The effects of temperature changes on the flagellar growth rate of *Chlamydomonas reinhardtii*

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Abstract

Chlamydomonas reinhardtii is a commonly used organism in biological laboratory research. The purpose of our study is to determine the impact of temperature on flagellar regrowth in C. *reinhardtii*. Our mode of experimentation involves inducing a brief pH shock, to force deflagellation, followed by observations of the time for flagellar regrowth. In our experiment, we test the importance of temperature on flagellar regrowth. Our alternate hypothesis states that increasing or decreasing the optimal temperature (20°C) has an effect on the flagellar regeneration rate of C. *reinhardtii*. Prior research suggested that flagellar regrowth rates would be optimal at temperatures of around 20°C, with variations from this temperature resulting in decreased growth rates. We utilize several treatments and replicates to conduct the experiment for a duration of 15 minutes, making observations at 5 minute intervals. Ultimately, our results were not significant in which we failed to reject our null hypothesis that temperature does not have an effect on flagellar regrowth rates in *Chlamydomonas reinhardtii*.

Introduction

Flagella are one of the most common locomotive structures utilized by unicellular life. Our research organism, *Chlamydomonas reinhardtii,* a unicellular green alga, is no exception, possessing two anterior flagella (Harris 2001). Without these structures, normally motile organisms are rendered severely handicapped in their ability to find resources, avoid predators, and signal mating (Mitchell 2000). In the case of *Chlamydomonas reinhardtii*, this is no exception as their flagella enable them to perform their signature “breaststroke” swimming action (Childress 2012).

In this study, our objective was to investigate the effects of temperature changes on flagellar growth rate of *C. reinhardtii*. Despite our goal, it is often difficult to measure re-growth rates for organisms of any sort due to the limited regenerative ability of most organisms on this

planet. Fortunately, *C. reinhardtii* are able to regrow their flagellum and are capable of doing so relatively quickly, making them an ideal organism for our experiment.

To measure regrowth, we must first remove the flagella of our *C. reinhardtii* population by subjecting them to a brief pH shock. Such stresses are of particular significance as they can affect the allocation and proportion of lipids contained in each *C. reinhardtii* individual (Siautt *et al*. 2011). Specifically, reductions in levels of nitrogen in their environment causes *C. reinhardtii* to grow larger stores of lipid and starch (Siautt *et al*. 2011). Another study found that reductions in nitrogen levels lead to significant reductions in pH (Liang *et al*. 2013). As they are a food source for growing salmon fry, this has a potential to impact salmon on a macroscopic level. If decreased nitrogen levels promote increased nutrient store formation in *C. reinhardtii* and reduce pH to levels that could knock off flagella, the resulting individuals would be immobilized and richer in nutrients than normal individuals. This posits a potential for increased nutrient access to salmon born in nitrogen poor areas though the significance of this is not well understood, any increases in nutrient level of *C. reinhardtii* could potentially have no impact as well.

After subjecting our experimental populations to de-flagellation via a pH shock, we will time their regrowth and take samples from each population at specific time intervals. We will then calculate rates of flagella regrowth using the samples we obtained to form averages for each time interval. Past research has suggested that temperature can significantly impact the rate of flagella growth. Results from past experiments suggest that optimal flagella regrowth rates occur at 20°C and that they decline as the temperature diverges from this optimum (Lien and Knutsen 1979). Our null hypothesis is that variations from the optimal temperature will have no impact

on flagellar regrowth rates in *C. reinhardtii*. Our alternative hypothesis is that variations from the optimal temperature will have an impact on flagellar regrowth rates in *C. reinhardtii*. In accordance with research by Lien and Knutsen (1979), we would predict flagella regrowth rates to be greatest in our 20°C treatment population.

Methods

Before starting the experiment, three Dinoxcopes were connected to microscopes and laptops with proper calibration for each of the three replicates (Figure 1). To connect the Dinoxscope camera to the microscope, one of the microscope eyepieces were removed. Next, we opened the associated Dinoxcope program to see a reduced portion of the visible stage of the microscope.

Figure 1. The setup of the Dinoxscope connected to microscopes and laptops used for viewing of the *C. reinhardtii* used during the experiment.

After setting up the Dinoxcopes at 40X magnification, three water temperature baths in glass dishes were prepared at 10°C, 20°C and 30°C using ice, room temperature water and a hot plate, respectively. We placed a thermometer in each bath to monitor the temperatures and adjust when needed. Next, we transferred 40 mL of *C. reinhardtii* into a 100 mL beaker containing a

magnetic stirrer. Then, we transferred the beaker into the 20°C bath and placed the beaker with the *C. reinhardtii* sample on top of the stirring plate. After turning on the stirring plate, we de-flagellated the *C. reinhardtii* by adding 0.5M acetic acid dropwise to the sample using a pipette. A pH meter was kept inside the 100 mL beaker to monitor instant changes in pH. After each drop of acid, we waited 10 seconds to ensure adequate time was given for the pH meter to respond to the change in pH and then read the recording. Once the pH reached 4.5, we immediately started a 30 second timer and took out a sample of culture with a micropipette to check for deflagellation under the Dinoxcope. At the same time, a couple drops of 0.5M KOH were added to our culture to avoid killing the *C. reinhardtii* cells. Once deflagellation was observed and confirmed, additional 0.5M KOH was added to restore the culture to its original pH. After, 10 mL of the culture was transferred from the beaker into three test tubes and placed it inside the designated temperature bath (20°C) for the run. A group member was assigned to a test tube to extract samples from during the 15-minute time range designated for flagellar regrowth. At 5-minute intervals, each group member took a sample from their test tube, viewed it under the microscope, and took pictures of individual cells (and their flagella) using the Dinoxscope. This allowed us to have three replicates at for each time interval. We obtained pictures of a minimum of five samples at each time 5-minute interval during the 15 minute time range. Then, we prepared three new test tubes and another beaker with 40 mL of *C. reinhardtii* for the next temperature treatment. We repeated the de-flagellation and regrowth procedures above for each culture under 10°C and 30°C. Once all Dinoxscope pictures of the samples at each time interval for all three temperature treatments we obtained, ImageJ software was used to determine the length of each flagella. An image of the stage micrometer slide was taken with the Dinoxscope to use as a scale

for ImageJ (unit length set to mm). After collecting the flagella length using ImageJ for each replicate, the growth rate of the flagella per minute was calculated and then averaged separately for each temperature treatment. To confirm the significance of our findings, a one-way ANOVA test was used on our data.

Results

For each temperature treatment of 10°C, 20°C, and 30°C the average growth rates calculated were found to be 0.841 μm/min, 1.842 μm/min, and 0.630 μm/min respectively. the 95% confidence intervals for each temperature treatment of 10°C, 20°C, and 30°C were calculated to be ± 0.82, ± 0.59, and ± 1.2 respectively. The highest growth rate was found at 20°C followed by the growth rate at 10°C and 30°C.

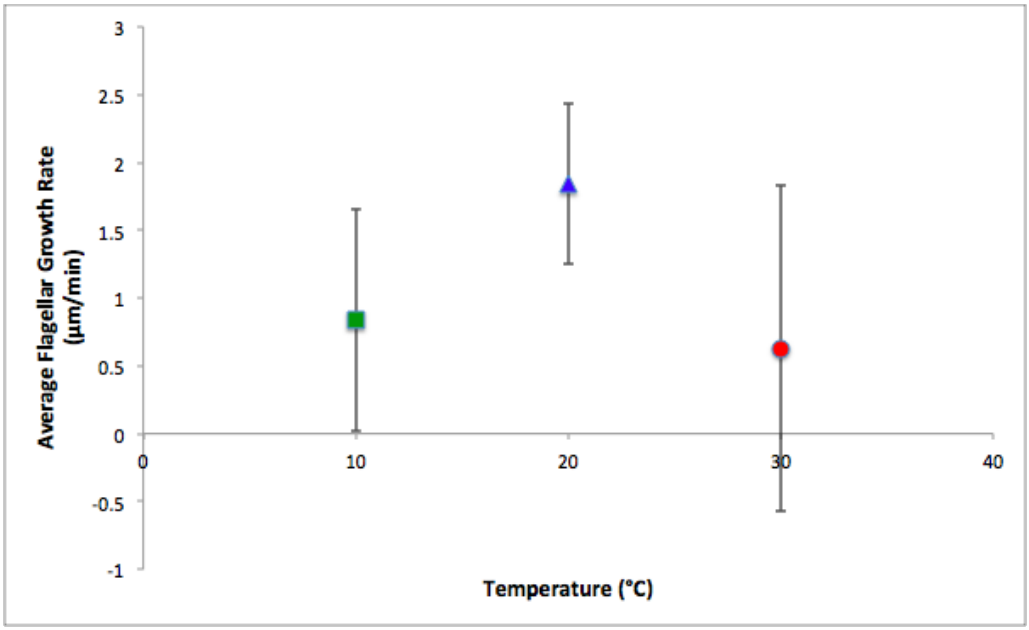

Figure 2. The average flagellar growth rates of wild-type *C.reinhardtii* at temperatures 10°C, 20°C, and 30°C with units (μm/min). Bars represent 95% confidence intervals, n=3 for each temperature. Data collected in November 2017, UBC.

According to Figure 2., it is visible that the error bars overlap when comparing the average growth rate at the temperature 10°C to the optimal growth temperature 20°C. Likewise, it is also visible that the error bars also overlap when comparing the average growth rate at the temperature 30°C to the optimal growth temperature 20°C as well as when comparing the growth rate at 10°C to the 30°C growth rate. Due to the overlap of both of the error bars for all scenarios, we assume that there was no significant difference when looking at the 95% confidence intervals when the temperature was decreased to 10°C or increased to 30°C from the optimal growth temperature (20°C). In order to further confirm the findings, a one-way ANOVA test was performed on the results. From the one-way ANOVA test, the calculated p-value was 0.229936. Since the p-value was greater than 0.05, it was found that the data was not significant. Overall, all of the 95% confidence intervals overlapped with one another and the one-way ANOVA test suggested that there was not a statistically significant difference between the average growth rates for our 10°C, 20°C, and 30°C treatment groups.

Discussion

A change in temperature from the optimal flagellar growth temperature (20°C) did not have a significant effect on the flagellar growth rate of *C. reinhardtii.* Based on the one-way ANOVA test (with a p-value of 0.23), we fail to reject our null hypothesis stating that increasing or decreasing the optimal temperature (20°C) has no effect on the flagellar regeneration rate of *C. reinhardtii*. We do not support our alternative hypothesis, and conclude that the growth rate of flagella in *C. reinhardtii* is not dependent on temperature.

Flagella provide *C. reinhardtii* with both mobility and structural support for the organism

to thrive (Mitchell and Nakatsugawa 2004) and the aim of this study helps determine the best environment for *C. reinhardtii* to thrive. Our findings suggest that *C. reinhardtii* had an observed growth rate of 0.841 μm/min (2nd highest) at 10°C, had the highest observed growth rate of 1.842 μm/min at 20°C, and had the lowest observed growth rate of 0.630 μm/min at 30°C. With the assumption that all *C. reinhardtii* cells were living when images were taken through the Dinoxscope, we observed longer flagella at 20°C compared to 10°C and 30°C. This trend correlates with previous literature, that flagella regrowth decrease outside the optimal threshold range of 20-25°C (Harris 2001). Although results show a variation between flagellar regeneration rates between 10°C, 20°C, and 30°C, there is insufficient evidence to conclude that there is a significant difference in our results.

Flagellar regeneration occurs instantly after de-flagellating *C. reinhardtii* (Rosenbaum *et al.* 1969), there is no lag time between amputation and regrowth. In this study, images were taken at 5, 10, and 15-minute increments but full reassembly of *C. reinhardtii* takes 120 minutes (Stolc *et al.* 2005). Upon comparing our data with similar experiments that have tested the regrowth rate of *C. reinhardtii* flagella, the results from this experiment do not correlate with those done in the past. Since it takes longer for flagella to grow to completion (120 minutes) than the amount of time we observed in lab (15 minutes), our experiment did not observe the full growth of the flagella. It is plausible that flagellar regrowth at 120 minutes could have led to different results than those observed through this experiment. For future studies, images should be taken for longer time frame of 120 minutes for possible observation of the full growth of *C. reinhardtii* flagella. Due to time constraint because of procedural errors and IKI fixative killing of our cells, we were unable to carry the experiment for a longer duration of time.

Other than temperature, all external variables such as light were controlled. Throughout our experiment, there were possible sources of error that may have affected our data. One potential source of error was the sudden, large imbalance in pH caused by the amputation of *C. reinhardtii* flagella. A pH meter was used to confirm the acidity of the *C. reinhardtii* solution. A pH=4.5 was required to de-flagellate the *C. reinhardtii;* however, the lag time in the pH meter resulted in pH decreasing below 4.5 at one point during our experiment which had the potential to kill some of the *C. reinhardtii* present in the solution. Although lag time was taken into consideration during the experiment, fluctuations in the pH reading resulted in excess acid being added to the *C. reinhardtii* solution. Also, in order to bring the pH back up to 7, the pH meter was used again, in which an inappropriate lag time length could have resulted in a very basic solution harming the *C. reinhardtii.* For future studies, it is important for the pH to be controlled throughout the duration of the experiment (not moving too low or high). To help control this, more time and precision should be allocated towards de-flagellating *C. reinhardtii* to avoid the risk the killing off cells. Another source of error that occurred during data collection is our repeatability and accuracy when taking pictures with the Dinoxcope. To improve this, during each repeatable trial, one should take more pictures to have a greater data set for analysis. For repetitions of this experiment in the future, it would also be more efficient to have more individuals involved in the experimental process to allow for more accurate results.

Conclusion

To conclude, we found that our alternate hypothesis which states that increasing or decreasing the optimal temperature from 20°C to 10°C and 30°C has an effect on the flagellar regeneration rate of *C. reinhardtii* was not supported. The information obtained throughout the

entirety of the experiment was not completely different than that found in scientific literature and similar past research experiments; however, our statistical analyses rendered our data to be insignificant. A possible reason for this was the many sources of error that could have affected our results when conducting the experiment on a time constraint.

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