

Effect of varying light intensity on cell abundance of *Chlamydomonas reinhardtii*

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Abstract

Chlamydomonas reinhardtii is a unicellular alga often used as a model organism in various studies involving cell cycle control. A number of factors are involved in the reproduction of these unicellular organisms, including light intensity. Previous research has demonstrated that light intensity influences the growth, abundance and metabolic processes of cells. This paper investigates the effect of light intensity on abundance of *C. reinhardtii*. Three groups of the organism with four replicates in each were exposed to three different intensities of light including 5000 Lux, 970 Lux and 0 Lux. The experimental setup was kept for 7 days, with alternating periods of 14 hours of light and 10 hours of dark. The abundance of *C. reinhardtii* was measured on the first, 3rd, 6th, and 8th day of the experiment (t=0, 2, 5, 7). The results indicated an initial decrease in abundance of all treatments. The 5000 Lux and 0 Lux treatments experienced an increase in abundance after t=2. The 970 Lux treatment group underwent an increase in abundance after t=5. The unexpected results obtained in t=5 could be due to changes in metabolic rates of the organism, sample contamination, mutation, and adaptation to changes in energy sources. There is also the possibility of *C. reinhardtii* undergoing photooxidative stress.

Introduction

Cell cycle control in various eukaryotic organisms is a primary focus in many studies worldwide. The studies' findings are extrapolated and used to answer fundamental questions regarding the reproduction of life. Various biotic and abiotic factors, such as light intensity can alter cell division in unicellular and multicellular organisms. Light intensity is responsible for influencing growth, metabolism, and other key elements of reproduction.

Chlamydomonas reinhardtii is a unicellular, green alga that is often used as a model organism in many experiments concentrating on cell cycle control. It is found worldwide in various environmental conditions such as oceans, soils and fresh bodies of water. Its main source of energy is photosynthesis, but it is able to use acetate as a carbon source in light scarce conditions (Johnson and Alric 2012). This allows it to be categorized as both a phototroph and a heterotroph. Cells of *C. reinhardtii* are haploid and consist of a cell wall, chloroplast, a pair of flagella for movement and an eyespot responsible for perceiving light. It reproduces both sexually and asexually due to being an ancestor to both animals and plants (*Chlamydomonas* connection 2011).

Previous studies have shown that green algae cells exposed to higher light intensity are larger prior to cell division (Sorokin and Krauss 1968). This is important, because unicellular algae need to reach a certain size to reproduce. A larger cell is more likely to divide because it synthesized all the necessary components that are needed for division. Light is also required for the cell to go past the G1 phase of the cell cycle, at which the cell grows in size and synthesizes necessary components before transitioning into another phase and progressing to mitotic division (Matsumura et al. 2010).

According to Spudich and Sager (1980), the mechanism behind *C. reinhardtii* reproduction lies in alternating light and dark periods. It is also known that *C. reinhardtii* can continue reproducing in complete darkness, provided the necessary nutrients such as organic carbon or nitrogen are accessible (*Chlamydomonas* connection 2011).

This study aims to investigate the differences in abundance under different light conditions, provided no other nutrients are involved. The importance of this research

lies beyond the value of light detection; it can also provide an idea of the cell's capabilities of alternating its metabolic processes and alternating sources of energy in order to survive in light scarce conditions.

This paper describes the effects of light intensity on the cell cycle of *C.reinhardtii*. The study focuses on whether increasing amounts of light causes an increase in abundance. The null hypothesis states that increasing light intensity decreases or has no effect on the abundance of *C. reinhardtii*. While the alternate hypothesis states that an increase in light intensity increases the abundance of *C. reinhardtii*.

Methods

A stock solution of *C. reinhardtii* in Sager and Granick media was obtained for this experiment (approximately 80mL). On day 1 we made observations on colour and clarity of the solution. Each member then micropipetted 100µL of the stock solution into a microcentrifuge tube, and then fixed the solution with 10µL IKI (Gram's Iodine solution). The stock solution was thoroughly mixed before each sample was taken for homogeneity. We then transferred 50µL of the fixed solution onto a haemocytometer slide for organism counts. We counted cells in each large corner square (each large square contains 1×10^{-4} mL), and the large center square of the haemocytometer. We then determined the cell concentration in the stock solution and obtained 3×10^5 cells/mL (starting cell concentration obtained from literature). We labeled 12 test tubes with A, B, or C treatments (3 treatments, 4 replicates of each) and transferred 6mL of stock solution into each test tube. Treatment A tubes were placed on a shaker at a light intensity of 5000 Lux. Treatment B tubes were placed on a shaker at 970 Lux, and

treatment C tubes were covered in black plastic and placed with treatment B (refer to Figure 1 and 2 for experimental setup). The experimental setup was kept in an incubator at 17^o C, on a light cycle of 14 hours of light and 10 hours of dark. On the third day of the experiment (t=2 days) each replicate from each treatment was first observed for clarity and colour and then sampled, fixed, and counted following the same procedure from day 1, and then placed back on the experimental setup. We then repeated this method on day 6 and 8 of the experiment (t=5 and 7, respectively). The experimental data was collected over 8 days. For statistical comparison we used 95% confidence intervals when analyzing our data.



Figure 1: Experimental setup during fixing and counting procedures in lab.



Figure 2: Experimental setup under separate light intensities in incubator. 5000 Lux samples on top shelf, 970 and 0 Lux on bottom shelf.

Results

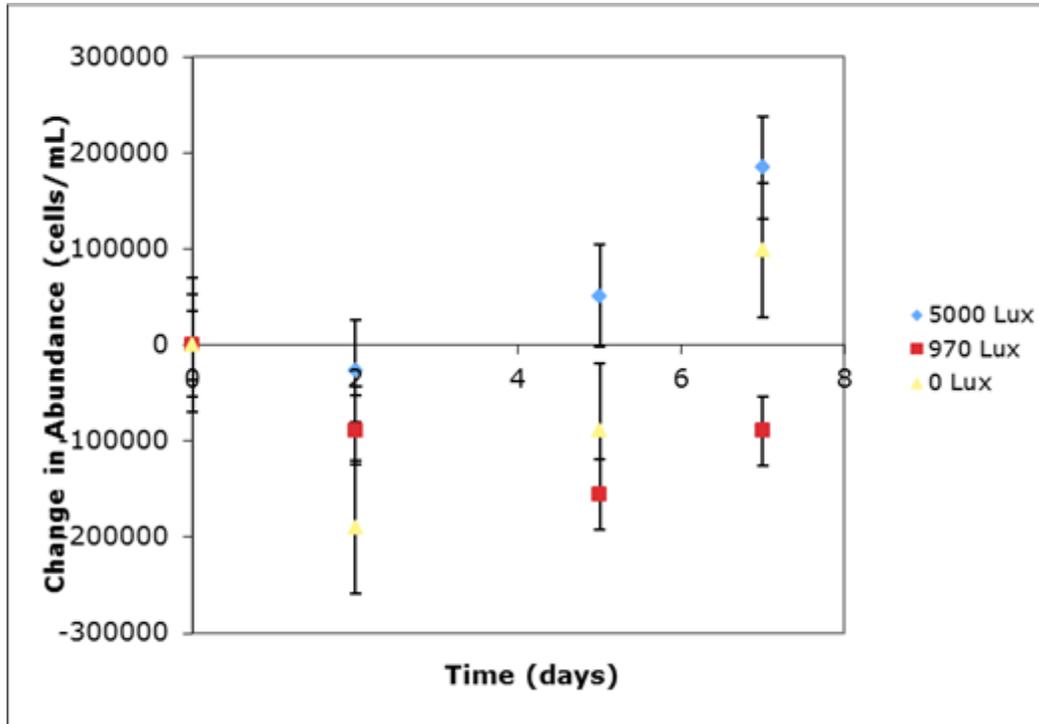


Figure 3: The change in the abundance of cells compared to time t=0. The three different trends depict the changes in abundance of the three separate treatments (at 5000, 970, and 0 Lux, respectively) over time. At t=2, and t=5, significant differences were found between the 5000 and 0 Lux treatments. At t=5, and t=7, the differences between 5000 and 970 Lux were also found to be significant. At t=7, there was a significant difference between the 970 and 0 Lux treatments and the 5000 and 970 Lux respectively. All treatments had an initial decrease in abundance, with the 5000 and 0 Lux treatments increasing in abundance steadily from t=2 to t=7.

We determined *Chlamydomonas* cell abundance by calculating the average cell number per large haemocytometer square, and then dividing by 1×10^{-4} mL/grid and multiplying by 1.1 to account for the addition of the IKI. We then obtained the change in abundance by subtracting each measurement of abundance at each treatment level on each day, by the abundance of cells at t=0 (i.e., abundance at t=5 for treatment of 5000 Lux = 361625 cells/mL - starting abundance of 310200 cells/mL = change of +51425

cells/mL). 95% confidence intervals were calculated for at ± 53280 cells/mL for 5000 Lux, ± 36074 cells/mL for 970 Lux, and ± 69759 cells/mL at 0 Lux.

Formula for obtaining Confidence Interval:

$$\begin{aligned} \text{Lower endpoint} &= \bar{X} - 1.96 \frac{\sigma}{\sqrt{n}}, \\ \text{Upper endpoint} &= \bar{X} + 1.96 \frac{\sigma}{\sqrt{n}}. \end{aligned}$$

where: \bar{X} =mean

σ =standard deviation

n =population size

Figure 3 shows that at $t=2$ only the 5000 Lux and 0 Lux treatments were significantly different from each other in growth, with the 5000 Lux having less of a decrease in overall change in abundance. At $t=5$ the 5000 Lux treatment was significantly different from both the 970 Lux treatment and the 0 Lux treatment; however, the 970 Lux and 0 Lux treatments were not significantly different from each other. At $t=7$ the 970 Lux treatment was significantly different from the other two treatments; however, the 5000 Lux and 0 Lux treatments were not significantly different from each other.

Qualitative observations that we noted throughout the experiment included both the 5000 Lux and 970 Lux exposed samples becoming greener over time; however, the 5000 Lux treatment samples were always greener and darker than the 970 Lux treatment (see figure 4). The 0 Lux treatment remained practically clear throughout the experiment (refer to figure 4 for colour change observed). We also observed that cells in the 0 Lux treatment over time lost (or didn't produce) their green colour, and were much smaller than the cells in the other two treatments.

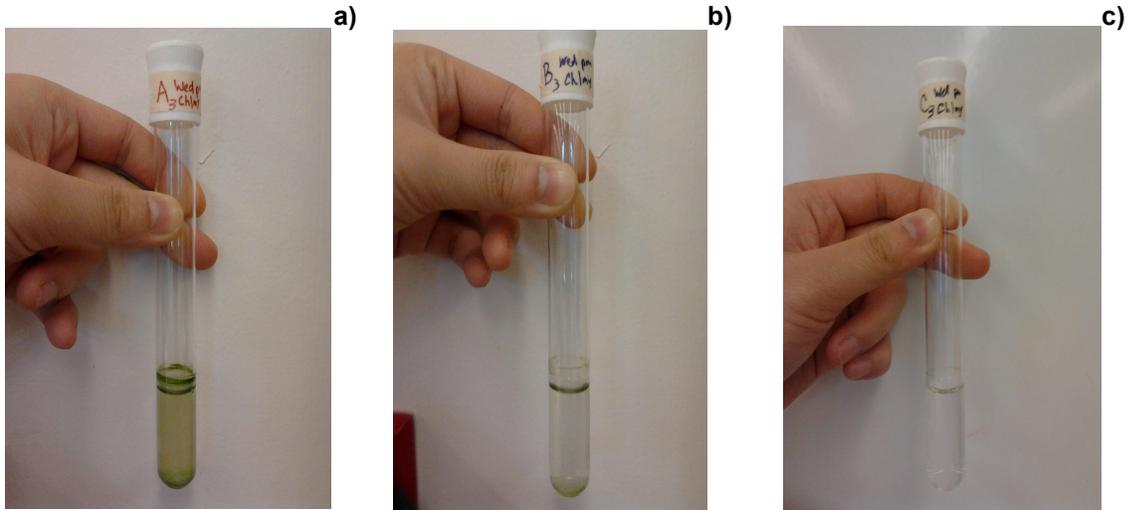


Figure 4: Colour of samples kept at the three different treatments: a) 5000 Lux, b) 970 Lux, and c) 0 Lux. Pictures were taken on the final day of the experiment.

Discussion

Based on our statistical analysis, we can reject our null hypothesis and support our alternative hypothesis; the confidence interval of treatment C (no light) on $t=7$ does not overlap with treatment B which was exposed to some light, but not in the highest intensity.

There was an initial decrease in abundance of all three treatments. The 5000 Lux treatment and 0 Lux treatment populations decreased to their lowest points at $t=2$, and then began increasing in abundance at a similar rate. The 970 Lux Treatment decreased in abundance until $t=5$ before increasing in abundance. Starting at $t=5$, all three treatment populations were increasing at an even more rapid rate. We may argue that the original stock culture could have been exposed to brighter light intensity, which then caused a decrease in all treatment populations until the populations adapted into dimmer light by changing their metabolic rate and mechanism.

Our data indicated a rapid increase in *C. reinhardtii* abundance under 0 Lux (treatment C) over seven days (from $t=0$ to $t=7$), but this contradicts the findings of the

existing literature, as treatment C should not have been viable under dark light intensity because the environment lacked both accessible carbon source and growth requirement to initiate reproductive phase (Harris 2001, Matsumura *et al.* 2010). Sodium acetate hydrate ($\text{NaOAc} \cdot 3\text{H}_2\text{O}$) is known to be the main carbon source of *C. reinhardtii* under light-deprived heterotrophic condition (Harris 2001). Other carbohydrates such as glucose and its derivatives are not well processed by the organism (Harris 2001). We did not add sodium acetate hydrate in our dark treatment, so *C. reinhardtii* cells in treatment C should not have had a carbon source to sustain themselves (Harris 2001). We may argue that citrate could have been taken up by *C. reinhardtii* as citrate is involved in the organism's heterotrophic mechanism (Johnson and Alric 2012). However, in treatment C, non-photosynthetic *C. reinhardtii* individuals cell cycles could be arrested if they are deprived of light energy at G_1 phase (see Figure 5) (Matsumura *et al.* 2010). Therefore, *C. reinhardtii* should have never reached the reproductive stage except the individuals who were in S or M phase of their cell cycles (Spudich and Sager 1980, Matsumura *et al.* 2010). Evidently, experiments using photosynthesis mutant *C. reinhardtii* report that dark treatment *C. reinhardtii* culture undergoes a drastic decrease in population within 196 hours due to prolonged G_1 phase of individual cells (Spudich and Sager 1980).

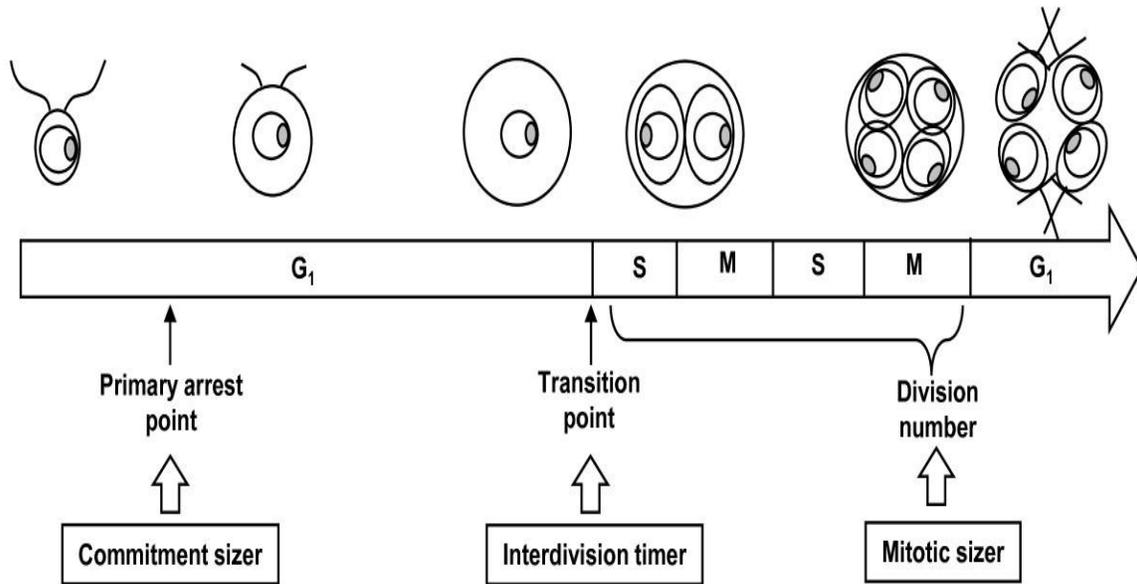


Figure 5: Cell cycle of *C. reinhardtii* in comparison to various life stages (gametes, individuals). At Primary arrest, the organism needs light and a specific cell size in order to proceed to transition point between G₁ and S phase, the borderline between growth and reproductive stage (Matsumura *et al.* 2010)

The cells observed in treatment C at t=7 were noticeably smaller and looked darker compared to the large and bright green *C. reinhardtii* cells observed in treatments A and B. There is some evidence that the cells we have observed in treatment C at t=7 are *C. reinhardtii* zygotes. *C. reinhardtii* zygotes lay down a thick, tough, multilayer wall along their cell wall in the first stage of their life cycle. The outer layer is dense, granular, and fibrous, which may explain why we saw smaller, less green, and darker cells in treatment C at t=7 (Vanwinkle-Swift *et al.* 1998). Furthermore, the individuals we have observed cannot be bacteria because unstained bacteria should not be visible under 100x magnification under compound microscope.

Should the dark treatment have been contaminated, the contaminant would likely have used citrate as its energy source under nonphotosynthetic conditions. The individuals observed in the dark treatment on t=7 appeared to be cocci in shape, the same as *C. reinhardtii* observed under 100x magnification. Since the cells in the dark

treatment consistently looked similar to another, we may assume the contaminant would have had outcompeted *C.reinhardtii*, but these cells appeared very similar to *C.reinhardtii* at first glance. We believe the contaminant would have been an organism capable of heterotrophic metabolism with citrate, which appears to be the only possible carbon source in Sager and Granick media (1953). Yet, the presence of the contaminant in the treatments is highly unlikely as we always used sterile technique when handling each treatment.

The unusual cell count pattern in our treatment replicates may have been due to procedural errors such as the method of mixing or counting errors. For example, each replicate test tube in t=7 was vortexed before the cell count unlike the other days whereas the experimenters mixed the replicates by tapping each test tube or pipetting. Vortexed replicates reduced the occurrence of cell clumps which could have critically affected the number of cells visible in each haemocytometer reading. Also, the cells in treatment A and B were noticeably bigger than those at Treatment C in t=7. This may have caused the observers to overlook clumped cells in treatment A and B in this experiment.

Another source of error may be due to the 0 Lux treatment being exposed to small amounts of light when we were extracting cells from the test tubes for counts. *Chlamydomonas* has evolved mechanisms that help it adapt to changes in irradiance, such as a competing species shading it from sun (Bonente *et al.* 2011). This adaptation may have allowed the cells to gain enough energy to grow and divide in treatment C.

For future studies on effect of light intensity, we recommend setting two dark treatments with and without sodium acetate; this will assure that there is no

contamination in the stock culture. We also recommend DNA analysis of the replicates grown for >200 hours in dark treatment since the stock may contain mutated individuals or species other than *C. reinhardtii*.

If the 0 Lux treatment were to be removed, we could see a trend with the 5000 and 970 Lux data respectively. The higher light intensity increases abundance, as the lower light intensity slowly decreases in abundance, which would have been the expected results.

Conclusion

The results indicated that the abundance of *C. reinhardtii* does increase with increasing light intensity, with 5000 Lux Treatment experiencing the highest increase in abundance. However, there was an increase in abundance of cells under 0 Lux treatment, which is contradictory to the expected results. The possible explanations of the results include *C. reinhardtii* switching to alternative energy source, sample contamination, mutation, or the organism undergoing a cyst stage. Possible future research can focus on determining how long *C. reinhardtii* can survive without light with and/or without other carbon source nutrients.

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Literature Cited

Bonente, G., Pippa, S., Castellano, S., Bassi, R., and Ballottari, M. 2011. Acclimation of *Chlamydomonas reinhardtii* to different growth irradiances. The Journal of Biological Chemistry, **287**(8): 5833-5847.

- Chlamydomonas* Connection. 2011. A gateway to resources for *Chlamydomonas* research. Available from <http://www.chlamy.org/info.html/> [accessed 13 November 2012].
- Harris, H. *Chlamydomonas* as a model organism. 2001. Annual Review of Plant Physiology and Plant Molecular Biology. **52**:363-406.
- Ishiura, M. 1976. Gametogenesis of *Chlamydomonas* in the dark. Plant Cell Physiology, **17**(6):1141-1150.
- Johnson, X., and Alric, J. Interaction between starch breakdown, acetate assimilation, and photosynthetic cyclic electron flow in *Chlamydomonas reinhardtii*. 2012. The Journal of Biological Chemistry. 2012. **287**(31):26445-26452.
- Matsumura, K., Yagi, T., Hattori, A., Soloviev, M., and Yasuda, K. Using single cell cultivation system for on-chip monitoring of the interdivision timer in *Chlamydomonas reinhardtii* cell cycle. 2010. Journal of Nanobiotechnology. **8**:23.
- Mcateer, M., Donnan, L., and John, P. 1985. The timing of division in *Chlamydomonas*. New Phytologist, **99**(1): 41-56.
- Muller, P., Li, X. P., and Niyogi, K. K. 2001. Non-photochemical quenching. A response to excess light energy. Plant Physiology, **125** (4): 1558-1566.
- Sager, R. and Granick, S. 1953. Nutritional studies with *Chlamydomonas reinhardtii*. Annual New York Academic Science. **56**: 831-838.
- Sorokin, C., and Krauss, W. B. 1958. The effects of light intensity on the growth rates of Green Algae. Plant Physiology, **33** (2): 109-113.
- Spudich, J., and Sager, R. 1980. Regulation of the *Chlamydomonas* cell cycle by light and dark. Journal of Cell Biology, **85**:136-145.
- VanWinkle-Swift, K., Baron, K., McNamara, A., Minke, P., Burrascano, C., and Maddock, J. 1998. The *Chlamydomonas* zygospore: mutant strains of *Chlamydomonas monoica* blocked in zygospore morphogenesis comprise 46 complementation groups. Genetics. **148**:131-137.