Effect of Varying Light Intensity on the Photosynthetic Rate of Wild Type and Mutant *Chlamydomonas reinhardtii*

De Luca, L.,Gibson, A., Luo, R.

Abstract

Chlamydomonas reinhardtii is a species of photosynthetic green alga that has many different mutant strains that are readily available for experimental study. The purpose of our study was to measure the amount of oxygen produced by the wild type *C. reinhardtii* and by the immobile mutant strain CC-3913-*pf9*-3, during photosynthesis at different light intensities. Three replicates of both wild type and mutant cells were placed at 0-watt, 60-watt, 100-watt, and 150-watt treatment levels for one hour and oxygen levels were measured before and after the replicates were exposed to the different light levels. The wild type trials showed a statistically significant difference in oxygen production between all four treatment levels (0-watt 5.57 +/- 0.06 mg/L of 0_2, 60-watt 6.40 +/- 0.10 mg/L of 0_2, 100-watt 6.83 +/- 0.06 mg/L of 0_2, 150-watt 7.00 +/- 0.00 mg/L 0_2), the mutants also showed a statistically significant difference between the treatments (0-watt 5.60 +/- 0.00mg/L of 0_2, 60-watt 6.43 +/- 0.06 mg/L of 0_2, 100-watt 6.9 +/- 0.1 mg/L of 0_2, 150-watt 6.87 +/- 0.15 mg/L of 0_2). However, when the results from both the wild type and mutant trials were compared there was no significant difference in the data. From this data we were able to conclude that the rate of photosynthesis increased as light intensity increased.

Introduction

Chlamydomonas reinhardtii is a unicellular, photosynthetic, green alga, having a diameter of approximately 10 micrometers. Some distinct features are the presence of two flagella on the posterior end of the cell, and organelles such as the chloroplast, equipped with a pyrenoid, that allows photosynthesis to occur. A cell wall surrounds these organelles and the nucleus. A single eyespot is present on the surface of the cell, which is used to sense light. In the wild type organism, the two long, whip-like flagella are fully motile, displaying asymmetric and rhythmical bending patterns that aid primarily in movement (Harris 2001). However, the mutant type (CC-3913-*pf9*-3 strain) possesses non-motile flagella, due to genetic defects in the central-pair microtubule structure of the supporting structure of the flagella (REF).

Light is a critical component for photosynthesis. The amount of oxygen produced by *C. reinhardtii* can be used as a reference to its sensitivity towards different light intensities. This is an important area of study because it helps us understand how *C. reinhardtii* behaves in larger blooms in the oceans; this is especially important when looking at the mutant. Increasing the light intensity accelerates the growth rate of *C. reinhardtii* until the maximum capacity is reached at 5380 lux. In addition, *C. reinhardtii* can survive in the dark but it can only cover its loss above 646 lux (Sorokin and Krauss 1958). It has been shown that *C. reinhardtii* can grow at within the range of 0 lux to 107639 lux. In our experiment, we used 17000 lux. In the flagella of mutant *C. reinhardtii*, slower movement, smaller shear amplitude for bending pattern, and slower beat frequency were discovered due to unique mutations in proteins, such as dynein (Brokaw and Kamiya 1987). Currently, there are no articles which have investigated how this mutant flagella affects the photosynthetic rate of *C. reinhardtii*. Therefore, the objective of our experiment is to demonstrate not only the relationship between light intensity and photosynthetic rate of both wild and mutant type *C. reinhardtii*, but also the difference between the photosynthetic rates of wild type and mutant.

Ha1: Increasing light intensity increases the photosynthetic rate of wild type and mutant *C. reinhardtii.*

Ho1: Increasing light intensity decreases or has no effect on the photosynthetic rate of wild type and mutant *C. reinhardtii.*

Ha2: Increasing light intensity has a greater effect on the photosynthetic rate of wild type *C. reinhardtii* than that of mutant *C. reinhardtii*.

Ho2: Increasing light intensity has the same or a decreased effect on the photosynthetic rate of wild type *C. reinhardtii* than that of mutant type *C. reinhardtii*.

Methods

For our experiment, we obtained both wild type and mutant *C. reinhardtii* strain CC-3913-*pf9*-3. The media we used was similar to that of Sager and Granick (Sager and Granick 1953). *C. reinhardtii* organisms were kept in the dark with no exposure to light, prior to the start of our experiment. We used 10 µL of 99% cells of each organism type and determined cell count using a haemocytometer and a compound microscope. We then diluted the wild type and mutant organisms to a final concentration of 250,000 cells/mL using media. When organisms were not in use, we kept them covered under large Styrofoam containers, as to prevent any excess exposure to light.

To test different light intensities on each organism type, we chose four different treatments, each characterized by a fluorescent lamp with varying bulb wattages. There was a control treatment where organisms were exposed to no light. In the other three treatments, organisms were placed under lamps with bulbs of 60 watts, 100 watts, and 150 watts, respectively. Light intensity was measured for each treatment level using a light meter. . Three replicates of wild type and three of mutant *C. reinhardtii* were placed in each treatment. Four additional containers were used as procedural controls; these were prepared with no *C. reinhardtii* cells, and only contained media solution. One of these was placed under each of the four treatments.

Each replicate was contained in a 27mL plastic, transparent vial and sealed with a lid. For the control treatment (no exposure to light), we used tin foil to completely cover the vials. To prepare each replicate, we transferred the diluted organism and media solution using sterile graduated cylinders into the vials, and filled them to about a centimetre below the brim. Before

we placed the vials under in the treatment, we measured the initial oxygen level in each of the replicates using an oxygen meter and recorded the value. Initial temperatures of the replicates were also measured using a thermometer and recorded. We topped off each vial with additional solution, filling them to the brim leaving no room for air, and sealed them with plastic lids.

Once the replicates were prepared, we placed them under their respective lamps for a duration of one hour (See Figure 1). Start times were recorded to ensure each replicate received a full hour under its treatment level. After this time, we removed the replicates from under the lamps. We immediately recorded final oxygen levels with the oxygen meter, and recorded these values. Final temperatures were also recorded to see if any change had occurred. We analysed our data by calculating means and 95% confidence intervals for each organism type under each treatment level. We represented our data in scatter plots of light intensity (in watts) versus photosynthetic oxygen levels (in mg/L) and used error bars.

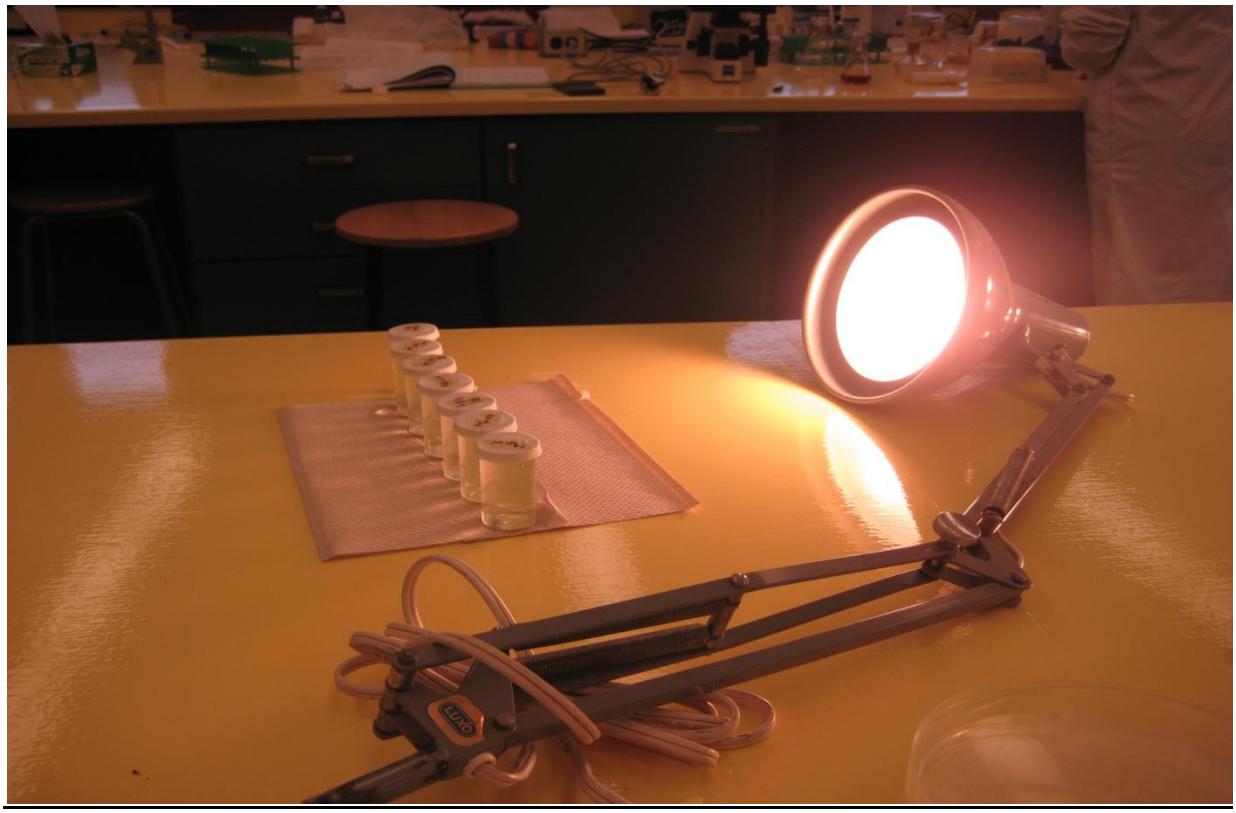

Figure 1. **Experimental set up**. Replicates are placed under fluorescent lamp for duration of one hour.

Results

The temperatures of samples exposed to 150-watt light bulb were measured to be 31 (^{o}C) while others were all constant at 24 (^{o}C). After being static for a period of time, dark green dots gathered at the center of the vials containing cells. In our experiment, all samples started with the similar oxygen concentration (5.73 mg/L-6.00 mg/L) in the trials, only the oxygen concentration in the no-light treatments had decreased after the final measurements. For our procedural control, which contained no cells, the oxygen levels stayed constant at 4.5 mg/L before and after the treatment. Figure 2 shows that oxygen levels had a statistically significant increase as light intensity became higher. The photosynthetic rate was highest for wild-type *C.*

reinhardtii in the 150-watt(17,020 lux) treatment (7.00 +/- 0.00 mg/L) when compared to those in the 100-watt(5,450 lux), 60-watt(3,800 lux), and 0-watt(0 lux) treatments (6.83 +/- 0.06 mg/L, 6.40+/-0.10 mg/L, 5.57 +/- 0.06 mg/L).

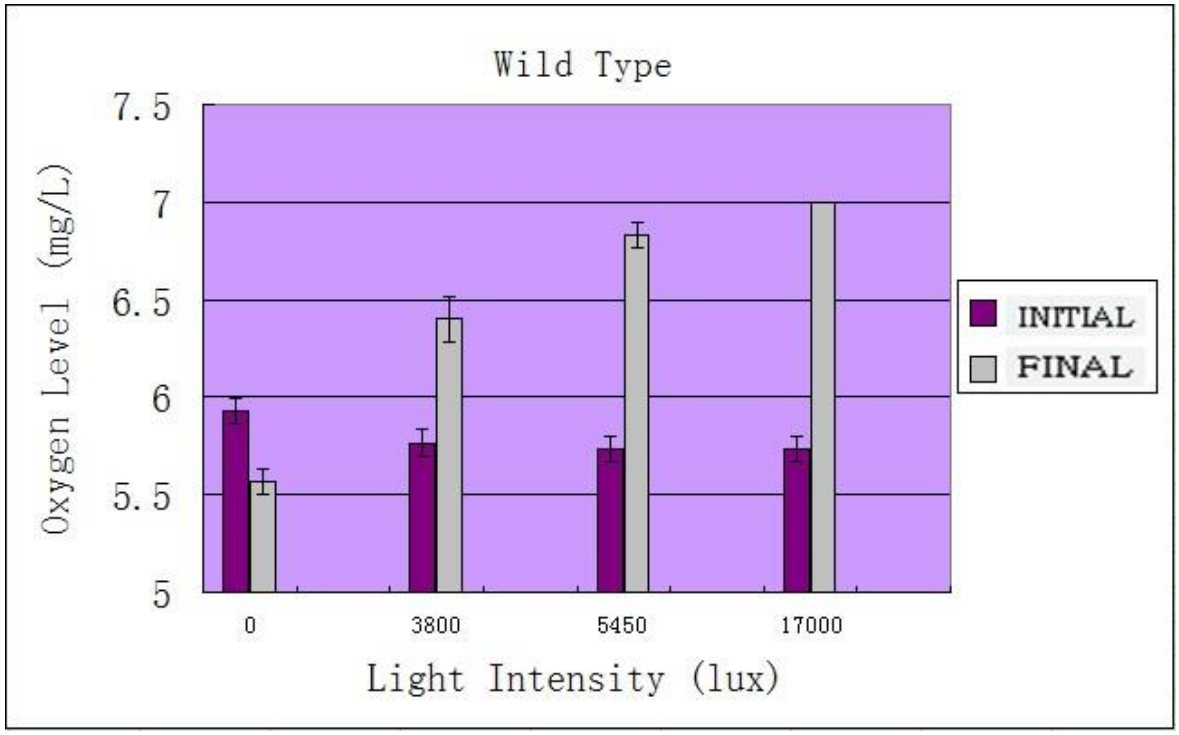

Figure 2. Mean O_2 concentration (mg/L) of wild type *C. reinhardtii* before and after various light intensity treatments (0 watts, 60 watts, 100 watts and 150 watts) in an hour. n= 3 per treatment group.

Figure 3 shows that when oxygen levels in the 0-watt, 60-watt, and 100-watt treatments were compared, their confidence intervals did not overlap with each other. However, when 100-watt and 150-watt treatments were compared their confidence intervals did overlap. Therefore, the photosynthesis rate was significantly higher for mutant *C. reinhardtii* in the 150-watt(17,020) and 100-watt(5,450 lux) treatments (6.87 +/- 0.15 mg/L, 6.9 +/- 0.10 mg/L) compared to those in the 60-watt(3,800 lux), and 0-watt(o lux) treatments (6.43+/-0.06 mg/L,

5.6 +/- 0.00 mg/L). In Figure 4 it is evident that the oxygen levels produced by photosynthesis did not show a statistically significant difference between wild type and mutant type for any of the treatments.

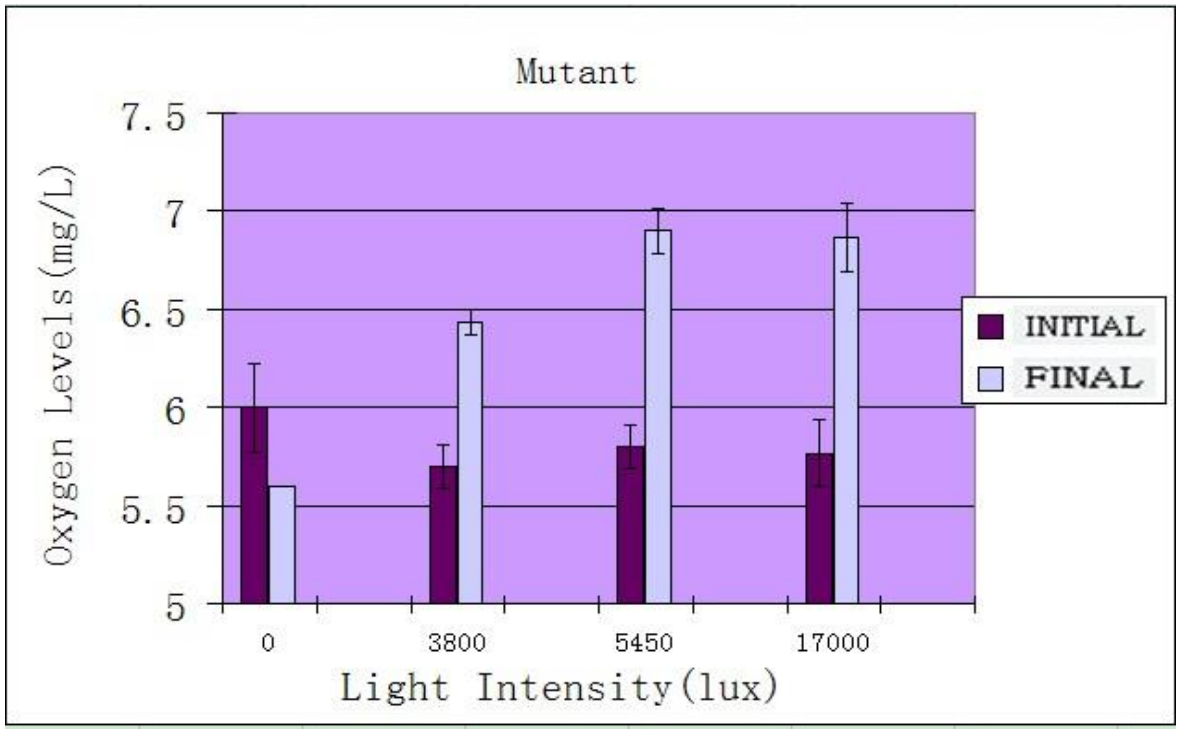

Figure 3. Mean O_2 concentration (mg/L) of mutant type *C. reinhardtii* before and after various light intensity treatments (0 watts, 60 watts, 100 watts and 150 watts) in an hour. n= 3 per treatment group.

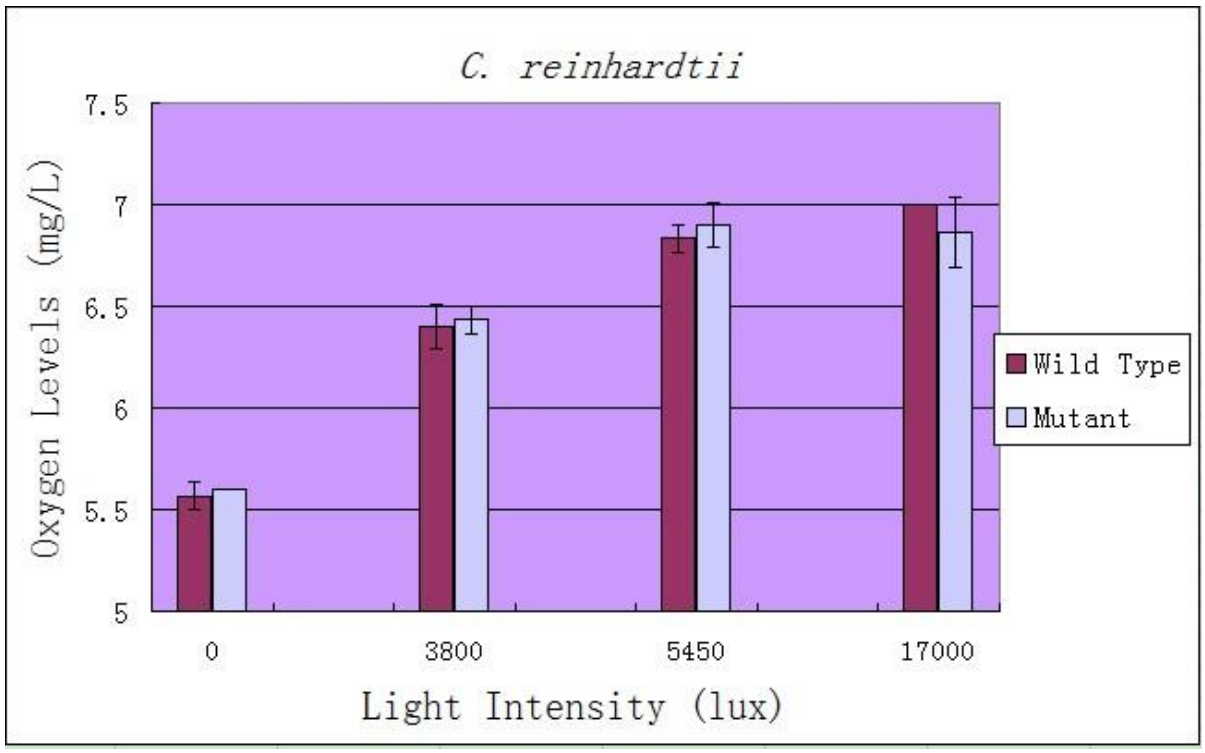

Figure 4. Mean Final O_2 concentration (mg/L) of wild and mutant *C. reinhardtii* after various light intensity treatments (0 watts, 60 watts, 100 watts and 150 watts) in an hour. n= 3 per treatment group.

Sample Calculation for 0 watt in Wild Type:

Mean = (5.6+5.6+5.5)/3= 5.57

Standard deviation: ((5.6-5.57)^2+(5.6-5.57)^2+(5.5)^2)/3 = 0.057735027

95% confidence interval value: 1.96*(standard deviation/ square root of n)

=1.96*(0.057735027/(3)^(1/2))= 0.065333333

Discussion

The trend shown by our results showed a significant difference between treatment levels in both the mutant and wild type *C. reinhardtii*. However, when the results from the wild type experiment were compared to those of the mutant type experiment there was no significant difference. Based on our statistical analysis, our results caused us to reject Ho1 and and resulted in our failure to reject Ho2. As the light intensity increases, the number of photons that are available for photosynthesis also increase. At a certain light intensity a cell's chloroplast reaches its saturation point and can no longer increase the rate of photosynthesis (Sorokin *et al*. 1958). Our results clearly show the trend described by Sorokin *et al*. 1958, although our particular experiment did not provide enough data for us to determine the light intensity at which photosynthesis was at its maximum.

The results that are shown when both experiments were compared to each other can be explained by a few factors. The particular mutant strain used was immobile and did not contain any mutation that directly pertained to photosynthesis. It was our thought that this mutation would inhibit the mutant's ability to move towards the light source, which would lead to a decrease in photosynthesis compared to the wild type. However, this was not the trend that was observed in our results. This could be attributed to the fact that our experiment was performed in such a limited space that the mobility of the mutant strain did not affect the amount of light it received. To further investigate this, future experiments should be performed in larger containers to better investigate if the immobility of the mutant strain would have any effect on its ability to photosynthesize under different experimental conditions. This would

provide more relatable data to real world conditions in the open ocean and could possibly provide some insight to if mutant *C. reinhardtii* are able to survive without the ability to escape shade produced by large algae blooms.

In our experiment we controlled for the amount of cells in each replicate by means of diluting both cultures down to approximately 250,000 cells per mL in both the mutant and wild type cultures. The solutions were fully and continuously mixed to ensure equal amounts of cells were transferred each time. We were not, however, able to control for temperature in all of our treatment levels. The temperature held steady in all of the treatment levels except at the 150-watt level. The temperature at this level jumped from 24 degrees Celsius at the beginning of the experiment to 31 degrees Celsius after the final reading. This could have caused the observed increase in oxygen concentration at the 150-watt treatment level. It was shown in the experiment by Sorokin *et al.* (1958) that with some species of green algae, an increase in temperature to 39 degrees can produce an increase in photosynthesis by as much as 300% when compared to trials done at 25 degrees under the same light intensity. The light intensity at 150 watts was measured to be approximately 19,000 lux, which is approximately equal to the measured light intensity at which photosynthesis was at its maximum point in Sorokin's experiment. This increase in temperature could have affected our results, but as far as we can conclude from comparing our results to those of Sorokin *et al.* (1958), the increase in photosynthesis should be attributed to the increase in light intensity and not the increase in temperature. To further explore this, further data needs to be collected from an experiment in which trials are done at different temperatures and under the same light intensity.

One of the treatments in our experiment was done under low light conditions and resulted in a decreased amount of oxygen in solution from initial to final readings. This can be explained by the fact that when the cells are not receiving adequate light they are producing enough oxygen to offset the amount they are using up through respiration, and the light level where *C. reinhardtii* is producing more oxygen than it is using has been measured by Sorokin *et al.* (1958) to be at approximately 600 lux. The low light treatment level was completely covered by tinfoil as to not allow the cells to receive any light. This explains the results that showed a decreased level of oxygen from start to finish under low light conditions. In our experiment we also performed procedural controls to determine if the fluctuations in temperature and light intensity cause any natural changes in the measured oxygen concentration. The procedural controls were placed at each treatment level and contained only the growth media with no cells present; the measured oxygen concentrations did not differ at all between the initial and final measurements, so it can be concluded that the different treatments levels did not cause any changes in the oxygen level. Therefore all of the final results can be directly attributed to cells that were present in all of the trials.

Conclusion

The purpose of this experiment was to investigate the effect of varying light intensity on the oxygen production of both wild type and mutant type *C. reinhardtii*. Based on our results, we provide support for our alternate hypotheses (Ha_1), that increasing light intensity increases the oxygen production of both wild type and mutant type organisms. We therefore reject our null hypotheses, Ho_1. We were unable to reject our second null hypothesis Ho_2, in comparing wild type to mutant type organisms, due to a lack of significant difference in our results.

Acknowledgments

We would like to extend our deepest gratitude to the following people, for not only providing us with the proper tools and instructions to make our experiment a success, but for offering endless support and making the process an enjoyable one.

To Niki Holden and Diana Rennison, for always offering assistance, even when unasked, and for their wise words of reassurance. To Mindy Chow, for providing us with excellent organisms, equipment, and expert advice to make our experiment seamless. To Carol Pollock, for her support, patience, and years of knowledge, and for helping us develop the best possible experiment.

We would also like to thank UBC for providing us with exceptional facilities, and also with the opportunity to take Biology 342.

Literature Cited

Brokaw, C.J., and Kamiya, R. 1987. Bending patterns of *Chlamydomonas* flagella: IV. Mutants with defects in inner and outer dynein arms indicate differences in dynein arm function. Cytoskeleton, **8**(1): 68-75.

Harris, E.H. 2001. *Chlamydomonas* as a model organism. Annual Review of Plant Physiology and Plant Molelcular Biololgy, **52**: 363-406.

Melis, A., and Neal, P.J. 1986. Algal photosynthetic membrane complexes and the photosynthesis-irradiance curve: a comparison of light-adaptation responses in *Chlamydomonas reinhardtii* (chlorophyta). Journal of Phycology, **22**: 531-538.

Sager, R., and Granick, S. 1953. Annals of the New York Academy of Science*,* **56**: 831-838

Sorokin, C., Krauss, R. W. 1958. The effects of light intensity on the growth rates of green algae [online]. Plant Physiology*,* **33**(2): 109-113.