The effect of temperature on the growth rate of wild-type and mutant *Chlamydomonas reinhardtii*

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Abstract

Chlamydomonas reinhardtii is a unicellular green alga with two flagella that facilitate its movement. This study investigates the effect of temperature on the growth rate of *C. reinhardtii*, as well as the differential effects on the CC-3913-*pf9-3 mt-* mutant compared to the CC-1690 wild type. Our method consisted of setting up samples of each strain under three temperature treatments (17°C, 20°C, 25°C) over 15 days. The data were obtained by measuring cell counts with a haemocytometer every three days, and the growth rate was calculated. A two-way ANOVA test followed this to obtain *p* values for each of our hypotheses. Our results of p_1 = 0.0001 provided support that increasing temperature will have an effect on thegrowth rate of *C. reinhardtii*. This was consistent with the literature, which suggests that higher temperatures cause *C. reinhardtii* to reach their maximum growth rate over a shorter time. The value p_2 = 0.03 provided support that the presence of a mutation will have an effect on the growth rate, because mutants without flagella have difficulty swimming to light sources for photosynthesis. Lastly, p_3 = 0.07 did not provide support that increasing the temperature would affect mutant and wild-type *C. reinhardtii* differently. This can be explained by the similarities between the mutant and wild-type cell cycles.

Introduction

Chlamydomonas reinhardtii is a haploid, unicellular green alga that lives in freshwater ecosystems (Kong et al. 2009). The organism has two flagella which facilitate its movement and it has a light-sensitive eyespot that allows it to grow autotrophically in the light. The *C. reinhardtii* cell cycle has also been found to be related to growth rate, which can be affected by abiotic factors such as temperature (Vitová et al. 2011).

In the last decade, *C. reinhardtii* has served as a valuable source for the development of low-cost, robust, and safe vaccines for humans (Rosales-Mendoza 2013). Therefore, it is important to investigate the optimal abiotic conditions in which we can maximize the growth rate of the organism under laboratory settings. In our study, we used wild-type CC-1690 and mutant CC-3913-*pf9-3 mt-*. The mutant strain lacked the ability to assemble flagella which resulted in

impaired motility. The objective of our study is to investigate the effect of temperature on the growth rate of both wild-type and mutant *C. reinhardtii*.

Our null (Ho) and alternate (Ha) hypotheses are:

Ho_1: Temperature has no effect on the growth rate in *C. reinhardtii.*

Ha_1: Temperature has an effect on the growth rate in *C. reinhardtii.*

Ho_2: Presence of the *pf9-3 mt-* mutation has no effect on the growth rate in *C. reinhardtii.*

Ha_2: Presence of the *pf9-3mt-* mutation has an effect on the growth rate in *C. reinhardtii.*

Ho_3: The effect of temperature on the growth rate of *C. reinhardtii* is the same in wild-type and mutant.

Ha_3: The effect of temperature on the growth rate of *C. reinhardtii* is not the same in wild-type and mutant.

Vitová et al. (2011) performed an experiment comparing the effects of temperature on the *C. reinhardtii* cell cycle and growth rate phases. The authors grew the cells at several temperatures from 15 to 37°C and under three various light intensities to obtain changes in mean cell volume and number of cells. Therefore, this study provides a model on which to base our investigation. Using findings from Vitová et al. (2011), we will conduct a similar experiment by exposing our *C. reinhardtii* strains to three temperature treatments and observing the effects of temperature on growth rate. In comparison to our control, we will be able to determine how an increase in temperature will affect the growth rate. We predict that an increase in temperature will have a negative effect on growth. Mutant strains used in the Huang et al. (1977) study, were discovered to populate at a slower rate when they lacked flagella; therefore, we predict that the presence of the *pf9-3 mt-* mutation will cause a reduction in growth. Furthermore, because of the

pf9-3 mt- mutation (impaired motility) an increase in temperature will affect the mutant differently compared to the wild-type strain.

Methods

To observe the effects of temperature and the presence of the *pf9-3 mt-* mutation on the growth rate of *C. reinhardtii,* we conducted a 15-day study (Adams et. al 1982). The setup of our experiment required us to first dilute the cell concentration of our wild-type and mutant strains with sterile medium to an initial concentration of 50,000 cells/mL (Vance & Spalding 2005).

We added 2 mL of each strain to twelve 6 mL test tubes giving us a total of 24 replicates (Figure 1).

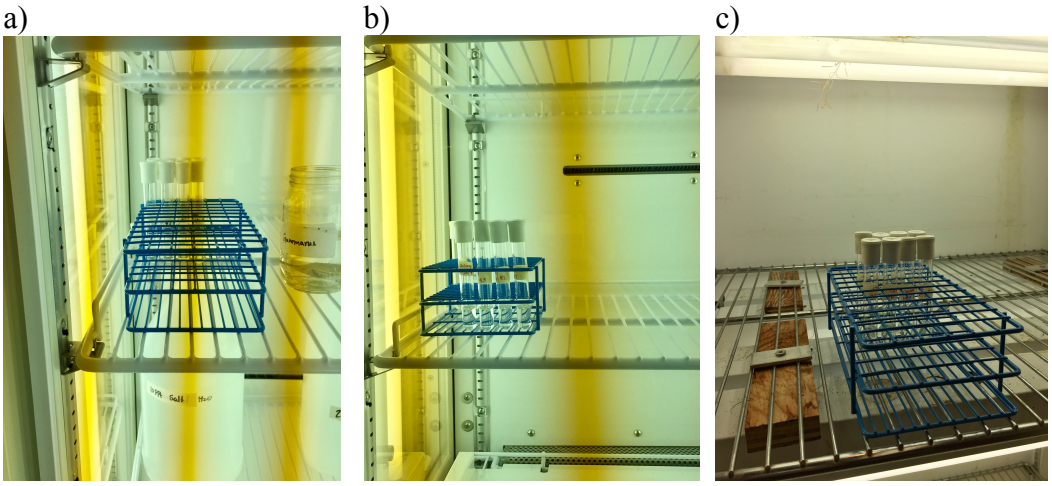


Figure 1. Setup of our apparatus in the a) 17°C (control), b) 20°C, and c) 25°C incubators. Four wild-type and four mutant tubes were held in test tube racks, providing each incubator with 8 replicates. The samples were placed on the left side of the incubators and labeled accordingly.

Before placing the tubes in their respective incubators, we collected a 100μL sample from each tube and fixed them with 10μL of IKI to count our cells and calculate the starting concentrations of each replicate (i.e., time 0). Four mutants and four wild-type test tubes were then placed in each of the three incubators that were previously set to three different temperatures: 17°C (control), 20°C, and 25°C (Figure 1). The light intensities at each of these temperature treatments were 7.7 x 10^3 lux, 7.2 x 10^3 lux, and 6.7 x 10^3 lux, respectively. The

amount of light exposure was kept constant, with each incubator receiving light for the same time period of 12 hours each day.

Daily, with the exception of weekends, we finger vortexed the samples to prevent any aggregation of the cells. We retrieved and fixed our samples four times, with a three-day interval between each sampling day (Figure 2).

a) b) c)

Figure 2. The samples taken on Nov 10, 2016, day 15 of the experiment. a) 17°C (control), b) 20°C and c) 25°C after vortexing.

Each time samples were collected, the cell concentration was determined using the haemocytometer viewed under a compound microscope (Figure 3).

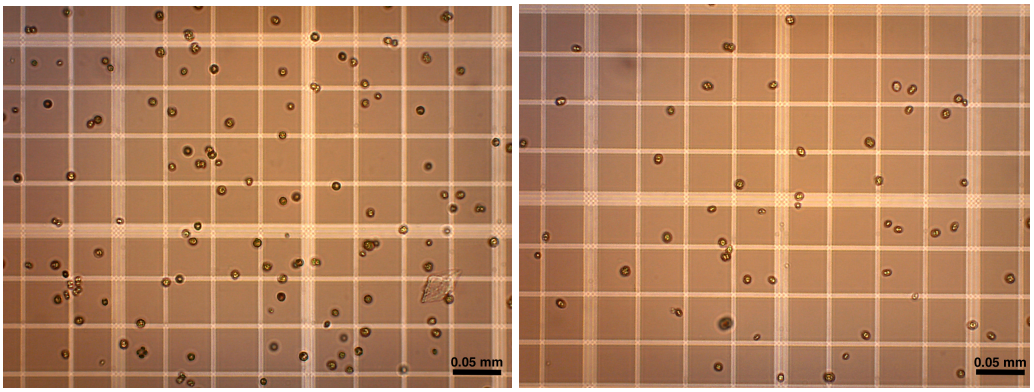


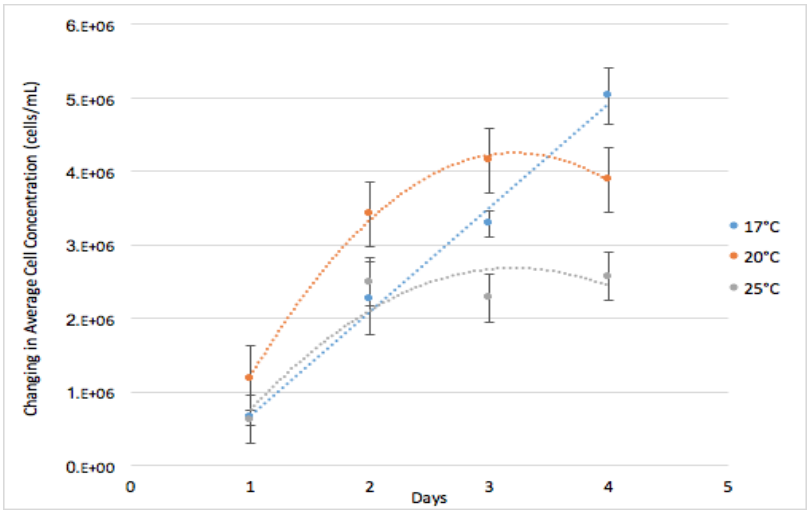
Figure 3. Image of wild-type sample 1 (left) and mutant sample 3 (right) *C. reinhardtii* at 20°C on a haemocytometer. Taken on day 15 of the experiment using a DinoXcope. Magnification at 100X.

Our data were obtained by determining the cell density of the fixed samples and comparing them with the initial concentrations calculated from time 0. A two-way ANOVA test (p value critical = 0.05) was performed to obtain p values for each of our three sets of hypotheses to determine the significance of our results.

Results

In Figure 4 a), at 17°C, the cell population grew linearly; therefore, a maximum cell concentration of 5.03 x 10^6 ± 3.88x 10^5 cells/mL was obtained. A concentration of 3.89 x 10^6 ± 4.42 x 10^5 cells/mL was obtained at 20°C and a minimum concentration of 2.57 x 10^6 ± 2.67 x 10^5 cells/mL was obtained at 25°C. It should be noted that the cell concentration began to decrease after day 3 for both at 20 and 25°C.

a)



b)

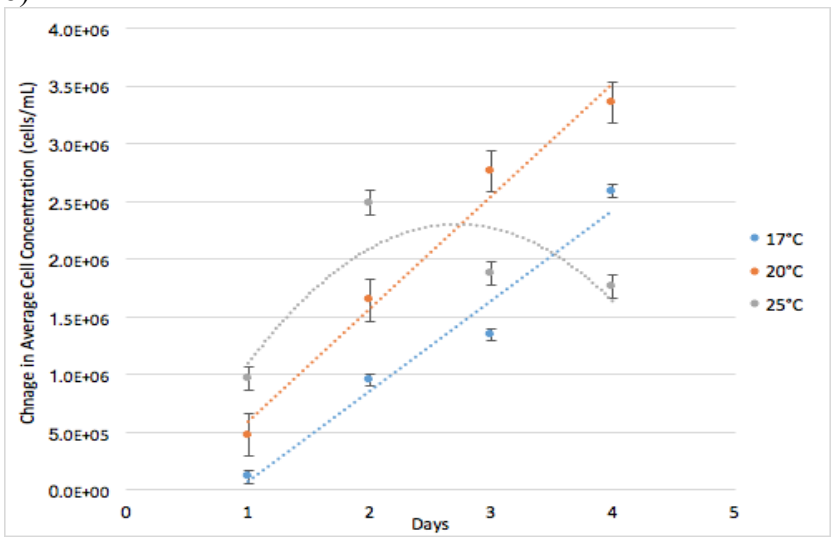
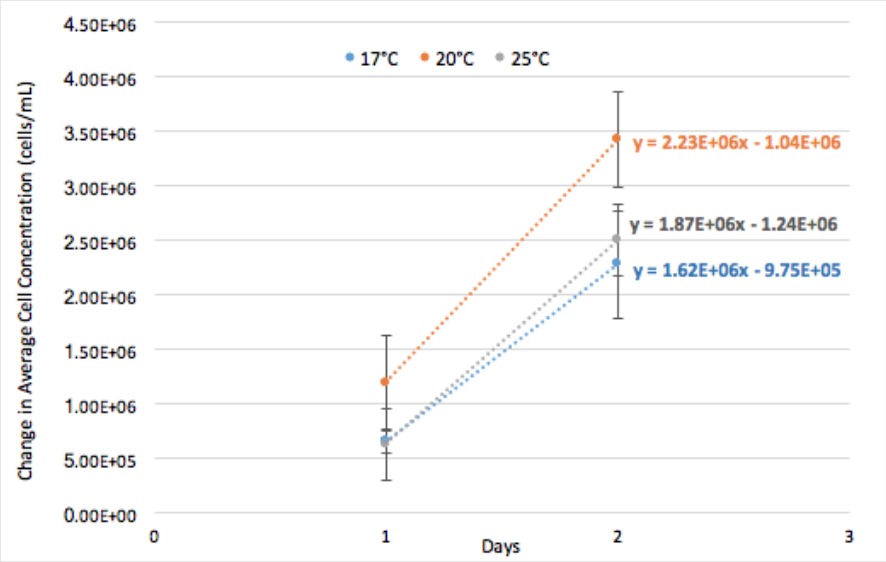


Figure 4. Effects of temperature on the average change in cell concentration (cells/mL) for a) wild-type and b) mutant *Chlamydomonas reinhardtii* at 17, 20 and 25°C taken on four different sampling days. Error bars represent 95% C.I., n=4.

According to Figure 4 b), unlike wild-type, a maximum mutant cell concentration of 2.76 x 10^6 ± 2.20 x 10^5 cells/mL was obtained at 20°C in which cell concentration increased linearly throughout. The same trend was observed at 17°C, where concentration increased up to a value of 2.59 x 10^6 ± 3.14 x 10^5 cells/mL, but at a more gradual rate. At 25°C, cell concentration started to decrease after day 3, and; therefore, resulted in a minimum value of 1.76 x 10^6 ± 1.19 x 10^5 cells/mL.

From Figure 5 a), the steepest slope was 2.23 x10^6 cells mL^{-1} day^{-1} at 20°C. In figure 5 b), the steepest slope was 1.52 x10^6 cells mL^{-1} day^{-1} at 25°C. However, from Figures 5 a) and b), 17°C shows the most gradual slope for both wild-type and mutant.

a)



b)

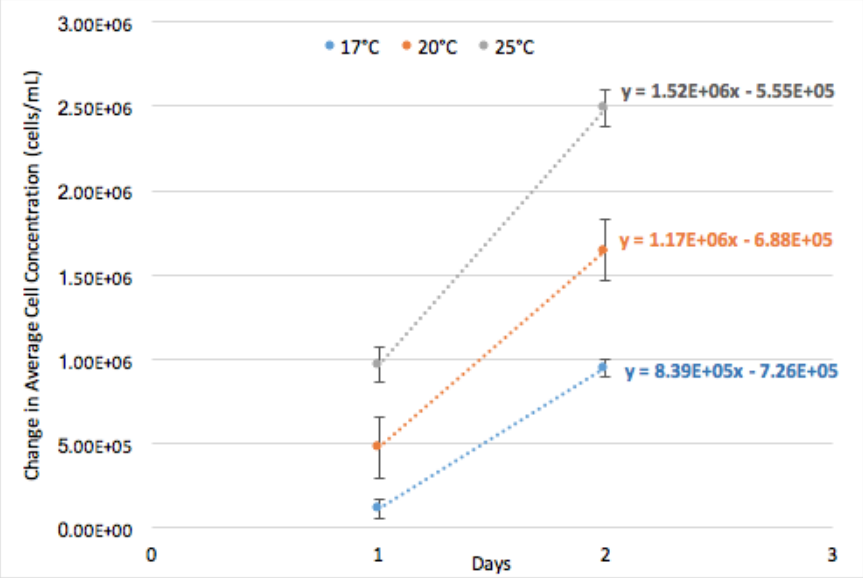


Figure 5. Initial slopes of exponential growth phase (cells/mL) for a) wild-type and b) mutant *Chlamydomonas reinhardtii* at 17°C, 20°C and 25°C taken on different days. Error bars represent 95% C.I., n=2.

Based on Figure 6, growth rate at 25°C for wild-type was $1.71 \times 10^5 \pm 1.78 \times 10^4$ cells mL^{-1} day^{-1} and for mutant was $1.18 \times 10^5 \pm 7.88 \times 10^3$ cells mL^{-1} day^{-1}. Ultimately, the growth rate of wild-type was higher than mutant by 1.62×10^5 cells mL^{-1} day^{-1} (17°C), 3.54×10^4 cells mL^{-1} day^{-1}(20°C) and 5.36×10^4 cells mL^{-1} day^{-1} (25°C). From the two-way ANOVA test, with a p value of 0.05, p_1 was 0.0001 for our first hypothesis, p_2 was 0.03 for our second and p_3 was 0.07 for our final hypothesis.

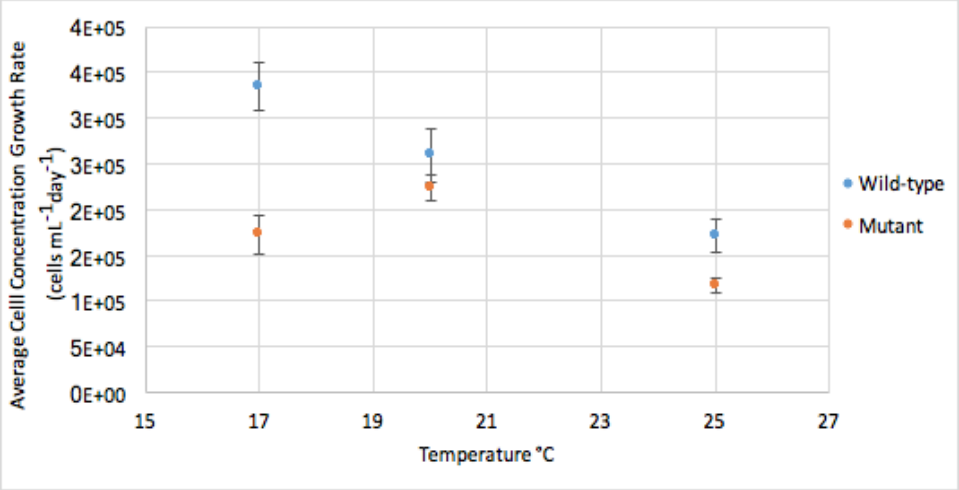


Figure 6. Effects of temperature on the average growth rate (cells mL^{-1} day^{-1}) for wild-type and mutant *Chlamydomonas reinhardtii* at 17, 20 and 25°C. Two-way ANOVA test, *p* value = 0.05, p_1=0.0001, p_2=0.03, and p_3=0.07. Error bars represent 95% C.I., n=3.

Discussion

We rejected our first null hypothesis because p_1 had a value of 0.0001, which was less than 0.05. We also rejected our second null hypothesis as the p_2 =0.03 (<0.05). However, we failed to reject our third null hypothesis because our p_3 = 0.07 which was greater than p = 0.05.

We have determined that an increase in temperature will have an effect on concentration of *C. reinhardtii* cells. This is evident in Figure 4 in which the overall trend shows that an increase in temperature will result in a decrease in cell concentration. However, Figure 5 indicates that initial growth rate increased with higher temperatures (20 and 25°C). According to Vitová et al. (2011) the algal cell cycle can be viewed as two distinct phases that are affected by abiotic factors, specifically light intensity and temperature. The authors analyzed the cell populations at several temperatures within 15 to 37°C. After graphing the cell number with time, Vitová et al. (2011) determined 28°C to be the optimal temperature. This result was due to the fact that the higher temperature caused the doubling time of *C. reinhardtii* to decrease, leading to increased cell division. This shortened the length of the cell cycle and thereby allowed them to reach their maximum growth rate over the shortest period of time (steepest slope) (Vitová et al.

2011). From these findings, we can suggest that a similar observation was seen in our results (Figure 5) and we can infer that an increase in temperature will increase the *C. reinhardtii* growth rate. Falk et al. (1990) also discovered that *C. reinhardtii* would have increased photosynthetic rates at higher temperatures because the cells would become light saturated more quickly. This provided another explanation as to why Figure 5 showed steeper slopes for the higher temperatures (20 and 25°C) in the initial exponential phase of *C. reinhardtii*, which was taken from the overall concentration curves (Figure 4).

Figure 6 shows that the overall growth rate was lowest for mutant ($1.18 \times 10^5 \pm 7.88 \times 10^3$ cells mL^{-1} day^{-1}) and wild-type ($1.71 \times 10^5 \pm 1.78 \times 10^4$ cells mL^{-1} day^{-1}) in 25°C. This was due to the stationary phase of their growth being reached sooner (maximum growth on day 2), and therefore allowed both cell types to plateau ahead of the cells at 17 and 20°C (Figure 4). As previously mentioned, Falk et al. (1990) found that higher temperatures resulted in faster photosynthetic rates; however, this also meant earlier exposure to photoinhibition. The authors determined that lower temperatures made *C. reinhardtii* become less susceptible to photoinhibition, and thus would allow the cells to increase in numbers for longer periods of time; this is indicated in Figure 4.

In comparing the mutant and wild-type strains in Figure 6, it is evident that the *pf9-3 mt-* mutation has a greater effect on the growth rate. Figure 6 shows that the growth rate was greater in wild-type than mutant *C. reinhardtii* at each of our temperature treatments. These results can be explained through the study performed by Huang et al. (1977) in which they used several temperature-sensitive mutant strains of *C. reinhardtii*, that were grown at 20°C, and then exposed them to a higher temperature of 32°C to test their flagellar regenerative potential. Huang et al. (1977) found that cell concentrations of the mutant strain, *dd-l-14*, had no flagellar

regenerative potential and grew more slowly than wild-type at 20°C.As a result, these mutants reached a plateau in cell division at a lower cell concentration (Huang et al. 1977). These findings are consistent with the results of our study and can be, in part, due to the lack of flagella in the mutant and their inability to swim to optimal light levels to perform photosynthesis (Silflow & Lefebvre 2001).

Our results were unable to show that the effect of temperature on the growth rate of *C. reinhardtii* differs in the wild type and mutant as we were unable to reject the third null hypothesis. Due to the inability of most plants and algae to escape unfavorable conditions such as high or low light and hot or cold temperatures, it is essential for them to acclimatize to these types of environments in order to survive (Minagawa et al. 2015). Schroda et al. (2014) explains that a heat stress response occurs when environmental temperatures increase to prevent the disturbances that are forced upon the cell's metabolism, enzyme activities and most relevant, cell division. In another study, Hemme et al. (2014) expressed that *C. reinhardtii* experience heat stress and arrest the cell cycle in the S phase due to lack of histone biosynthesis, a process necessary for DNA to be packaged into nucleosomes. Due to the overall decrease in growth rate seen at higher temperatures for each cell type (Figure 6), the general trend of an increased cell cycle in both strains is observed.

Sources of uncertainty for our experiment may have resulted from several factors, accounting for our large standard deviations. After making our stock solution, the dilutions of our samples ranged from 2.64×10^4 to 1.03×10^5 cells/mL. This range of cell density may have stemmed from an inaccuracy in counting when using the haemocytometer and the lack of mixing before micro-pipetting our samples. However, to reduce the effects of these random errors, we adjusted our results accordingly by subtracting our time 0 concentrations from each of our

samples. The data seen in Figures 4 and 5 are based on the change in cell concentration from time 0, thereby reducing the effect of our range in the initial cell concentrations.

Another main source of uncertainty originated from the amount of time the replicates spent out of the incubator as we prepared our fixed samples. The exposure to air temperature was not controlled as the time was not recorded; therefore, there could be several variations in time of exposure. Our final major source of uncertainty was the variation in light intensity in each incubator. Since our samples were exposed to the highest light intensity at 17°C, it may have increased the preferential growth conditions at this temperature, resulting in an increased overall cell concentration for the wild type at 17°C. A study done by McCombie (1960) discovered that high light intensity was a more suitable environment for the growth of *C. reinhardtii* as they are autotrophic. By having a higher light intensity in the 17°C incubator, *C. reinhardtii* were able to produce more food and nutrients due to the improved growth conditions (McCombie 1960). These findings are consistent with our results for wild-type *C. reinhardtii.*

Conclusion

Our hypotheses consisted of the effect of temperature on the growth rate of *C. reinhardtii*, the effect of the *pf9-3 mt-* mutation on growth rate, and if temperature would affect mutant and wild-type strains the same way. We rejected the first two null hypotheses and failed to reject the third hypothesis. We correctly predicted that an increase in temperature would result in decreased growth rate overall; however, in the initial phase, temperature enhanced the grpwth rate. Furthermore, we predicted correctly that the presence of the *pf9-3 mt-* mutation would cause a decrease in growth rate; this is evident in Figure 6. Due to the similar trends seen in the mutant and wild-type graphs (Figure 4), we incorrectly predicted that an increase in temperature would affect mutant and wild-type strains differently. Overall, 20 and 25°C increased growth rate in the

initial exponential phase of the wild-type and mutant growth curves, respectively; however, in the long-term, cell concentration was highest at 17°C.

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