Measuring the Effect of Decreasing Light Intensity on the Cell Density of the Unicellular Algae *Chlamydomonas reinhardtii* at 17^{0}C

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Abstract

The purpose of conducting the experiment was to find the optimal light intensity range for growth of the unicellular, photosynthetic algae, *Chlamydomonas reinhardtii*. Since the rate of photosynthesis is dependent on light intensity and availability, light directly impacts the growth of *C. reinhardtii*. Over a period of 10 days, we counted cell density of *C. reinhardtii* exposed to four different light intensities: 8020 Lux (control), 5700 Lux, 3180 Lux and 0 Lux. We found that there was no statistical significance between the control group and the 5700 and 3180 Lux groups. We suggest that clumping of the algae observed after 144 hours is the reason behind the largely variant cell densities. It is also possible that we grew *C. reinhardtii* at a lower than optimal temperature, giving similar cell densities in the control group and 5700 Lux and 3180 Lux groups. Through our results and observations, we are unable to reject our null hypothesis.

Introduction

The motile unicellular green alga, *Chlamydomonas reinhardtii* (*C. reinhardtii*) is an aquatic organism found in a variety of habitats (Raven 2005). *C. reinhardtii* has two flagella for movement and a chloroplast to perform photosynthesis (Raven 2005). Since photosynthesis is vital in the algae's growth and survival, we aimed to learn how *C. reinhardtii* growth would respond to different light intensities, the driving force of photosynthesis. This investigation is important, because if we know the optimal light intensity range, we would gain a better understanding of its required growth conditions. Given its use as a model organism in a variety of experiments (Harris 2001), it is important to know how *C. reinhardtii* can be maintained and grown.

<u>Hypotheses</u>

Ho: Decreasing light intensity has no effect or increases the cell density of *Chlamydomonas reinhardtii*.

Ha: Decreasing light intensity decreases the cell density of *Chlamydomonas reinhardtii.*

C. reinhardtii often swims in the direction of light, and gathers around light sources to maximize its exposure to light (Harris 2001; Harris 2009). McCombie (1960) describes that as illumination decreases, the organism's growth rate decreases as well. A decrease from 1614 Lux to 1183 Lux, and 807 Lux, results in the growth rate decreasing from 0.11 to 0.093, and to 0.071 respectively (McCombie 1960; Miller *et al.* 2008). In another study, it was seen that *C. reinhardtii* growth was limited when exposed to light intensities below 5380 Lux. Growth rate was unaffected between 5380 Lux to 8608 Lux, and minimally affected up to a light intensity of 21520 Lux) (Sorokin and Krauss 1958). These findings support our alternate hypothesis that decreasing light intensity will result in lower growth rates of our organism.

Methods

Organism

We used *C. reinhardtii* CC-1690 -wild type mt+ 21gr cells. We measured the initial concentration of the *C. reinhardtii* culture using a haemocytometer under a light microscope. The culture was then diluted using a *Chlamydomonas* growth medium so that each replicate started with a cell density of 50,000 cells/mL. We started with 15 mL of diluted culture in sixteen different test tubes, so that we had 4 replicates in 4 different treatments. Based on the findings of Fadaghi *et al.* (2011), it was concluded that enough growth medium was added to each test tube so that the nutrients were in excess; the aim was to prevent nutrient competition in the test tubes.

Treatment Design

Our experiment was conducted in an incubator, where *C. reinhardtii* were grown at 17^{0} C, under a constant light source. The sources of light were two fluorescent lights. To test the effect of light intensity on the growth of *C. reinhardtii*, we used black plastic and cheesecloth as light filters. We recorded light intensity values under these filters in the incubator. We used the following four treatments: control treatment (no filter) at 8020 Lux, single layer of cheesecloth (5700 Lux), four layers of cheesecloth (3180 Lux) and one layer of black plastic (0 Lux).

Figure 1 *C. reinhardtii* cells in their respective treatments test tubes on a shaker set at 80 rpm, inside the 17ºC incubator. Cultures are under fluorescent light bulbs. Right: single layer of cheesecloth (5700 Lux); black plastic (0 Lux). Left: control, no filter (8020 Lux); four layers of cheesecloth (3180 Lux).

Experiment Setup

We wrapped the tubes with their respective filters: unwrapped control (tubes 1-4), one layer of cheesecloth (tubes 5-8), four layers of cheesecloth (tubes 9-12), and one layer of black plastic (tubes 13-16). These filters were taped on the tubes using as small pieces of masking tape as possible on one side of the tubes, so that the masking tape would not act as a light filter during the experiment. The test tubes were placed on a shaker in the incubator, directly underneath the fluorescent lights (Figure 1). The shaker speed was set at 80 rpm.

Counting

We ran the experiment for 10 days, calculating the cell density/mL roughly every 24 hours when possible. There was a two-day gap in our data over the weekend, since we did not have access to the lab then, for a total of 8 sampling days. The test tubes were kept in the incubator at all times, except when we needed to take samples; we tried to minimize the time they spent outside of the incubator. We took 100 μL of culture from each tube, and added 10 μL of IKI fixative to each sample. Before sampling, all test tubes were carefully mixed on the vortex for a minimum of 30 seconds, and checked for clumps to make sure the cell concentrations within the tubes were overall balanced. For each replicate, we took 50 μL to use on a haemocytometer. Cell density was calculated using the haemocytometer instructions. All procedures were done under sterile conditions to prevent contamination of the cultures.

After all the sampling was done, we calculated the means of the replicates in each treatment. These means were plotted in a scatter plot to look for any trends, using 95% confidence intervals to look for any statistical significance in our results.

Results

Sample calculations for cell density (using a haemocytometer)

Cell number ÷ (volume of grid* × number of grids used) = cell density/mL

e.g. 117 cells ÷ (1×10^{-4} mL × 4) = 292,500 cells/mL

*Volume of grid can be found in the haemocytometer usage instructions.

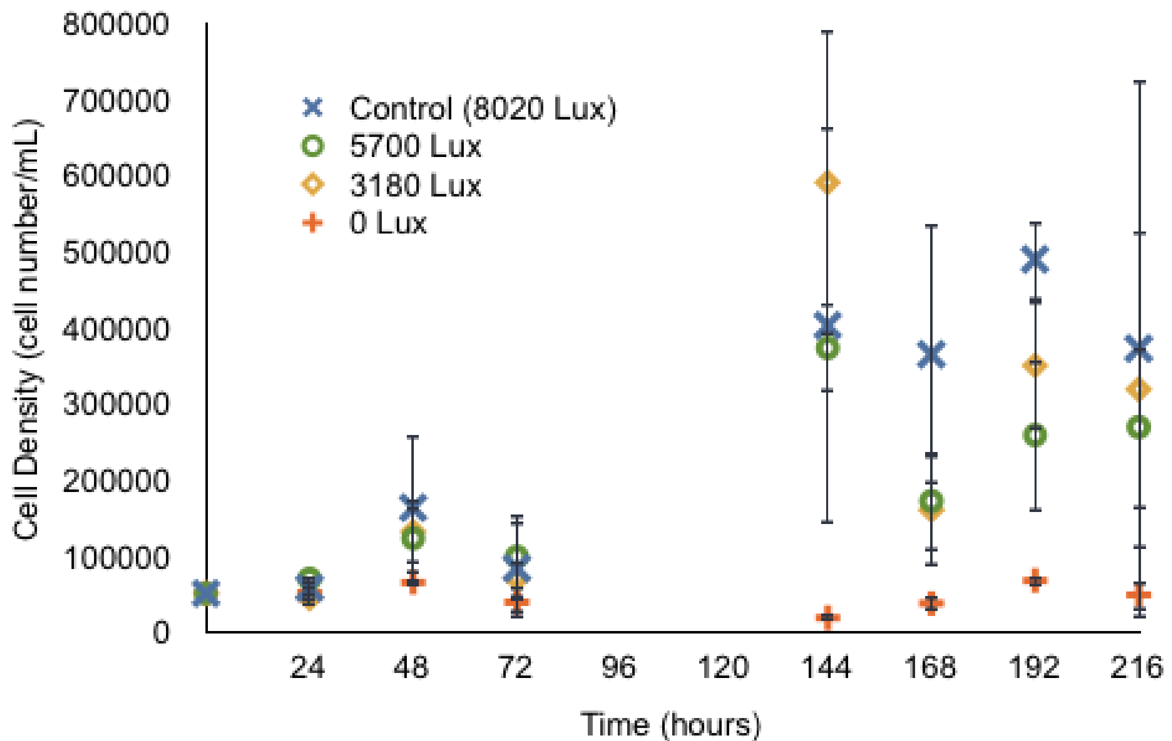

Figure 2 Average cell density of *C. reinhardtii* grown under different light intensities after 10 days, grown at 17°C. Bars represent 95% confidence intervals, n=4.

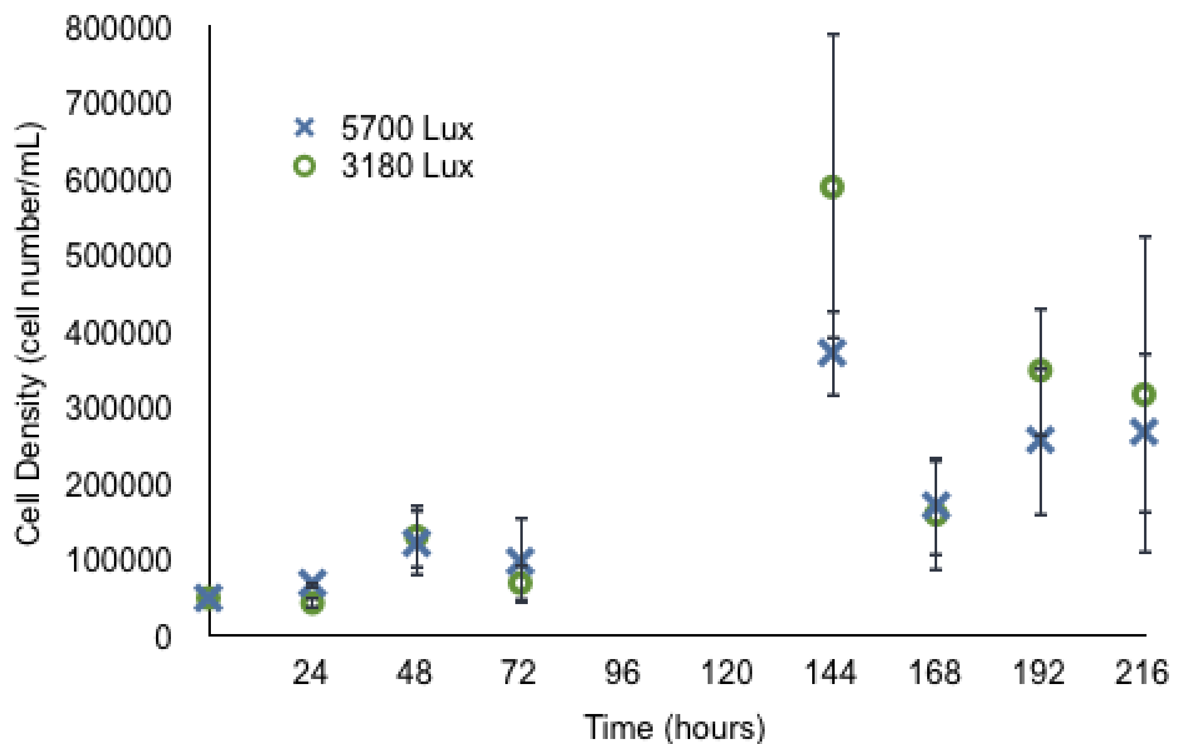

Figure 3 Comparison of the mean cell densities between treatment of *C. reinhardtii* grown at 5700 Lux and 3180 Lux, after 10 days at 17°C. Bars represent 95% confidence intervals, n=4.

There was an overall trend of increasing cell density when *C. reinhardtii* was exposed to light (Figure 2). As the experiment progressed, the test tubes with *C. reinhardtii* culture showed lots of clumping, this was especially seen from 144 hours into the experiment onwards. During the first 72 hours, the cell densities in all treatments were close to each other, without too much variation; after this point, there was much more variation in the cell densities (Figure 2). There was no significant differences in *C. reinhardtii* cell densities between the control, 5700 Lux and 3180 Lux treatments, shown by the wide 95% confidence intervals (C.I.), which had a lot of overlap (Figure 2, Figure 3).

As shown in Figure 2, the mean cell density of treatment at 0 Lux stayed fairly constant, without too much variation, and was significantly different from the other treatment groups after 144 hours. Overall, the control group had a higher cell density than all of the other treatment groups, but did not show a statistically significant difference. The 5700 Lux and 3180 Lux treatments showed the cell densities between the two groups closely matching the other; at some point after 72 hours, a trend for higher cell growth in the 3180 Lux treatment was seen (Figure 3).

Discussion

According to Figure 2, our data has large CI values, which show great overlap throughout and at the end of the experiment. Therefore we fail to reject that null hypothesis and as such cannot support the alternate hypothesis.

Considering this lack of statistical significance, one is forced to consider the trends of the data. From 144 to 192 hours (Figure 2), our data is consistent with McCombie's (1960) findings, as it shows that elimination of light negatively affects

population growth. Light is a limiting factor because it controls photosynthetic rate (McCombie 1960). As a mixotrophic organism, *C. reinhardtii* has the ability to use either light or acetone as a source of energy, being able to grow in the dark in the presence of acetone (Sager and Granick 1953). Since the medium in which the *C. reinhardtii* was grown was devoid of acetone, *C. reinhardtii* in the 0 Lux treatment was perhaps unable to perform necessary metabolic functions, hence the constant low cell density (Sager and Granick 1953). However, this light intensity is too extreme to be able to draw any conclusions on the intermediate light intensities.

There was no significant difference in cell density observed between the control (8020 Lux), and the treatments at 5700 and 3180 Lux (Figure 2). A similarity between the 5700 Lux and the control treatment was expected because both of these are considered to be above the saturation intensity of 5380 Lux (Miller *et al.* 2008). Therefore light intensity would no longer limit *C. reinhardtii* growth (Sorokin and Krauss 1958). This does not satisfactorily explain why there were no statistical differences between the 8020, 5700 and 3180 Lux treatments, as expected. This lack of differentiation could be due to the fact that Sorokin and Krauss (1958), and McCombie (1960), both used water baths at 25ºC, as well as cotton-batting to promote gas exchange. This temperature is 8ºC higher than our incubating temperature and therefore could also affect population growth (Fadaghi *et al.* 2011). Furthermore, our design did not include cotton; although this reduced the risks of contamination, it limited the potential for gas exchange.

At 72 hours, the data points were very close together. However, at the 144 hour mark, the 3180 Lux treatment had the largest cell density (Figure 3). This could have

been due to unobserved growth and declines in the population over the weekend, when we did not collect any data. Since the generation time of *C. reinhardtii* has been noticed to be as low as 5 hours (Harris 2001), it is possible that the population of the algae in the faster growing groups, 8020 and 5700 Lux treatments, grew exponentially during this period; if a population grows too fast, (e.g. exponentially), overexploitation of the resources can cause a sharp decline in the population before it levels off (Smith and Smith 2009). Delayed growth in the 3180 Lux group would show this peak (and slower decline) in the population later in the experiment.

One possible explanation for the statistical similarity between the three non-zero treatments, could be due to insufficient mixing of the treatment tubes before a sample was drawn. The *C. reinhardtii* strain that was studied is motile (Fadaghi *et al.* 2011) and therefore able to move to maximize resource availability (Harris 2009). This could lead to stratification in the substrate. If the test tube was insufficiently vortexed, the sample that was drawn would not be representative of the actual cell density. This misrepresentation would result in larger confidence intervals, which decreases statistical significance. Although the proper level of mixing in the control tubes could be determined visually, the experiment setup precludes visual examination of other samples and thus it is possible that insufficient mixing occurred.

Another possible explanation for our lack of statistically significant data is the fact that after the 168 hour mark, clustering was frequently observed at both the 8020 and 5700 Lux treatments, as well as occasionally observed at the 3180 Lux treatment. The clusters are also known as palmelloids (Lurling and Beekman 2006). Their formation is possibly used as a defense mechanism against herbivory (Lurling and Beekman 2006),

or as a result of salt concentrations as well as alkaline conditions (Iwasa and Murakami 1969). The experiment measured neither salt concentration nor pH. These factors would be good areas of further study. Palmelloids present a problem when attempting to obtain an accurate count of cell density. This is due to the fact that accurately counting the cells in an intact cluster is very difficult. To mitigate the necessity for this, the samples were vigorously mixed using a pipette before being mounted on the haemocytometer. This occasionally introduced small air bubbles into the sample, which could negatively affect the readings by mounting an inaccurate volume on the haemocytometer, and an inaccurate cell density calculation.

Conclusion

Through our results and observations, we were unable to reject our null hypothesis, as there was too much variance in the data. The 3180 Lux group showed the highest cell density of any group during the experiment, despite having a lower light intensity than the control. Though the aim of the experiment was to find the optimum growing light intensity, for *C. reinhardtii*, our results did not show any relevant trends.

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