The effect of different wavelengths of light on the population growth of mutant and wild type *Chlamydomonas rheinhardtii*

Lisa E. Lee, Kelly C.Y. Liu, Natasha D.M. Parkinson, Qianhui Sun

Abstract:

Chlamydomonas rheinhardtii, a unicellular photosynthetic alga, has been previously studied to determine their reproductive rates at varying wavelengths of light. In this study, we proposed to shed light on the effect of different wavelengths of light including red (~620 nm), green (~520 nm) and blue (~450 nm) light on the population growth of *C. rheinhardtii* in a 12-day period, within a temperature controlled room. Cell counts were performed on Day 0, 4, 7 and 12 in order to determine the cell concentrations. There was no significant difference among populations under different wavelengths.

Introduction

Chlamydomonas rheinhardtii is a unicellular, photosynthetic green alga that has two anterior flagella for locomotion (Li *et al.* 2003). The organism has an elliptical shape with a diameter of 10 micrometers (Atwell *et al.* 1999). *C. rheinhardtii* undergoes autotrophic growth in sunlight by performing photosynthesis, and experiences heterotrophic growth in the dark. It is known that photosynthetic efficiency varies with different wavelengths of light even under the same light intensity (Brown 1969). Atwell *et al.* (1999) have found that algal species tend to grow more rapidly under red light than blue light, which is closely associated with the absorption spectrum of the photosynthetic pigment, Chlorophyll a.

The mutant strain is CC-3913-pf9-3 mt- with the lack of ability to assemble flagella, and the wild-type strain is CC-1690. This study would allow us to understand if lack of motility could have an effect on the population growth of *C. rheinhardtii* under different wavelengths of light by comparing the wild type and mutant. *C. rheinhardt*ii is wildly used in pharmaceutical research, and it can be modified to produce vaccines (Demurtas *et al.* 2013). Therefore, by

understanding the optimal population growth under different wavelengths of light, we can maximize the population of *C. rheinhardtii* in a research lab.

Our null and alternative hypotheses are:

H_o: Longer wavelengths of light would decrease or have no effect on the population growth of wild type and mutant *Chlamydomonas rheinhardtii.*

H_a: Longer wavelengths of light would increase the population growth of wild type and mutant *Chlamydomonas rheinhardtii.*

A previous study conducted by Münzner and Voigt (1992) showed that cell division of *C. rheinhardtii* would slow down in blue light and remain unaffected in red or far-red light. If cell division were delayed, we would expect the growth cycle to be longer and that the population would decrease at a later time.

Methods

Initial Setup - Upon receiving *Chlamydomonas rheinhardtii* wild type and mutant stock cultures, cell counts were performed to determine initial cell concentrations. This was done by taking 10 μL of the sample and performing a count with haemocytometer and compound light microscope. We then determined the cell concentrations using the following equation:

cell density (per mL) = (number of cells counted)/(grid volume) x corrective factor

(where grid volume = 9×10^{-4} mL if counting all 9 squares (all 9 squares were counted only if there were fewer than 30 cells in one square), otherwise grid volume = 1×10^{-4} mL if counting 1 square; correction factor = 1.1 to account for the added IKI fixative).

Once we acquired the initial concentrations for each culture, we diluted the samples to

1×10^4 cells/mL in order to ensure that we started the experiment with wild type and mutant samples at similar concentrations. We did this by mixing medium with the wild type sample, and the medium and the mutant sample into separate 250-mL flasks. From this dilution, we created 20 wild types samples and 20 mutant samples, each containing 5-mL of the culture and medium mix. The samples were each placed into 6-mL test tubes. Four test tubes from the wild type samples and four test tubes from the mutant samples were wrapped in one of the determined colour of acetate filters and black plastic (clear at 400-700 nm (all visible light colours), red at ~630-700 nm, green at ~550 nm, blue at ~470 nm, black at ~0 nm). There were four wild-type samples wrapped in clear; four wild type samples wrapped in red; four wild-type samples wrapped in green; four wild-type samples wrapped in blue; and four wild-type samples wrapped in black plastic. The same applied for the mutant samples. Once all the test tubes were wrapped, they were placed into test tube racks (spaced to minimize the amount of shadowing by other test tubes), and placed into a room held at a constant 17ºC, at light intensity 5550 LUX; as seen in Figure 1. The resulting light intensities were: clear 5550 LUX, red 140 LUX, green 180 LUX, blue 30 LUX, and black 0 LUX. The samples were allowed to sit, while occasionally being shaken by hand.

Figure 1. 40 test tubes wrapped in blue, green, red and clear acetate paper and black plastic under the same light intensity in 17 degree Celsius controlled room.

Day 0 - We removed 100µL of *C. rheinhardtii* wild type and mutant from respective test tubes with a micropipette and then fixed each sample with 10 µL of IKI. We determined initial cell concentration by performing a cell count, using haemocytometers and compound light microscopes (see Figure 2). Once extraction for the counts was completed, we placed the samples back into 17ºC controlled room, to ensure temperature remained as constant as possible.

Figure 2. The initial setup for cell count on Day 0.

Day 4 - *C. rheinhardtii* samples were mixed and then individually pipetted from their respective test tubes and counted. 100 µL of sample was removed via pipetting and added to 500µL centrifuge tube. 10 µL of IKI was then added to each mixing tube to act as a fixative, and we mixed the samples by pipetting up and down. A coverslip was placed over the haemocytometer, and 10 µL of fixed sample was pipetted onto the haemocytometer loading groove. Cell counts were done by counting cells from each replicate to get an average cell count for each light sample.

Day 7 and 12 - Day 4 procedures were repeated in order to determine a trend in cell growth over time.

For each replicate, we counted cell number using a haemocytometer. If less than 30 cells were counted in a square, we counted all 9 squares, which is equivalent to the volume of 3 *x* 3 *x* 0.1 mm grid. If more than 30 cells were counted in a square, we counted the 4 squares at the corner plus the middle square. Mean cell concentration was then determined by taking the mean cell count from the 4 replicates. We divided mean cell count by 9×10^{-4} mL if all 9 squares were counted. If 5 squares were counted, we took the cell number average and divided that number by 10^{-4} mL. We then multiplied these values by 1.1 IKI fixative correction factor. 95% confidence interval was then calculated from individual cell concentration for that replicate using the formula 95% CI = $1.96 \, x \, \frac{\sigma}{\sqrt{n}}$, where σ is standard deviation and n is population size. We compared confidence intervals for cell concentrations under each wavelength of light for wild type (Figure 3) and mutant (Figure 4).

Results:

Sample calculation using cell cultures under red light on Day 4:

Day 4	Red light – wild type
Replicate	Cell count
1	15
2	107
3	44
4	103

We calculated cell concentrations for each replicate. For replicate 1, cell concentration would be:

Cell concentration = $\frac{15}{9 \, x \, 10^{-4}}$ = 18333 cells/mL

We then calculated mean number of *Chlamydomonas rheinhardtii* cells. Rounding to the nearest whole cell:

Mean cell count = $\frac{15+107+44+103}{4}$ = 67 cells

We took the mean cell count and calculated mean cell concentration:

Mean cell concentration = $\frac{67}{9\,x\,10^{-4}}$ = 82194 cells/mL or 8.22 x 10^4 cells/mL.

From individual cell concentrations, we calculated standard deviation using $\sigma = \sqrt{\frac{\Sigma(x-\bar{x})^2}{N}}$, where x is each value in the population, $\bar{x}$ is mean of the values, and N is number of values (population).

$$\sigma = \sqrt{\frac{[(18333+130778+53778+125889)-82194]^2}{4}} = 55242.78$$

We could then calculate 95% confidence interval = $1.96\,x\,\frac{55242.79}{\sqrt{4}}$ = 174

Again, using scientific notation, 95% CI is expressed as 1.74E+02

Thus, wild type *C. rheindardtii* cell concentration on Day 4 would be 8.22E+04 ± 1.74E+02 cells/mL

We repeated same calculations for all wild type samples on Days 0, 7, and 12 for all 5 treatments. We repeated the same calculations for the *C. rheinhardtii* mutant.

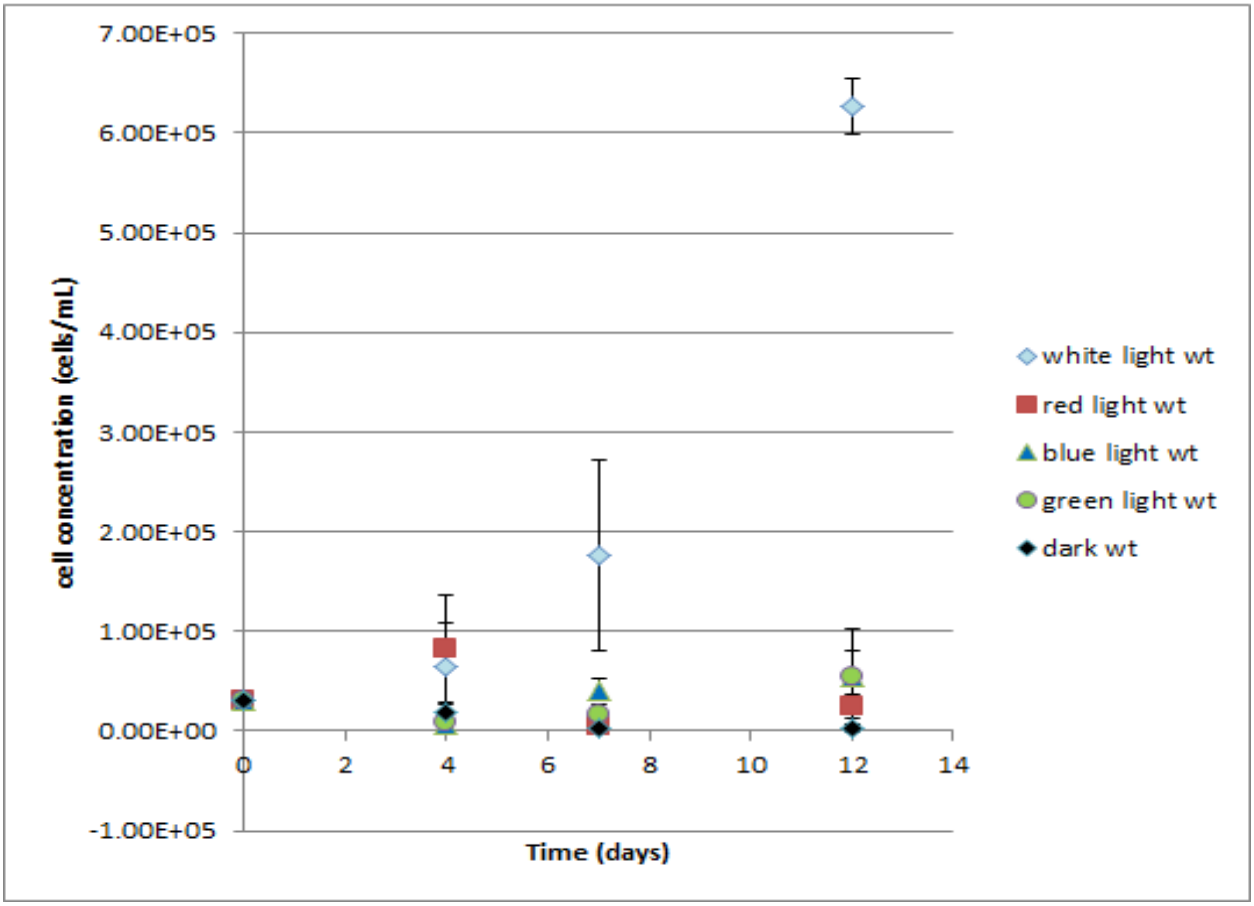

Figure 3. Average *Chlamydomonas rheinhardtii* cell concentration for wild type culture over 12 days, n = 4. Cell concentrations for cultures under white light (light control) were significantly higher on Days 7 and 12. There was no significant difference for cultures under red, blue, and green lights, and the dark control.

Figure 3 shows that we started the experiment on Day 0 with the same cell concentration of $3.12 \times 10^4 \pm 2.27 \times 10^3$ cells/mL for *C. rheinhardtii* wild type. Over the 12-day period, only cultures under clear light (light control) had significantly higher cell concentrations on Day 7 ($1.76 \times 10^5 \pm 9.63 \times 10^4$ cells/mL) and Day 12 ($6.27 \times 10^5 \pm 2.78 \times 10^4$ cells/mL), because there was no overlapping of confidence intervals with other samples on the same day. On the other hand, no significant difference was observed over the 12 days for cell concentrations under red, blue, and green light, and the dark control because confidence intervals overlapped on all the days that the data was collected.

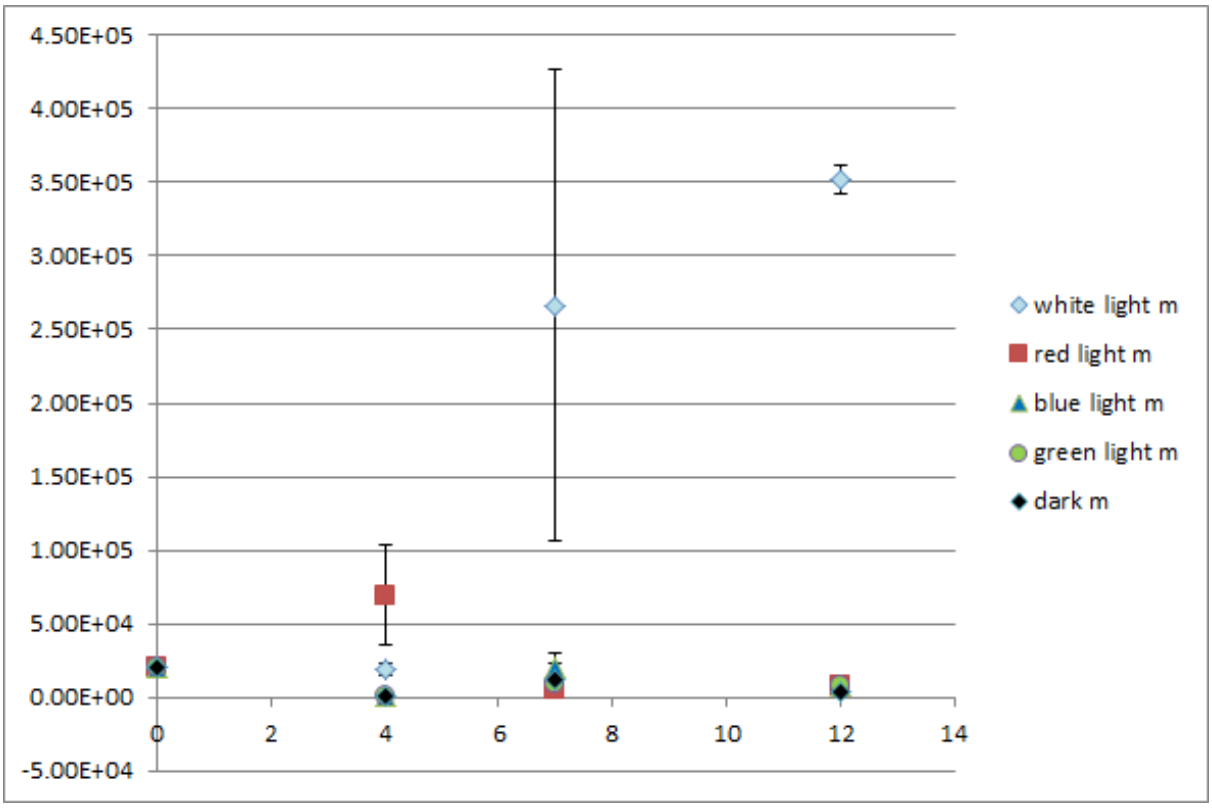

Figure 4. Average *Chlamydomonas rheinhardtii* cell concentration (cells/mL) for mutant culture over 12 days, n = 4. Cell concentration of mutant culture under red light was significantly higher on Day 4. Cell concentration under white light (light control) was significantly higher on Days 7 and 12. On Day 4, cultures under light control had cell concentration that was significantly lower than cultures under red light, and significantly higher than cultures under blue and green lights, and dark control.

Figure 4 shows that we started the experiment on Day 0 with constant cell concentration of $2.02 \times 10^4 \pm 7.36 \times 10^2$ cells/mL for *C. rheinhardtii* mutant. Cultures under white light (light control) exhibited significantly higher cell concentrations on Day 7 ($2.66\ 10^5 \pm 1.60\ 10^5$ cells/mL) and Day 12 ($3.52 \times 10^5 \pm 9.80 \times 10^3$ cells/mL) because there was no confidence interval overlap with other samples on those days. The mutant culture under red light had a cell concentration that was significantly higher on Day 4 ($6.9156 \times 10^4 \pm 3.39 \times 10^4$ cells/mL). In addition, on Day 4, cell concentration for cultures under light control ($1.8639 \times 10^4 \pm 4.19 \times 10^4$ cells/mL) was significantly lower than cultures under red light, and significantly higher than other cultures.

There was no significant difference over the 12 days for cell concentrations under blue and green lights, and the dark control.

Discussion:

In this study we failed to reject the null hypothesis, and failed to lend support to the alternate hypothesis. Our findings showed that longer wavelengths of light had no effect on the population growth of *Chlamydomonas rheinhardtii* wild type and mutant (Figures 3 and 4).

For wild type and mutant *C. rheinhardtii* under red light, there was an initial peak in cell concentrations on Day 4, and decreased cell concentrations on Day 7 and Day 12 (see Figures 3 and 4). *C. rheinhardtii* mutant population was significantly higher on Day 4; however, this observation was not important to the purpose of our study. The overall trends we observed for wild type and mutant were inconsistent with the studies by Kuwahara *et al.* (2011). Kuwahara *et al.* (2011) found that *C. rheinhardtii* cells grew and divided under 655 nm or 680 nm red light. However, we assumed that *C. rheinhardtii* grown under red light may have experienced photoinhibition after Day 4, where light-harvesting pigments in the cell absorbed more light energy than that can be utilized for carbon fixation (Elrad 2002). The excess excitation energy generated reactive oxygen species that could potentially damage cellular components and cause possible cell deaths (Elrad 2002). Therefore, excess light energy must be dissipated in order to prevent serious damage to photosynthetic apparatus such as photosystem II S protein (Elrad 2002).

C. rheinhardtii wild type under blue light had an increase in cell concentrations over 12 days (Figure 4). The change was statistically not significant due to overlapping confidence intervals with cultures under red and green lights, and dark control (Figure 4). Huang and Beck (2003) observed *C. rheinhardtii* population growth under blue light due to the presence of the

cryptochrome gene (*CPH1*) and the phototropin gene (*Phot*), which act as blue light photoreceptors. Beel *et al.* (2002) also found that animal-like cryptochrome (aCRY) in *C. rheinhardtii* could alter cell cycle control and allow cultures to maintain a stable population under blue light exposure. Munzer and Voigt (1992) further reported that application of blue light aided *C. rheinhardtii* respiration. Figure 3 showed an initial decrease in cell concentration on Day 4, which could be explained by delayed cell division and cell inactivity during lag phase that were typically observed in *C. rheinhardtii* under blue light (Munzer and Voigt 1992).

C. rheinhardtii wild type under green light increased in cell concentration from Day 4 to Day 12 (Figure 3). Brown (1969) found *C. rheinhardtii* to have lowest growth constant under green light in comparison to red, white, and blue light due to lower photosynthetic rate. However, the data collected from our study has failed to support this.

C. rheinhardtii wild type and mutant under white light (light control) showed significant growth on Day 7 and Day 12. Brown (1969) found that *C. rheinhardtii* experienced higher photosynthetic activity under white light, and that organic carbons dissolved more easily. In addition, Spudich and Sager (1980) showed that the *C. rheinhardtii* cell cycle is light dependent at primary arrest (A) point in early G1 phase in mitosis. Primary arrest point is critical for population regulation (Spudich and Sager 1980).

C. rheinhardtii wild type under no light (dark control) decreased in cell concentration over the 12 days (Figure 3). The observation was consistent with Lewin (1956), who observed that cells grown under dark condition would experience decrease in population. Thompson *et al.* (1985) further suggested that cells would swell and disintegrate when they were grown in the dark; however, such character was not observed in our study.

There were some limitations in our study. While performing cell counts, we had to account for the presence of dust particles on our slide samples. The dust particles varied in size and shape. While some were much larger, rectangular and colored green and brown, some had very similar color, size and shape as *C. rheinhardtii* cells. They were green, slightly larger than *C. rheinhardtii* cells, and had marginally deformed circular shape. We had tried our best to only count cells that were green and circular, but sometimes it was difficult to differentiate between *C. rheinhardtii* and dust particles when they were extremely small in size and were at close proximity.

In addition, even though the light intensity in the 17ºC incubator was controlled at 5550 LUX, the test tubes may not have been perfectly lined up under the fluorescent light bulb above. This may cause uneven light distribution amongst our samples, in which one sample may have access to more light than the other.

Also, the different acetate filters did not have corresponding light intensities. It would be expected that the red filter would have a higher light intensity than the green filter; however, in our study, the red light had an intensity of 140 LUX, whereas, the green light had a light intensity of 180 LUX. This difference in light intensity would be different from the real world setting of the water column that *C. rheinhardtii* would actually experience, in that red light would be more intense than green light.

Furthermore, *C. rheinhardtii* may not be distributed evenly throughout the entire solution in the test tube even though we have mixed the samples by pipetting up and down prior to taking them out for analysis. This may explain why there was some variation in our cell counts. In the future, more replicates would be useful in order to reduce variation in the data.

Conclusion:

Based on the results from our experiment, we have failed to the reject null hypothesis that longer wavelengths of light would decrease or have no effect on population growth for *Chlamydomonas rheinhardtii* wild type and mutant. Nonetheless, there was a trend that cells had higher population growth under white light.

Acknowledgements:

We would like to sincerely thank Dr. Carol Pollock (Department of Zoology, University of British Columbia) for her time and expertise in research using our model organism, *Chlamydomonas rheinhardtii*. Further gratitude goes to Katelyn Tovey and Mindy Chow for providing us with the necessary materials as well as important feedback, advice and constructive criticisms throughout our experiment.

Literature cited:

Atwell, B. J., Kriedemann, P.E., Turnbull, C.GN. 1999. Plants in action: adaption in nature, performance in cultivation. Macmillan Education, Australia.

Beck C.F. and Acker A. 1992. Gametic differentiation of *Chlamydomonas reinhardtii* – control by nitrogen and light. Plant Physiology, **98** (3):822-826.

Beel, B., Prager, K., Spexard, M., Sasso, S., Weiss, D., Muller, N., Heinnickel, M., Dewez, D., Ikoma, D., Grossman, A. R., Kottke, T., and Mittag, M. 2002. A flavin binding cryptochrome photoreceptor responds to both blue and red light in *Chlamydomonas reinhardtii*. Plant Cell, **24** (7): 2992-3008.

Brown, T. J. 1969. The effect of light quality on growth, photosynthetic and excretion rates, and the carbon metabolism of *Chlamydomonas rheinhardtii* dangeard. Simon Fraser University.

Demurtas, O. C., Massa, S., Ferrante, P., Venuti, A., Franconi, R., and Giuliano, G. 2013. A *Chlamydomonas*-derived human papillomavirus 16 E7 vaccine induces specific tumor protection. PLoS One, **8** (4): 1-9.

Elrad, D. 2002. Photoprotection and light harvesting in *Chlamydomonas reinhardtii*. UMI Dissertations Publishing.

Huang, K. and Beck, C. F. 2003. Phototropin is the blue-light receptor that controls multiple steps in the sexual life cycle of the green alga *Chlamydomonas reinhardtii*. PNAS, **100** (10): 6269-6274.

Kuwahara, S. S., Cuello, J. L., Myhre, G., and Pau, S. 2011. Growth of the green algae *Chlamydomonas reinhardtii* under red and blue lasers. Elsevier, **49** (3): 434-438.

Lewin, R. A. 1956. Control of sexual activity in *Chlamydomonas* by light. Microbiology, **15** (1): 170-185.

Li, J. B., Lin, S., Jia, H., Wu, H., Roe, B. A., Kulp, D., Stormo, G. D., and Dutcher, S. K. 2003. Analysis of *Chlamydomonas reinhardtii* genome structure using large-scale sequencing of regions on linkage groups 1 and 3. Journal of Eukaryotic Microbiology, **50** (3): 145-155.

Munzner and Voigt. 1992. Blue light regulation of cell division in *Chlamydomonas reinhardtii*. Plant Physiology, **99**: 1370-1375.

Spudich, J. L. and Sager, R. 1980. Regulation of the *Chlamydomonas* cell cycle by light and dark. Journal of Cell Biology, **85** (1): 136-145.

Thompson, R. J., Davies, J. P., and Mosig, G. 1985. 'Dark-lethality' of certain *Chlamydomonas reinhardtii* strains is prevented by dim blue light. Plant Physiology, **79**: 903-907.