The effect of temperature on the photosynthetic rate of wild-type and mutant C*hlamydomonas reinhardtii*

Maria Bui, Ie-Re Choi, Louisa Hadley, Holland Stuart, Jennifer Wang

Abstract:

The effect of temperature on the photosynthetic rate of *Chlamydomonas reinhardtii* wild type (CC-1690 - wild type mt+ 21 gr) and mutant (CC-3913 - pf9-3-mt-) was studied in this experiment by the measurement of oxygen production under treatments of 15°C, 22°C, and 27°C. We predicted that the mutant would produce less oxygen than the wild type due to the lack of flagella, and that decreasing temperature would decrease the photosynthetic rate due a decrease in kinetic energy (Lambers *et al*. 2008, Petrucci *et al.* 2011). Our results showed that the wild type produced significantly more oxygen than the mutant for the 15°C and 27°C treatments (0.27mg/L and 1.24 mg/L more oxygen, respectively), allowing us to reject Ho_1 for these treatments. We also found that decreasing temperature decreases oxygen production of the wild type with significant differences in the oxygen production between the 15°C (0.27±0.1mg/L) and 22°C (1.2±0.2mg/L) treatments, as well as between the 15°C (0.27±0.1mg/L) and 27°C (1.6±0.4mg/L) treatments. This trend, however, is not consistent with the results of the mutant; therefore, we were failed to reject Ho_2. The mutant's oxygen production peaked at 22°C with a production of 0.84±0.1mg/L, and then dropped at 27°C to a production of 0.36±0.1mg/L. These results suggest that mutant strain CC-3913 - pf9-3-mt- may be a temperature-sensitive mutant, and likely has reduced RuBisCO carboxylase activity at certain temperatures (Spreitzer *et al.* 1988).

Introduction:

Chlamydomonas reinhardtii is a photosynthetic unicellular eukaryotic alga that lives in fresh water ecosystems (Harris 2001). It has a diameter of approximately 50 micrometers and an eyespot that mediates light perception and helps in finding optimal light conditions for photosynthesis (Boyd 2011). The optimal temperature for photosynthesis of wild type *C. reinhardtii* is 28°C (McCombie 1960). Our experiment focused on the photosynthetic ability of *C. reinhardtii* wild type strain CC-1690 - wild type *mt*+ 21 gr and mutant strain CC-3913 - *pf*9-3-*mt*- under varying temperature conditions. A key difference between the wild type and mutant that may affect their photosynthetic rates is their flagella, as this affects their mobility. The wild

type has two flagella, resulting in faster movement, while the mutant has no flagella and is therefore non-motile. These movement patterns were observed when viewing the wild type and mutant under an Axio light microscope. This observation led us to predict that the wild type would have a higher photosynthetic rate due to its increased ability to move to areas of optimal light. Therefore, our first null and alternate hypotheses are:

Ho_1: Wild type *Chlamydomonas reinhardtii* will produce equal or less oxygen than mutant *Chlamydomonas reinhardtii*.

Ha_1: Wild type *Chlamydomonas reinhardtii* will produce more oxygen than mutant *Chlamydomonas reinhardtii*.

Photosynthesis, a process used by plants to convert light energy to chemical energy, consists of two main parts: the light reactions and the Calvin cycle (Nelson and Cox 2008). As oxygen is a by-product of the light reactions, we used it to measure of the rate of photosynthesis in our experiment. The light reactions also produce ATP and NADPH, which are used to drive the Calvin cycle, a process which consumes CO_2 and produces sugar (Fig. 1). It is important to note that the Calvin cycle also produces ADP and $NADP^+$, which are required for the light reactions. Therefore, the light reactions and Calvin cycle are dependent on one another. Temperature has an effect on the photosynthetic rate of organisms as increasing temperature increases kinetic energy, which has been found to increase the rate of photophosphorylation (Lambers *et al*. 2008, Petrucci *et al.* 2011). In our experiment, an increased rate of photophosphorylation was measured by an increase in oxygen production. This model leads to our second set of hypotheses:

Ho_2: Lower temperatures increase or have no effect on the oxygen production of *Chlamydomonas reinhardtii.*

Ha_2: Lower temperatures decrease the oxygen production of *Chlamydomonas reinhardtii.*

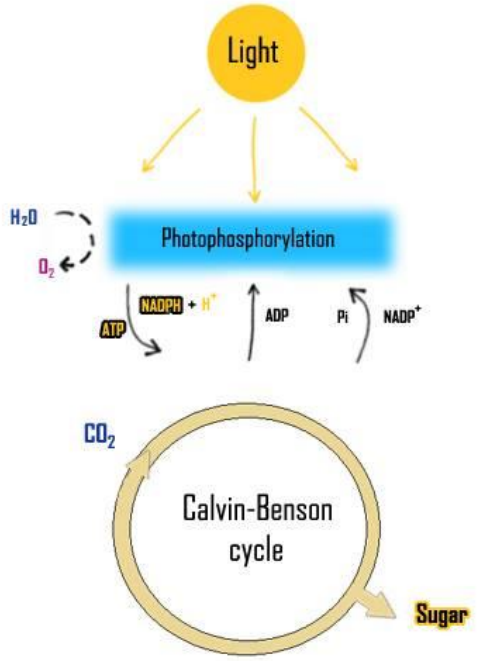

Figure 1. Overview of the two steps involved in photosynthesis. Image adapted from Purves *et al.*, Life: The Science of Biology, 4th Edition, by Sinauer Associates and WH Freeman.

Another factor that has been found to increase the photosynthetic rate of *Chlamydomonas reinhardtii* is increased light intensity. Therefore we will be sure to maintain constant light intensity over all three of our treatments (McCombie 1960).

The effect of temperature on the rate of photosynthesis of *C. reinhardtii* is an important factor to investigate as it is a primary producer at the base of the ecosystem; thus its productivity has an effect on the productivity of the ecosystem as a whole (Olga *et al.* 2012). As global climate change is a current issue we are facing, it is valuable to know if *C. reinhardtii* can photosynthesize at varying temperatures and produce sufficient amounts of oxygen to help maintain habitable environments for other freshwater organisms (Allan and Castillo 2007).

Knowing the effect of temperature on the photosynthetic rate of *C. reinhardtii* can also act as a model for how the photosynthetic rate of other plants may be affected at different temperatures.

Methods:

We performed our experiment over the course of one morning. First, we added 10 μL of IKI fixative to 90 μL of stock culture of each strain (wild type and mutant) in two mcf vials, and mixed them by micropipette. Next, we placed four 10 μL samples each of the fixed mutant and wild-type strains on haemocytometers and counted the number of cells within a 1 × 1 × 0.1 mm square grid under a light microscope (Fig. 3). We counted four replicates per strain. We then calculated the cells/mL ratio to determine initial concentrations of each solution. We used the formula $C_1V_1 = C_2V_2$ (C representing the concentration and V, the volume), to determine what the final volumes of each strain needed to be in order for the wild type and mutant solutions to be the same concentration. To achieve this concentration, we centrifuged each strain stock solution and discarded the supernatant. We then re-suspended the pellet of each strain to achieve a final cell concentration of 3,450,000 cells/mL (Fig. 2). Again, we added 10 μL of IKI fixative to 90 μL of the now more concentrated stock solutions in two more mcf vials, and mixed by micropipette. We then placed four more 10 μL samples of each the fixed concentrated wild type strain and mutant strain on haemocytometers to determine the new cell/mL ratio, and to make sure the concentrations of both strains were relatively close to our target concentration of 3,450,000 cells/mL.

Figure 2. Louisa proudly displaying our centrifuged and re-suspended *C. reinhardtii* mutant cells.

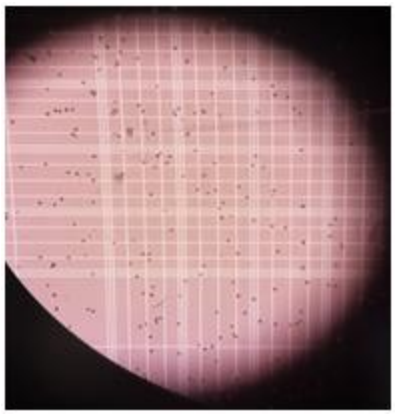

Figure 3. The partial view under a light microscope of the fixed concentrated *C. reinhardtii* mutant cells about to be counted on a haemocytometer under 100X magnification.

We prepared three replicates each of the wild type, mutant, and standard medium (the procedural control) for each of the three experimental conditions (15°C, 22°C, and 27°C), as well as three of each (the wild type, mutant, and standard medium) for an initial oxygen measurement. Each replicate consisted of a 27 mL sample in a 27 mL vial, sealed tightly to prevent air bubbles (Fig. 4). We filled three clear glass bowls with tap water for our water baths. Each was individually lit with their own 150W flood lamp. We recorded the light intensity reaching each water bath with a light intensity meter. To achieve the temperatures of 22°C and 27°C, we let the water warm up under the lamps, and then maintained the target temperature by the addition of ice. Similarly, the water bath of temperature 15°C was maintained by the addition of ice (Fig. 5).

Figure 4. *C. reinhardtii w*ild type replicate at time zero in the 22°C water bath, notice no air bubbles (left). *C. reinhardtii* wild type replicate at 22°C displaying the formation of oxygen bubbles after 1 hour (right).

We measured the initial oxygen concentration of three replicates of each of the wild type, mutant and procedural control using an oxygen meter, and then discarded them. We then placed nine replicates (three wild type, three mutant, and three procedural control) into the 15°C water bath. The vials were placed upside down to expose the transparent side of the vial to the light source, allowing for maximum light exposure. After five minutes, nine replicates were placed in the 22°C bath, and after another five minutes nine replicates were placed in the 27°C bath. This staggering of start times was to ensure all replicates would be exposed to their treatment for the same amount of time despite the time lag created by taking the final measurements. Throughout the experiment, we monitored the temperatures of the water baths with thermometers and added ice when necessary. After the replicates had been exposed to their treatment for eighty minutes, we measured their final oxygen concentrations using an oxygen meter.

Figure 5. Our experimental set-up. Three *C. reinhardtii* mutant, three wild type, and three procedural control replicates in each water bath (temperatures from left to right: 15^{o}C, 22^{o}C, and 27^{o}C) under 150W flood lamps.

After data collection, we subtracted the mean initial oxygen concentration from the mean final oxygen concentration for each of the wild type, mutant, and procedural control solutions, and calculated 95% confidence intervals. (Note: Since we had to discard the samples after taking the initial oxygen reading, the data were not paired; this is why we subtracted means instead of pairings.) The mean difference in oxygen concentration of the procedural control was then subtracted from the mean oxygen concentration differences of the wild type and mutant. For comparison between the wild type and mutant, we subtracted the mean oxygen production of the mutant from the wild type for each condition and performed a t-test between samples.

Results:

Table 1. Difference in oxygen production (mg/L) between *C. reinhardtii* wild type and mutant at 15°C, 22°C, and 27°C after 80 minutes.

Temperature Treatment	Wild Type [O_2] – Mutant [O_2]
15°C	0.27 mg/L (P-value 0.05)
22°C	0.36 mg/L (P-value 0.10)
27°C	1.24 mg/L (P-value 0.01)

As shown in Table 1, the wild type produced more oxygen than the mutant at each temperature treatment. Based on the P values for the t-test, we can conclude that there was a significant difference in the production of oxygen between the wild type and mutant at 15°C and 27°C, but not at 22°C. At 15°C, the wild type produced 0.27 mg/L more oxygen than the mutant, and at 22°C, 0.36 mg/L more the mutant. The largest difference in oxygen production was at 27°C, with the wild type producing 1.24 mg/L more than the mutant.

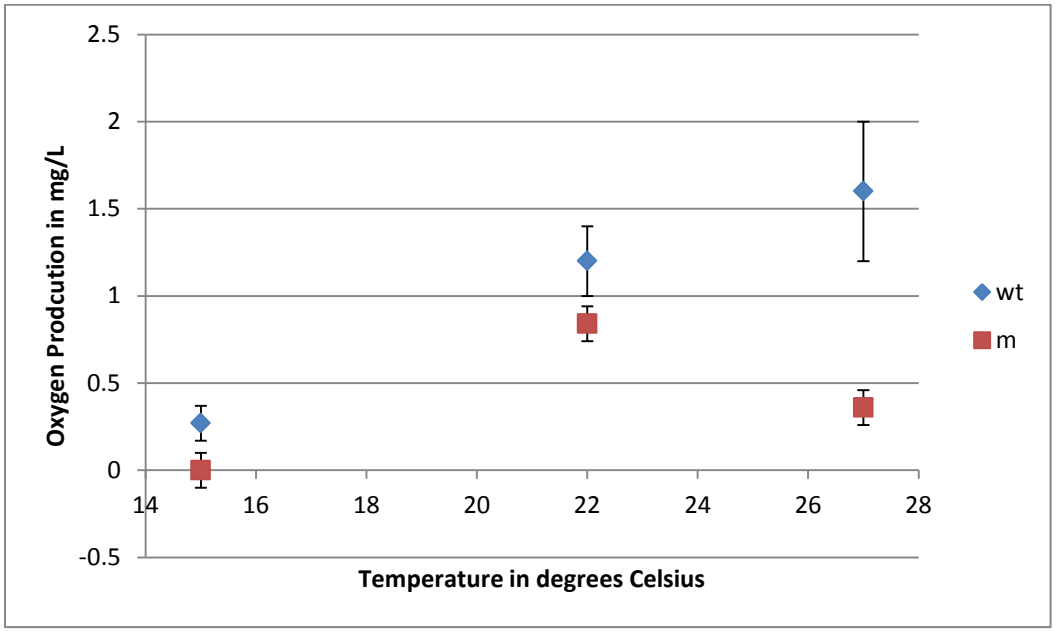

Figure 6. Mean oxygen production (mg/L) of *C. reinhardtii* mutant (m) and wild type (wt) at 15°C, 22°C, and 27°C after 80 minutes with 95% confidence intervals (n=3).

For the wild type, there was a significant difference between oxygen produced at 15°C and 22°C. There was also a significant difference between oxygen produced at 15°C and 27°C. While there was no significant difference between 22°C and 27°C, the 27°C treatment still had a mean higher than that of the 22°C treatment, thus showing an apparent trend. The trend is that with increasing temperature, there is an increase in oxygen production, with the 15°C treatment having the lowest production of oxygen, 0.27±0.1mg/L, the 22°C treatment having an oxygen production of 1.2±0.2mg/L, and the 27°C treatment having the highest production of oxygen, 1.6±0.4mg/L (Fig. 6).

Figure 6 also shows that for the *C. reinhardtii* mutant, there was a significant difference between oxygen concentrations in each temperature treatment. Oxygen concentration was lowest at 15°C, with a production of 0±0.1mg/L, and peaked at 22°C, with an oxygen production of 0.84±0.1mg/L. Oxygen concentration then dropped down to 0.36±0.1mg/L at 27°C.

Sample Calculation of [O_2] Produced by the Mutant at 22°C:

Initial Mean [O_2] Mutant: (8.3 + 8.4 + 8.4)/3 = 8.4±0.1 mg/L
Initial Mean [O_2] Procedural Control: (8.3 + 8.2 + 8.3)/3 = 8.3±0.1 mg/L
Final Mean [O_2] Mutant: (8.7 + 8.7 + 8.6)/3 = 8.7±0.1 mg/L
Final Mean [O_2] Procedural Control: (7.7 + 7.7 + 7.8)/3 = 7.7±0.1 mg/L
*used excel to calculate 95% confidence intervals:
Example of a confidence interval calculation for final mean of procedural control:

$=1.96\times \frac{s}{\sqrt{n}} = 1.96\times \frac{\sqrt{\frac{0^2+0^2+0.1^2}{2}}}{\sqrt{3}} = 0.08$ mg/L = 0.1 mg/L

Subtracted Initial [O_2] Means from Final Means:
[O_2] Production of Mutant: (8.7±0.1 mg/L) - (8.4±0.1 mg/L) = 0.30±0.1 mg/L
[O_2] Production of PC: (7.7±0.1 mg/L) - (8.3±0.1 mg/L) = -0.54±0.1 mg/L
Subtracted [O_2] Production of the PC from [O_2] Production of Mutant:
0.30±0.1 - (-0.54±0.1) = 0.84±0.1 mg/L

Sample t-test calculation between wild type and mutant O_2 production at 15°C:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{(s_1^2/n_1 + s_2^2/n_2)}}$$

$$t = \frac{1.6-0.36}{\sqrt{\frac{(0.09^2)}{3}+\frac{(0.35^2)}{3}}} = \frac{1.24}{\sqrt{0.0027+0.0408}} = \frac{1.24}{0.209} = 5.933$$

Degrees of freedom = 3+3-2=4
The value for a two-sided test with a p-value 0.01, and 4 degrees of freedom on the t-table is 4.604.
5.933 > 4.604, therefore we are 99% confident of a difference between the two means.

Discussion:

Based on statistical analysis of our results, we reject Ho_1 and Ho_2. Supporting Ha_1, the results from our experiment showed that the wild type *C. reinhardtii* produced significantly more oxygen than the mutant at 15°C and 27°C. While the difference was not significant at 22°C, the wild type still produced more oxygen than the mutant. This trend probably exists due to the fact that our mutant doesn't have any flagella. This is most likely the result of a genetic defect in the mutant's central-pair microtubule structure in the flagella's supporting structure (Adam *et al*. 1981). Due to this mutation (lack of flagella), the mutant's ability to move in the direction of a light source is restricted (Adam *et al*. 1981). While we applied constant light over all samples, the top of each vial most likely received the most light. Therefore, the wild type's ability to move to the top of the vial may have caused the higher oxygen production compared to the mutant. Observations during our experiment support this as in the wild type vials, we saw a concentrated green colour near the top of the vials, which represented *C. reinhardtii* gathering together in that location. This was not seen as much in the mutant vials, as the green colour was much more evenly distributed.

Decreasing temperature decreases the oxygen production of wild type *C. reinhardtii.* The decrease in temperature caused a significant decrease in oxygen from 27°C to 15°C, and from 22°C to 15°C. While the difference from 27°C to 22°C was not significant, it still followed the

same trend. Literature supports these findings as it states that 28°C is the optimal temperature for photosynthesis in wild type *C. reinhardtii*, suggesting that lower temperatures should result in less oxygen production (McCombie 1960). Because oxygen is a product of the light reactions of photosynthesis, our results show that increasing the temperature increases the rate of photophosphorylation. A possible explanation for this is that increasing temperature increases the kinetic energy of a system (Petrucci *et al*. 2011). Higher kinetic energy increases the number of collisions between the enzymes and substrates of the light reactions, ultimately resulting in a higher photosynthetic rate (Lambers *et al*. 2008).

The light reaction rate (and thus rate of oxygen production) is also affected by the rate of the Calvin cycle since the ADP and NADP+ produced in the Calvin cycle are required for the light reactions to take place. Therefore, another possible factor that may contribute to the trend seen in the wild type could be that RuBisCO, an enzyme responsible for carbon fixation, is sensitive to colder temperatures (Storey and Tanino 2012). If the Calvin cycle was slowed down, the rate of production of ADP and $NADP^+$ would slow down, and thus the rate of the light reactions would slow down accordingly.

When looking at the trend in the mutant, there is a peak in oxygen production at 22°C, with significantly less oxygen being produced at 27°C and 15°C. Spreitzer *et al*. (1988) states that many mutants are temperature-sensitive, with their defects being evident at varying temperatures. Our results suggest that the mutant strain CC-3913 - pf9-3-mt- is temperature-sensitive, since the difference in oxygen production between the wild type and mutant was significantly larger at 27°C, with a difference of 1.24 mg/L. This defect can be due to a number of factors including pigmentation, a defective electron transport chain, or a defect in RuBisCO (Spreitzer *et al.* 1988). The pigmentation of the wild type and mutant were both a pale green

colour, and defects in the electron transport chain are rare as they are usually lethal, so it is unlikely the temperature sensitivity in the mutant was due to either one of those factors. The mutant's temperature sensitivity is most likely caused by reduced RuBisCO carboxylase activity at certain temperatures, as this is the case for most temperature-sensitive mutants (Spreitzer *et al.* 1988). This reduced activity is generally caused by the defect in a gene that codes for the large subunit of RuBisCO (Spreitzer *et al.* 1988). As stated earlier, reduced RuBisCO activity would decrease the rate of the Calvin cycle, which would decrease production of ADP and $NADP^+$, and thus decrease the rate of oxygen production. Regarding the low oxygen production of the mutant at 15°C, this is most likely due to decreased kinetic energy at lower temperatures and the temperature sensitivity of RuBisCO, just as in the wild type (Petrucci *et al.* 2011, Storey and Tanino 2012).

One limitation to our study is that while these results are representative of the particular culture we tested, they may not be representative of the strain in general. The particular mutant and wild type cultures used were raised at 17°C, thus natural selection could have favoured individuals best suited for this temperature. It is possible cultures raised at different temperatures would lead to different results.

In addition to biological factors, sources of error in the execution of the experiment could have affected our results. For example, while we tried to keep our water baths at 15°C, 22°C, and 27°C, their temperatures fluctuated between 14-15°C, 20-23°C, and 26-30°C, respectively. Another source of error regarding the maintenance of abiotic factors is that although we tried to keep the light intensities constant by using bulbs of the same watts, there was still some variation. The light intensities were 46500 lux, 47500 lux, and 44100 lux in the 15°C, 22°C, and

27°C treatments, respectively. It is possible that part of the increase in oxygen production in the 22°C treatment was due to the increased light intensity, not solely due to the temperature.

Another factor that may have had an effect on our data is that although our target cell concentrations for the wild type and mutant was 3,450,000 cells/mL, there is likely to be some degree of error due to sampling variation. If the wild type or mutant ended up having a higher cell concentration, this would most likely result in higher oxygen production.

Conclusion:

In conclusion, we rejected H1 and Ho_2. We found significant differences showing that increasing temperature increases oxygen production. The wild type *C. reinhardtii* produced significantly more oxygen than the mutant at 15°C and 27°C.

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Literature Cited:

Adams, G., Huang, B., Piperno, G., and Luck, D.J. 1981. Central pair microtubular complex of *Chlamydomonas* flagella: polypeptide composition as revealed by analysis of mutants. Journal of Cell Biology, **91**(1): 69-76.

Boyd, J.S. 2011. Eyespot Assembly and Positioning in *Chlamydomonas reinhardtii.* Ph.D. Thesis, University of Arizona, Arizona, US.

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

Lambers, H., Chapin III, F.S., and Pons, T.L. 2008. Plant Physiology Ecology, 2nd Edition. Springer Science, New York, NY.

McCombie, A.M. 1960. Actions and Interactions of Temperature, Light Intensity and Nutrient Concentration on the Growth of the Green Alga, *Chlamydomonas reinhardii* Dangeard. Journal of the Fisheries Research Board of Canada. **17**(6): 871-894.

Nelson, D. L. and Cox, M. M. 2008. Lehinger Principles of Biochemistry, 5th Edition. Palgrave Macmillan, New York, NY.

Olga, B.K., Klassen, V., Doebbe, A., Kersting, K., Grimm, P., Wobbe, W., and Kruse, O. 2012. Cellulose degradation and assimilation by the unicellular phototropic eukaryote *Chlamydomonas reinhardtii*. Nature Communications. **3**(1214): 1.

Petrucci, R.H., Herring, F.G., Madura, J.D., and Bissonnette, C. 2011. Organic Chemistry, 6th Edition. Prentice Hall, Upper Saddle River, NJ.

Spreitzer, R.J., Al-Abed, S.R., Huether, M.J. 1988. Temperature-sensitive photosynthesis deficient mutants of *Chlamydomonas reinhardtii*. Plant Physiol. **86**(3): 773-777.

Storey, K.B., and Tanino, K.K. 2012. Temperature adaptation in a changing climate: nature at risk. RubisCo. CAB International, Cambridge, MA.