was obtained. Each member counted the cell concentration using a haemocytometer on an Axio microscope and found that the original cell count was, on average, 327,500 cells/mL. Then, the stock solution was diluted with *Chlamydomonas*

medium so that the initial cell density count was, averaged with four counts, 118,250 cells/mL. From here, 9 tubes were each filled with 10 mL of *C. reinhardtii* cells in their medium, placed in a rack and in a temperature controlled room at 17°C for the duration of the experiment. Cheesecloth was wrapped around the control and low light intensity tubes to achieve varying light intensities. As seen in Figure 1, there were three light intensities each with three replicates. The control, 2380 Lux, was the light intensity in which they were originally grown in the lab, while the higher intensity was at 4660 Lux with no layers of cheesecloth and the lower intensity was at 1330 Lux with three layers of cheesecloth. All tubes were in the same room, in a rack and on a shaker at 80 RPM for the entirety of the experiment.

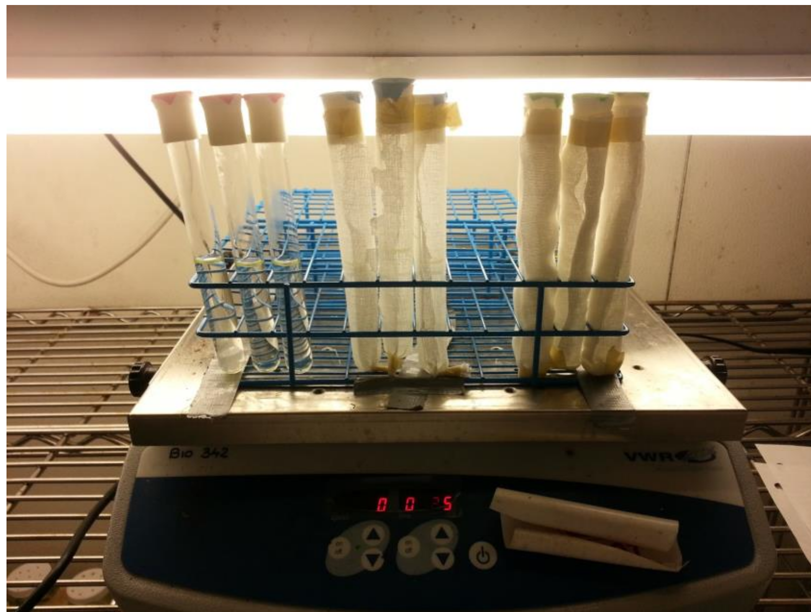

**Figure 1**. The experimental set-up. Left tubes = high intensity of 4660 Lux; middle tubes = control intensity of 2380 Lux; right tubes = low intensity of 1330 Lux.

*Sampling and Counting*

As seen in Figure 1, the control (middle) is denoted as having a blue cap. The higher intensity (left) was denoted with a red cap and the low intensity (right) was denoted

with a green cap. Every Monday, Wednesday and Friday the tubes were counted for a total of six counts, excluding the initial count. On each counting day, the tubes were taken off the shaker, brought out of the cold room and into the lab. A 100 µL sample was taken from each replicate and fixed using 10 µL of IKI (Gram's Iodine solution) in a microcentrifuge tube and mixed by micropipetting the solution. From this sample in the microcentrifuge tube, 10µL of the fixed samples were transferred onto a haemocytometer. A minimum of 100 cells of *C. reinhardtii* were counted with a clicker, and then this number was divided by the number of 1mm squares used (to average the count).

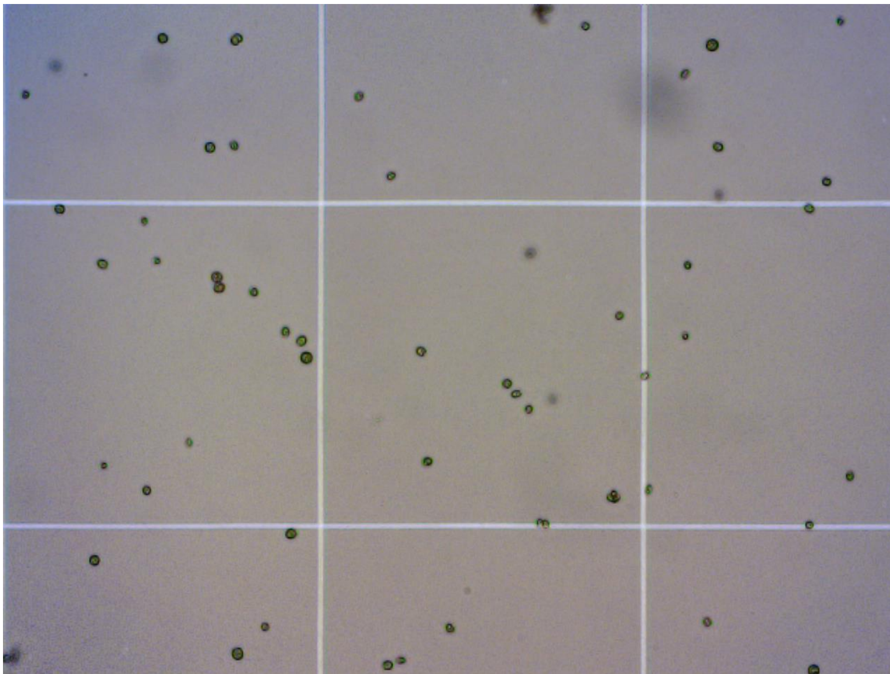

**Figure 2.** Image taken by Taylor Keraiff showing fixed *Chlamydomonas reinhardtii* cells from the 3rd replicate of the high light intensity treatment on the haemocytometer on 100X total magnification.

*Data Analysis*

From our raw cell counts, we evaluated the count in cells/mL using a 1.1 conversation factor (to account for the addition of IKI). Then the exponential growth

rate of each replicate was determined using a line of best fit. These growth rates were then statistically evaluated by one-way analysis of variance (ANOVA) test to determine the F and p values. Lastly, the growth rates of each replicate were averaged separately for each treatment and graphed as a function of light intensity. The finalized data can be seen in Figure 3.

**Results**

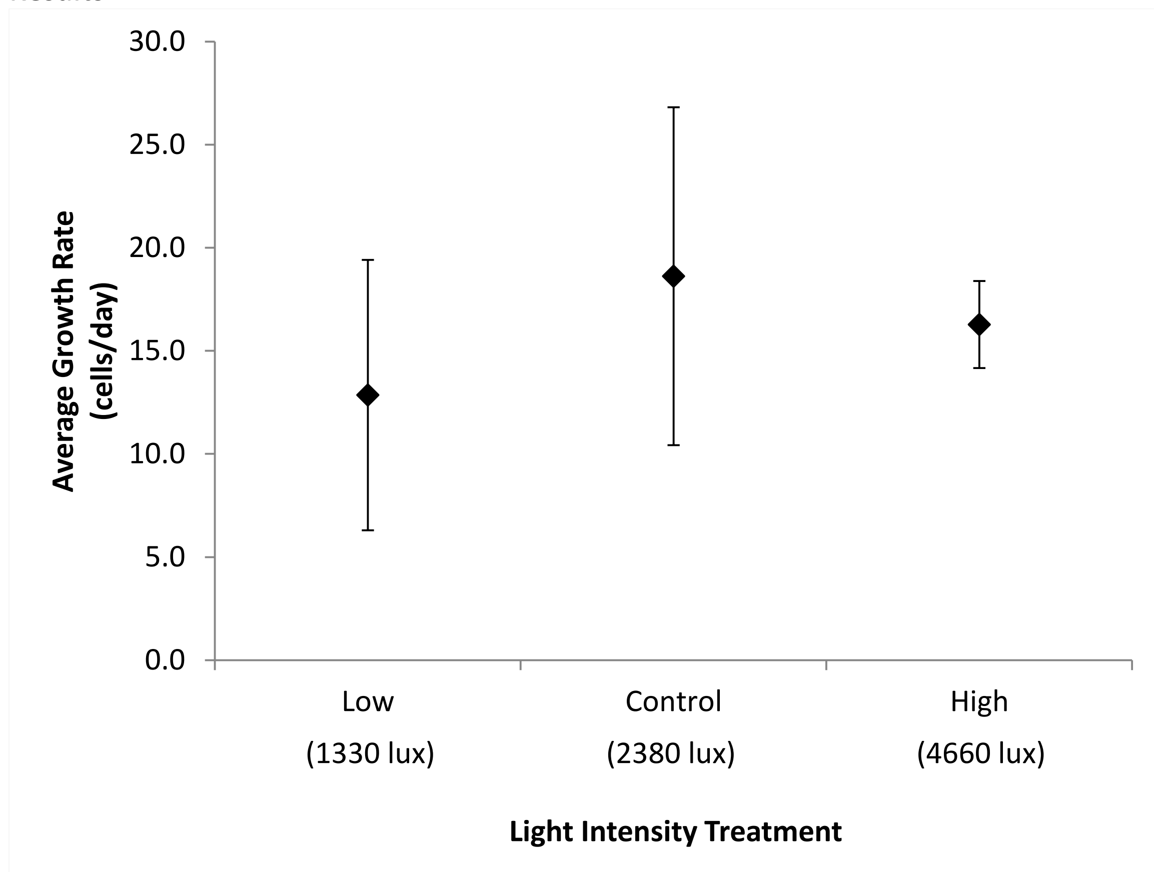


**Figure 3**. Effect of light intensity on average growth rate in cells/day of *Chlamydomonas reinhardtii* in low light intensity, control light intensity and high light intensity (1330 Lux, 2380 Lux, and 4660 Lux, respectively). Error bars represent 95% confidence intervals, n=3.

The general trend observed in Figure 3 was a decreased growth rate of 12.8572 ± 5.7973 cells/day for low light intensity at 1330 Lux when compared to the control growth rate of 18.6207 ± 7.2430 cells/day at 2380 Lux. There was also a slight decrease

in growth rate of 16.2760 ± 1.8620 cells/day for high light intensity at 4660 Lux when compared to the control light intensity, but these two intensities had a close average growth rate. As seen in the error in Figure 3, the control had the most amount of variation in growth rate, while the highest light intensity had the smallest variation. Another thing to note is that there was a peak in growth at 6 days for high and control light intensities, whereas the low light intensity took 8 days to peak during its initial growth.

The obtained *p*-value for the ANOVA test of the difference between replicates, their treatments and the length of time was $p = 0.48$. This means that there are no significant differences between the growth rates at the different light intensity treatments due to the p-value being higher than 0.05. This can also be more easily seen in Figure 3 due to the overlapping of error bars, suggesting no significant differences.

**Discussion**

The purpose of the experiment was to determine the effect of light intensity on the growth rate of *Chlamydomonas reinhardtii*. Our alternate hypothesis stated that increasing light intensity would increase the growth rate of *C. reinhardtii*. Our null hypothesis stated that increasing light intensity would either decrease or have no effect on the growth rate of *C. reinhardtii*. According to the calculated p-value and the results shown on Figure 3, the data did not support the alternate hypothesis. Hence, we failed to reject the null hypothesis because the calculated p-value is higher than 0.05, representing no significant difference. Moreover, all three 95% confidence intervals of

average growth rates overlap, also illustrating no significant differences between the treatments tested.

The growth rate of high light intensity replicates did not vary greatly compared to the other two light intensities. However, standard deviations of average growth rate of low light intensity and control were large because some replicates had counts that were far off from the average. The variation in cell count might be caused by pipetting error when we transferred 100µL of *C. reinhardtii* sample from the test tubes to the microcentrifuge tubes to fix them with IKI solution for cell counting. During the transfer of 10µL of fixed sample to the haemocytometer slides for cell counting, the number of cells pipetted could have varied greatly, which resulted in large variance in cell count for among the three replicates. One critical source of error was that some of the cells in a few of our replicates were still alive and motile after the cells were sampled and fixed using IKI solution. Some samples were not counted immediately after IKI fixation, therefore there is a possibility that these cells continued to reproduce until the sample was counted, and this may have contributed to a counting error. The observed decrease in growth rate at low light intensity was predicted and it may have been due to limiting the source of energy for photosynthesis. At low light intensity, photosynthesis would be very limited due to a decrease in the number of electrons that reach the reaction centers. This would decrease the amount of sugars produced in the Calvin cycle, and therefore provide less energy for the cells to divide (Forti 2008).

The observed decrease in growth rate at high light intensity did not agree with our alternate hypothesis that higher light intensity should result in higher growth rate.

This prediction was made because photosynthesis is the primary source of energy for *C. reinhardtii*, therefore a higher light intensity should provide the cells with more energy to use in reproduction and division (Funes *et al.* 2007). However, according to Muller *et al.* (2001), there is a possibility that the cells use non-photochemical quenching (NPQ) to control energy absorption when the excess light intensity is harmful to them. Since the quality of light can vary greatly in the natural environment, plants and algae adapt to changes in light intensity by altering the function of their photosynthetic apparatus (Rochaix 2001). High light intensity can be harmful to the organism because of the production of reactive chemical species, therefore the excess energy can be dissipated as fluorescence or heat through NPQ to protect the plant or algae (Rochaix 2001). According to Muller *et al.* (2001), in almost all photosynthetic eukaryotes the NPQ mechanism helps to regulate photosynthesis when the energy absorption is much higher than energy utilization. Based on this information, we should have observed the same growth rate in the control treatment and the high light intensity treatment, assuming that this light intensity is optimal. Also, this is under the assumption that at the control treatment, the NPQ process is not already induced and the cells are harvesting at an optimal level of energy, while the NPQ process is induced at high light energy. Another article by Allorent *et al.* (2013) uses a light intensity of 50 µmol $m^{-2}s^{-1}$, which equates close to 4,000 Lux. This study also supports the NPQ process in higher light intensities as well, suggesting there is an optimal level of light intensity for *C. reinhardtii* optimal growth rates.

Although the primary source of its energy comes from photosynthesis, this mechanism is not vital for its survival as these green algae can grow either photoautotrophically or heterotrophically on acetate (Funes *et al.* 2007). Too much light can be harmful to photoautotrophic organisms because it leads to production of damaging reactive species (Muller *et al.* 2001). This subject would be an excellent candidate for further experiments to determine exactly at which light intensity the light becomes harmful. In our study, our high light intensity was nearly double that of our control. This gives a large range of light intensity to study and pinpoint where the *C. reinhardtii* cells start to decrease their growth rate. Also, it is highly possible that the cells at high light intensity used up all the nutrients required very quickly, and therefore resulting in the lower counts when compared to the control. The Lux was nearly double that of the control, and as seen in Figure 1, it was placed very close to a light source. Because of this, the control cells likely had sufficient nutrition throughout the experiment. This suggests that future studies could observe concentrations of nutrients at the beginning and end of the experiment.

**Conclusion**

In conclusion, we failed to reject the null hypothesis, providing no support for our alternate hypothesis. The data in the high light intensity had little variation and had a lower growth rate than the control, whereas the control light intensity had the greatest growth rate. This suggests that our experiment could be the result of the NPQ process, and could give us more insight into the photosynthesis and the NPQ process in many other photoautotrophic organisms.

### *Acknowledgements*

We would like to thank the University of British Columbia for the funding and opportunity to perform this experiment in the Biology 342 course. Also, special thanks to Professor Carol Pollock, lab technician Mindy Chow and teaching assistant Katelyn Tovey for guidance throughout our study.

### *Literature Cited*

Allorent, G., Tokutsu, R., Roach, T., Peers, G., Cardol, P., Girard-Bascou, J., Seigneurin-Berny, D., Petroutsos, D., Kuntz, M., Breyton, C., Franck, F., Wollman, F., Niyogi, K., Krieger-Liszkay, A., Minagawa, J., and Finazzi, G. 2013. A dual strategy to cope with high light in *Chlamydomonas reinhardtii.* The Plant Cell, **25**(2): 545-557. doi: 10.1105/tpc.112.108274

Bonente, G., Pippa, S., Castellano, S., Bassi, R. and Ballottari, M. 2012. Acclimation of *Chlamydomonas reinhardtii* to different growth irradiances. The Journal of Biological Chemistry, **287**(8): 5833-47. doi: 10.1074/jbc.M111.304279.

Falk, S., Samuel, G., and Oquist, G. 2006. Temperature-dependent photoinhibition and recovery of photosynthesis in the green alga *Chlamydomonas reinhardtii* acclimated to 12 and 27. Physiologia Plantarum, **78**(2): 173-180.

Forti, G. 2008. The role of respiration in the activation of photosynthesis upon illumination of darkadapted *Chlamydomonas reinhardtii*. Biochimica et Biophysica Acta (BBA) - Bioenergetics, **1777**(11): 1449-1454. doi: 10.1016/j.bbabio.2008.08.011

Funes, S., Franzén L. G. and González-Halphen D. 2007. *Chlamydomonas reinhardtii*: the model of choice to study mitochondria from unicellular photosynthetic organisms. Methods in Molecular Biology, **372**: 137-149. doi: 10.1007/978-1-59745-365-3_10.

Griesbeck, C., Kobl, I. and Heitzer, M. 2006. *Chlamydomonas reinhardtii.* Molecular Biotechnology, **34**(2): 213-223.

Muller, P., Li, X., and Niyogi, K., 2001. Non-photochemical quenching. A response to excess light energy. American Society of Plant Physiologists, **125**(4): 1558-1566.

Rochaix, J. 2001. Assembly, Function, and Dynamics of the Photosynthetic Machinery in *Chlamydomonas reinhardtii*. Plant Physiology, **127**(4): 1394-1398.