The Effect of Temperature on Cell Density of Wild Type and Mutant *Chlamydomonas reinhardtii*

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Abstract:

Chlamydomonas reinhardtii is a unicellular green alga, which is used as a model organism in many fields of molecular biology. In order to understand more about the optimum conditions necessary for the growth of *C. reinhardtii* in a lab setting, we focused on how temperature can affect the abundance of both wild-type and mutant forms. We used the CC-1690 wild-type strain and the CC-3913 - *pf9-3* mutant strain. In our experiment, we used three temperature treatments: 11°C, 17°C, 25°C. We had three replicates at each treatment temperature for each and took cell density measurements over ten days. Based on observations and data that we collected, we observed that the wild type generally reaches the greatest cell density at 17°C, where as the mutant type has the highest cell density at 25°C. The results of these data allow us to reject both of our null hypotheses. We suggest that this is because the wild type *C. reinhardtii* are better capable of accumulating the energy necessary for growth due to the functions of flagella and the presence of certain enzymes, whereas the mutant type lacks these traits beneficial for their living (Falk *et al.* 2006).

Introduction:

Chlamydomonas reinhardtii is a unicellular green flagellated organism that has two anterior flagella that facilitate its movement. The mutant strain lacks flagella and therefore lacks movement. With this study, we aimed to learn more about the environmental factors that can affect the growth of *C. reinhardtii*, focusing on the temperatures that are conducive to optimal growth. It is important that we investigate this aspect because by determining the optimal temperature range, we can better understand the growth conditions necessary to obtain a culture with maximum density. These results will be useful since *C. reinhardtii* are widely used in lab studies, due to their rapid replication and sequenced genome, allowing their inclusion in a variety of molecular experiments (Falk *et al.* 2006).

Our null and alternate hypotheses are:

- H_{o1}: The density of wild-type and mutant *C. reinhardtii* does not change or decreases as temperature increases.

- H_{a1}: The density of wild-type and mutant *C. reinhardtii* increases as temperature increases.
- H_{o2}: The density of the wild type *C. reinhardtii* is not different from or is less than the density of the mutant *C. reinhardtii.*
- H_{a2}: The density of the wild type *C. reinhardtii* is greater than the density of the mutant *C. reinhardtii*.

Past studies have investigated the effect of temperature on *C. reinhardtii*, and it is generally believed that the wild type has a higher growth rate than the mutants when both are treated under the same environmental conditions. Falk *et al* (1990) suggest that reasonable temperatures for growth of *C. reinhardtii* were in the range from 12°C – 27°C, where photosynthesis was greatest at 27°C. *C. reinhardtii* are tolerant to temperatures between 6°C and 35°C, with optimal growth at 28°C, which is in accordance with our alternate hypothesis in a temperature range of 11°C - 25°C (McCombie 1960).

It has also been found that the mutant grows at a lower rate than the wild type because certain enzymes that are involved in photosynthesis are lacking, which would affect the growth rate since *C. reinhardtii* obtains its nutrients through photosynthesis (Adams *et al.* 1982). This supports our alternate hypothesis that the wild type would grow at a higher rate than the mutant cells.

Methods:

We conducted a ten-day-long experiment on the effect of three temperature treatments on the growth of *C. reinhardtii*. The wild-type strain we used was CC-1690 - wild type mt+ 21 gr

and the mutant strain was CC-3913 - *pf9-3 mt-*. We first put 10μL of each stock culture on a haemocytometer and counted the number of cells in the centre square under a light microscope (Figure 2) and extrapolated the amount for 1mL. We diluted our samples with the standard culture medium so that we had an initial approximate concentration of 50,000 cells/mL for both the strains, using the formula $M_1V_1 = M_2V_2$, where M is the molar concentration and V is the volume (Vance and Spalding 2005).

Figure 1: Materials used for experiment

Figure 2: Light microscope and lab notebook

We prepared three replicates for each of the three temperatures (11°C, 17°C, 25°C), for both wild type and mutant cells.. In order to start with the same number of cells in each replicate, we began with 10mL of our culture in each of the 18 test tubes (for a final concentration of 50,000 cells / mL).

We sampled one mL from each of the vials seven times over the course of ten days. Before sampling we re-suspended the cultures to ensure an even cellular concentration

throughout the vial. To keep the cells from moving, we added 100μL of fixative (Figure 1). We took a 10μL sub-sample from each vial and placed it on the haemocytometer, where we counted the number of cells (using clicker counters) in the central grid (as per instructions on the use of the haemocytometer). Since we did not have access to the lab during the weekend, our seven days of sampling was split into three and four day sequences.

We ensured sterile technique was used throughout the experiment (Figure 1) to avoid contamination. To minimize extraneous factors, we kept our samples in the incubators at all times, except when extracting the samples. The incubators had a set constant light and constant speed for the shakers, which were used for aeration.

After the seven days of data collection, we graphed the data using the means of the replicates, comparing wild type to mutant at each temperature (Figures 6, 7, 8), as well as graphs of just the wild type and mutant at all temperatures (Figures 4, 5). We used 95% confidence intervals to help determine whether our results were significant.

Results:

Both the wild type and mutant *C. reinhardtii* divided over the course of the experiment, which was evident on the fifth day (after 168 hours) when clusters of algae could be seen with the naked eye and the solution became a vivid green colour (Fig. 3). Furthermore, these characteristics were more evident in the wild type than the mutant.

By calculating mean population for each culture type (Figure 4), we can see that wild type generally has an optimum temperature of 17˚C. However, after 24 and 48 hours, the cell density was the greatest at 25°C. Confidence intervals of some temperatures overlapped after 24 and 216 hours. After 48 and 72 hours, the confidence interval overlapped for 17°C and 25°C. After 192

hours, the confidence intervals of 11˚C and 25°C overlapped. Figure 4 shows no overlap between the confidence intervals of the means for any of the temperatures on the fourth sampling day (after 96 hours).

The mutant cell density increased the most at 25˚C. *C. reinhardtii* population increased as temperature increased, and this trend shows consistency throughout the duration of the experiment. Confidence intervals of all temperatures overlap on the first day and the confidence intervals overlap on the seventh day (after 168 hours) between 11˚C and 17˚C.

Both the wild type and mutant *C. reinhardtii* grew over the 216 hours, and the wild type generally has a higher growth rate than the mutant at 11˚C. The confidence intervals overlap after 48 and 96 hours. There is no data point on the third day of sampling (after 72 hours) for the mutant, because the cells were lysed and therefore we were unable to count them. At 25 °C, the wild type cells increased in number more than the mutant for the first 72 hours, while the mutant population grew more between 168-216 hours. The population size was the same for both culture types, with a mean of sixty cells /ml after 96 hours. The confidence intervals of all population growth overlap on all days except the first sampling day (after 24 hours).

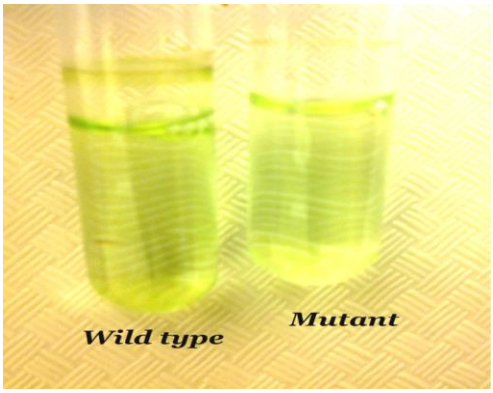

Figure 3: Wild type and mutant *C. reinhardtii* at 17˚C after 192 hours.

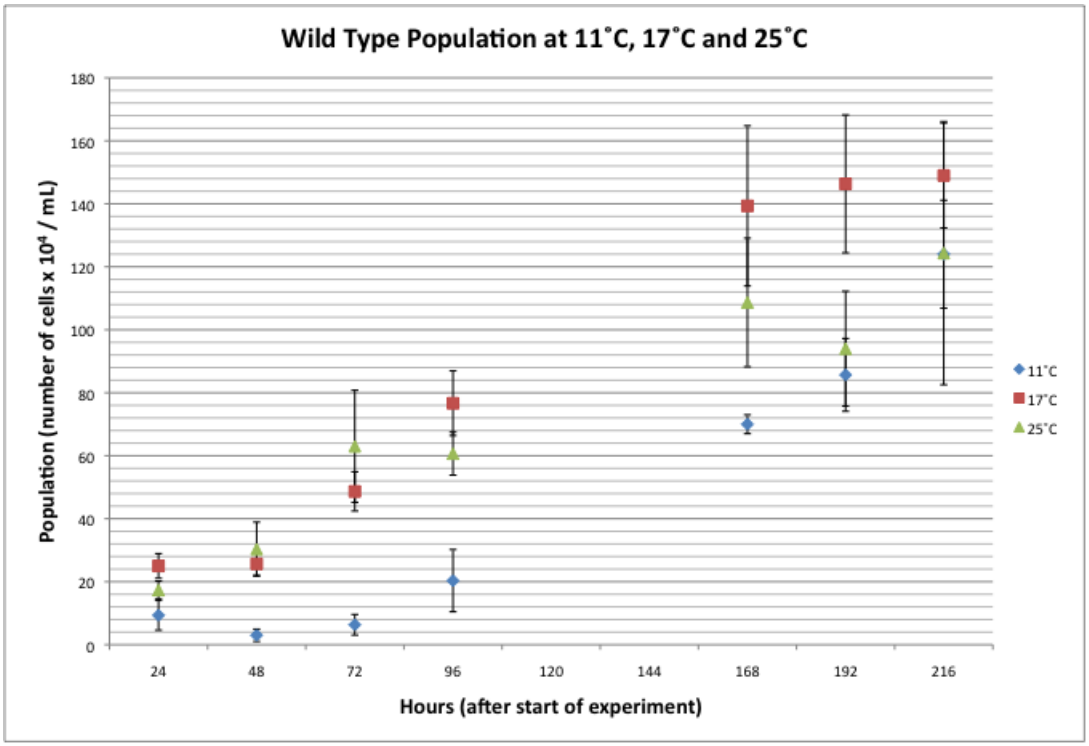

Figure 4: Mean density of wild-type *C. reinhardtii* over the course of 216 hours for three temperature treatments. The mean was calculated from three replicates and 95% confidence intervals are presented.

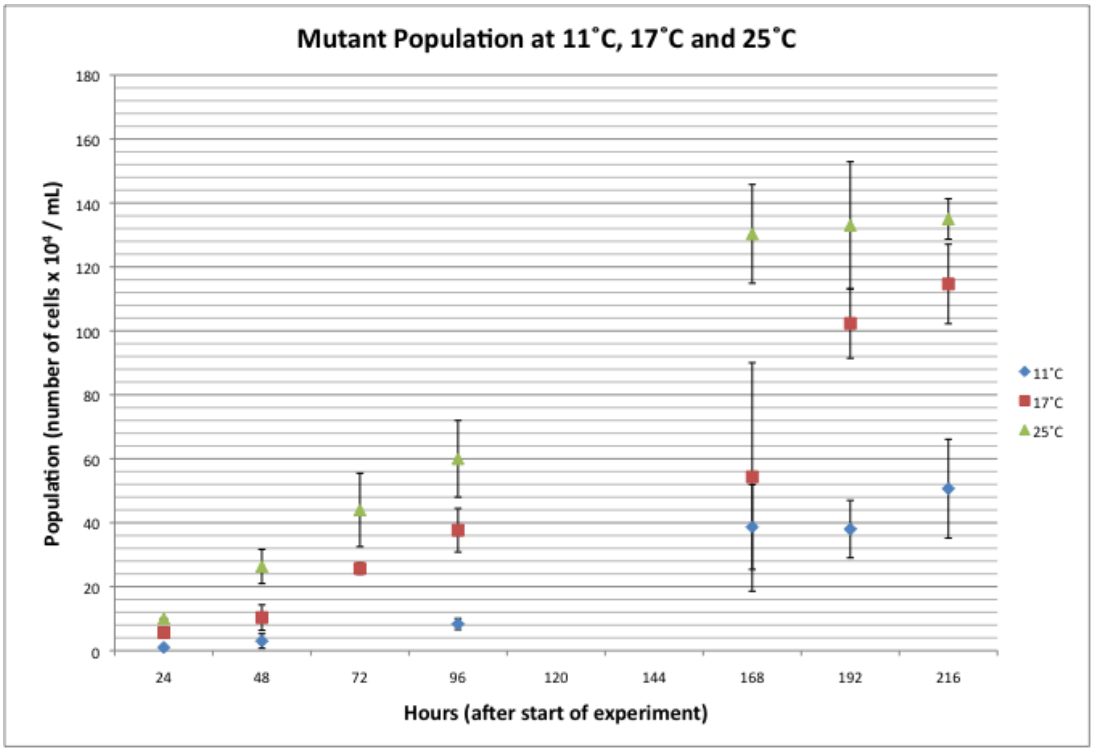

Figure 5: Mean density of mutant *C. reinhardtii* over the course of 216 hours for three temperature treatments. The mean was calculated from three replicates and 95% confidence intervals are presented.

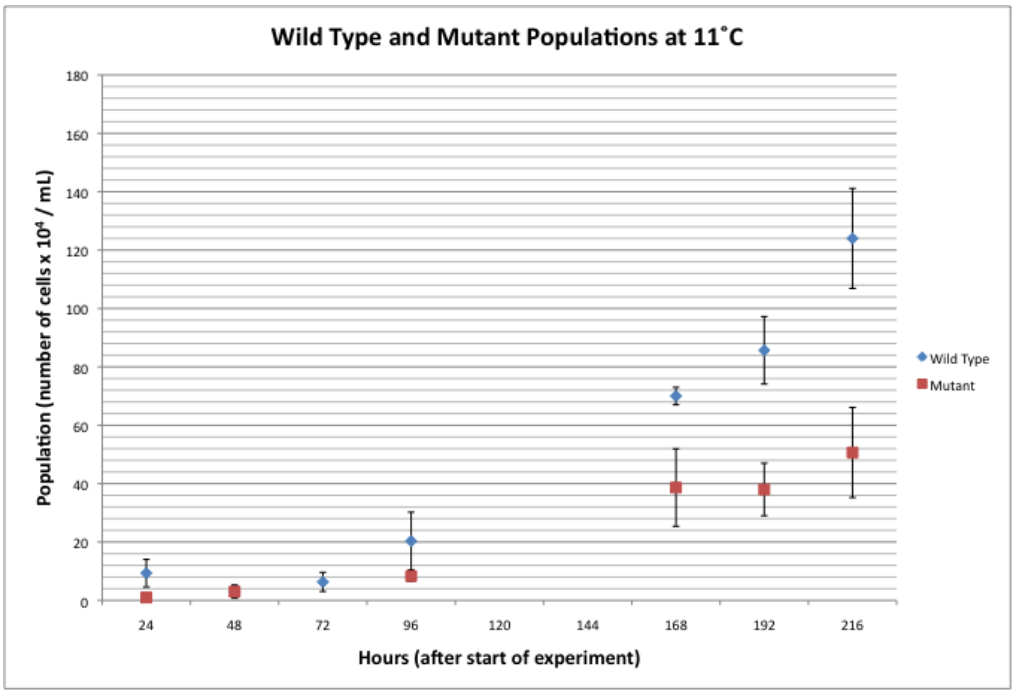

Figure 6: Mean density of wild-type and mutant *C. reinhardtii* over the course of 216 hours at 11°C. The mean was calculated from three replicates and 95% confidence intervals are presented.

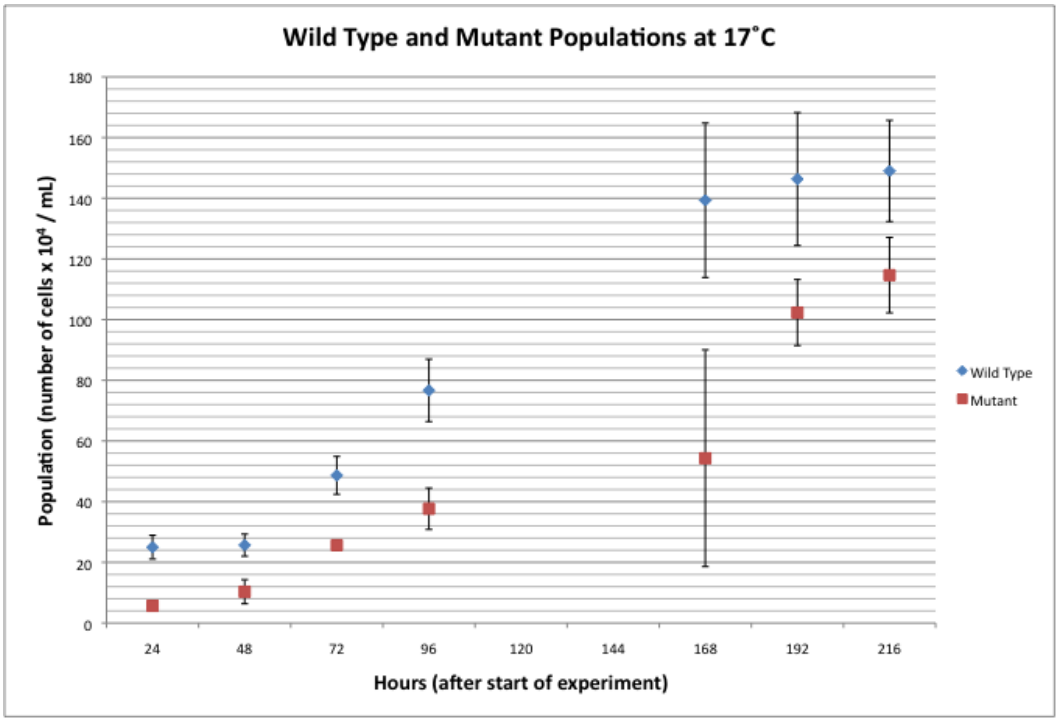

Figure 7: Mean density of wild-type and mutant *C. reinhardtii* over the course of 216 hours at 17°C. The mean was calculated from three replicates and 95% confidence intervals are presented.

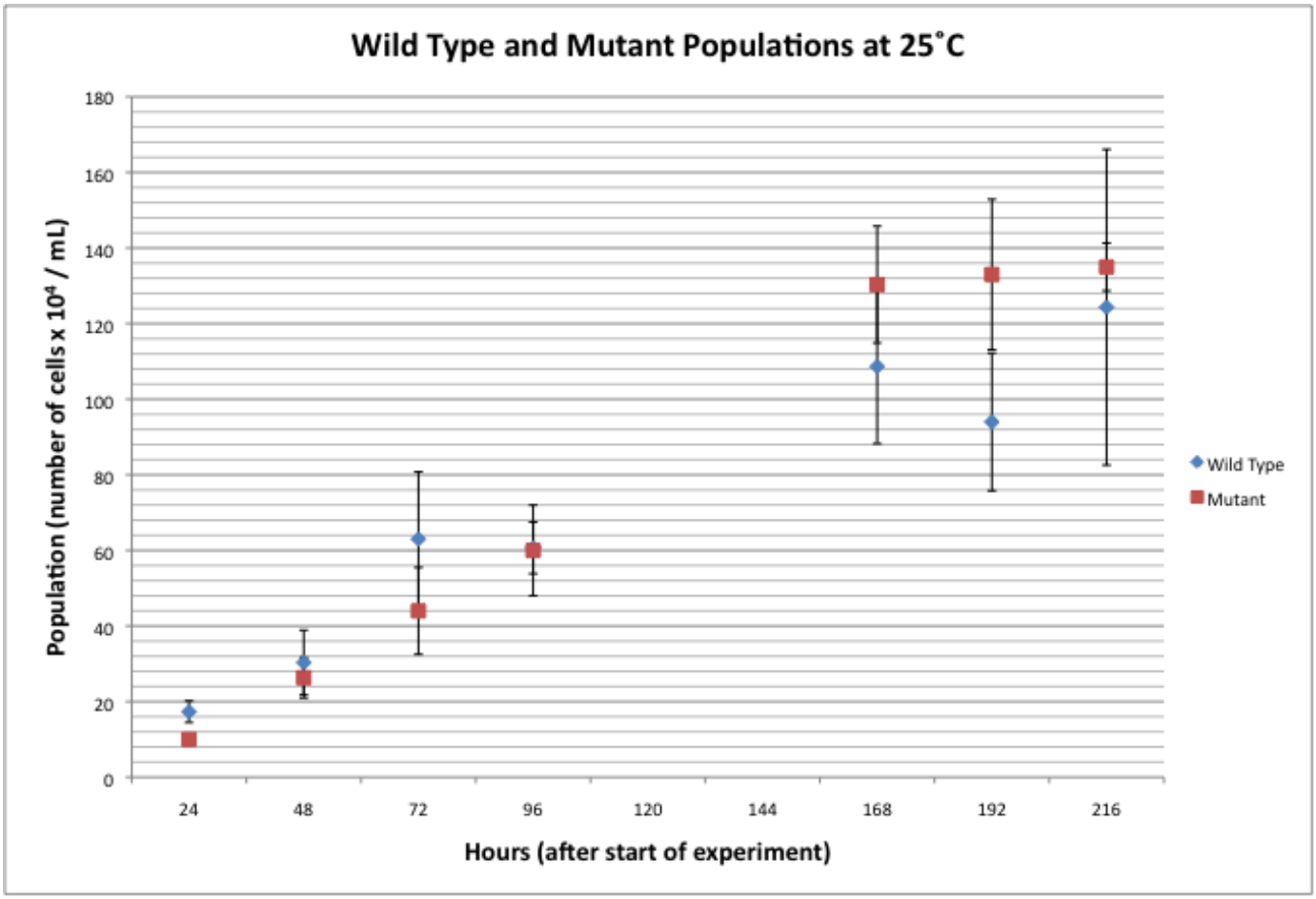

Figure 8: Mean density of wild-type and mutant *C. reinhardtii* over the course of 216 hours at 25°C. The mean was calculated from three replicates and 95% confidence intervals are presented.

Discussion:

Based on statistical analysis of our final results, we reject both our null hypotheses. The results from our experiment show a general trend of increasing population density each sampling day of each temperature, which is especially clear in the 11˚C and 17˚C treatments (Figs. 6 and 7). At these temperatures, it is clear that the wild type has had more growth than the mutant *C. reinhardtii*, which supports our second alternate hypothesis. However at 25˚C, the wild type had a lower growth rate than that of the wild type at 17˚C. The mutant grew at a higher rate than the wild type during the last three days (Fig. 8), which is most likely due to the malfunctioning of the

incubator which started from 168 hours into incubation and continued to the end of the experiment. One possible biological explanation for this trend is that the wild type is better able to reproduce than the temperature-sensitive mutants since the mutants lack motility and certain enzymes involved in photosynthesis (Adams *et al* 1982). This results in decreased availability of nutrition as photosynthesis is their primary source of nutrition.

Looking separately at each individual temperature, we observe that the wild type *C. reinhardtii* have a greater population than the mutants at 11°C and 17°C (Figs. 6 and 7). However at 25°C, the mutant has a greater population than the wild type (Fig. 8). This suggests that temperature may not be as large of a contributing factor in population growth as thought, which conflicts with our literature since previous studies show that the temperature of the environment does have an effect on and is essential to the length of the cell cycle and therefore the abundance of *C. reinhardtii* (Mihara and Hase 1971).

Previous research found that in general, the mutants reproduce slightly slower than the wild type, which is supported by our evidence at 11˚C (Fig. 6) and 17˚C (Fig. 7) (Spreitzer *et al* 1988). However, it contrasts with our results at 25˚C since we have a higher population density of the mutant compared to the wild type (Fig. 8). This is most likely because the temperature inside the 25°C degree incubator temperature did not stay at 25°C, but rose to 32˚C. This may explain the inconsistency at that temperature because 32˚C is the cutoff point at which the *C. reinhardtii* cells reabsorb their flagella due to the high temperature (Huang *et al.* 1977). Therefore, since the mutants are better adapted to surviving without flagella, they grow at a higher rate than the wild type.

We also noted that we were unable to obtain data for the mutant population at 11°C on our third sampling day (after 72 hours) because all the cells on the haemocytometer were lysed and abnormally shaped. This occurred for all the three replicates of the 11°C degree mutant sample. *C. reinhardtii* cell walls usually lyse during the mating period when cell fusion occurs between the plus end and the minus end of the gametes (Goodenough and Weiss 1975). However, since it was very specific to this temperature and all the three replicates were affected, it is more likely due to the inconsistency of the shaker (which was used to keep our populations evenly aerated throughout the test tube), which was found stationary (not spinning) on most of the days when we came to collect samples.

In addition to the malfunctioning of the 25˚C incubator, other sources of error such as sample preparation, counting and technical errors did exist in our experiment. Since the living environment for the cells was aqueous, the cells naturally moved toward the surface of the culture to get the most oxygen. During our experiment, we might have not been thorough in shaking the test tubes, which could have caused an unevenly distributed concentration of cells when extracting a sample. This would lead to obtaining a number of cells that does not accurately represent the true population in the original replicate cultures. Light source is another factor that may have affected the growth of our samples in the incubators. When we put the test tubes back into the incubators for further testing, we might not place them back to their original positions, which might lead to unequal light intensity between the individuals of each treatment, and could possibly affect our final results.

Conclusion:

Therefore based on our results, at 11˚C and 17˚C, we reject both our null hypotheses and have evidence to support our alternate hypotheses. As temperature increases, abundance of *C. reinhardtii* cells also increase. At these temperatures, when comparing the wild type and mutant, it is clear that the wild type grows at a higher rate than the mutant. However, at 25˚C we fail to reject both the null hypotheses, since the population at 17˚C was higher than the population at 25˚C for the wild type *C. reinhardtii*. In addition, during the last three days, the growth rate of the mutant population was higher than that of the wild type population at 25˚C.

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