Effects of Increasing Temperature on the Growth Rate of *Tetrahymena thermophila*: the impact of climate change and adaptation for survival

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Abstract

Tetrahymena thermophila is a unicellular complex ciliate organism that is able to undergo mitosis and meiosis. This protozoan makes up lower levels of the food web that includes salmon, a keystone species on the western coast of British Columbia. Increasing negative effects from climate change will have drastic consequences on marine ecosystems, altering the dynamics within and between populations. In order to test if temperature affected the cells' growth rate, *T. thermophila* was cultured in temperature treatments of 25°C, 35°C and 39°C to observe their average population growth rate at each temperature. This research is essential to investigate the consequences of climate change on the components of the salmon food web. The experimental findings were that there was little to no growth rate for all three temperature treatments and the growth rates for the three temperatures were also not significantly different ($p > 0.05$); resulting in us being unable to reject the null hypothesis that temperature does not affect the cells' growth rate. This finding contradicts our initial prediction that higher temperatures increase growth rate and implies that temperature does not have an overall effect on growth rate and that the cultured *T. thermophila* cells barely grew.

Introduction

Tetrahymena thermophila is a model organism for research due to its ability to alternate between sexual and asexual stages, its quick growth rate, and its ability to be cultured very easily in the laboratory (Cassidy-Hanley, 2012). *T. thermophila* is lined by many cilia and uses them to move through water, as well as to sweep bacteria and other small debris into its mouth (Bozzone, 2000). Moreover, they are also a key component of the oceanic ecosystem, playing a crucial role in the salmon food web. *T. thermophila* eat bacteria, then are eaten by zooplankton, who are later eaten by salmon. Over many years, researchers have cultured and conducted experiments using *T. thermophila* due to their simplicity to work with and ability to double in about two hours

(Frankel & Nelsen, 2001). Some researchers found the optimal living conditions for *T. thermophila* to be close to 35°C and others found it to be closer to 39°C or even 40°C (Holz et al., 1959; Frankel et al., 1976; Frankel & Nelsen, 2001). Different temperatures affect the ability for this ciliate to grow and reproduce; determining their ability to reproduce under various circumstances will allow further understanding into their ability to maintain a stable food web and provide structure to the oceanic ecosystem.

Due to increasing human activities, carbon dioxide has been increasing in the atmosphere. This carbon dioxide gets dissolved into the ocean, quickly increasing its acidity and slowly increasing its temperature (Genner et al., 2014). The temperature of the ocean has been rising over the last several hundred years, which will have effects on all organisms at all levels of food webs. Currently, the average sea surface temperature is about 20°C (Voyager, 2014) but is expected to rise by almost 1°C by 2050 (Genner et al., 2017). At this rate of ocean warming, *T. thermophila* likely will not encounter a temperature greater than 40°C for at least 600 years if human activities remain as they are today. Unfortunately, with the current ocean temperature being so much lower than their optimal, they aren't actually in a living condition that is the most beneficial for them. If temperatures did rise close to 40°C in the near future, *T. thermophila* might face intensifying exponential growth rates which will cause a bottom-up cascade effect. This will have an effect on the salmon populations and higher tier predators in the oceanic ecosystem. This study aims to compare, identify and confirm population growth at various temperatures and apply the data to understanding and predicting real-life scenarios.

After thorough analysis of literature, the following hypotheses were developed for the experiment. The null hypothesis was determined to be that temperature does not affect the growth rate of *T. thermophila*. In addition, the alternate hypothesis is thus stated as temperature does have a significant effect on the growth rate of *T. thermophila*. It was predicted that the growth rate would increase with the increase in temperature, before reaching supraoptimal temperature, as the optimal temperature was mentioned to be around 35°C in past research papers (Holz et al., 1959; Frankel et al., 1976).

Methods

Preparation of Culture

A culture of *T.thermophila* had been grown in a sterile environment prior to experimental handling. This stock culture had been stored in a 125 mL Erlenmeyer flask and the top was covered with aluminum foil to ensure no contamination prior to the experiment. Going forward, any time that the opening to a flask or tube was exposed to the air, it was flamed to ensure that it was kept sterile (Cassidy-Hanley, 2012). The stock culture was of an unknown cell concentration, so in order to determine the concentration, the culture was first thoroughly mixed by being re-suspended several times with a micropipette. Multiple samples were taken and placed into Eppendorf tubes and the fixative, 3% glutaraldehyde, was added to freeze the *T. thermophila* cells in place. Once thoroughly mixed, 20 microlitres of this stock culture was added onto a Fuchs-Rosenthal Scientific Haemocytometer. The cells were counted using a

compound microscope. The average cell concentration of the stock culture was found to be 98,400 cells per millilitre.

A serial dilution was performed to get the stock culture to a working culture with the optimal cell concentration of 20,000 cells per millilitre (Ducoff et al., 1964). In a 125 mL Erlenmeyer flask, 19.31 mL of stock culture and 75.69 mL of culture media were mixed together. After labelling nine 15 mL test tubes with their corresponding temperature treatment, 10 mL of working stock was added to each. There were three replicates per temperature treatments of 25°C, 35°C, 39°C (Fig. 1). The tubes were placed in test tube racks and left overnight in their corresponding incubators. Eppendorf tubes for sampling were prepared ahead of time. These were labelled with the sampling time, number of the replicate (1, 2, 3) and the temperature. Each tube received 20 microlitres of 3% glutaraldyhyde (at the time of each sampling treatment). These sampling tubes were stored in a 4°C refrigerator overnight.

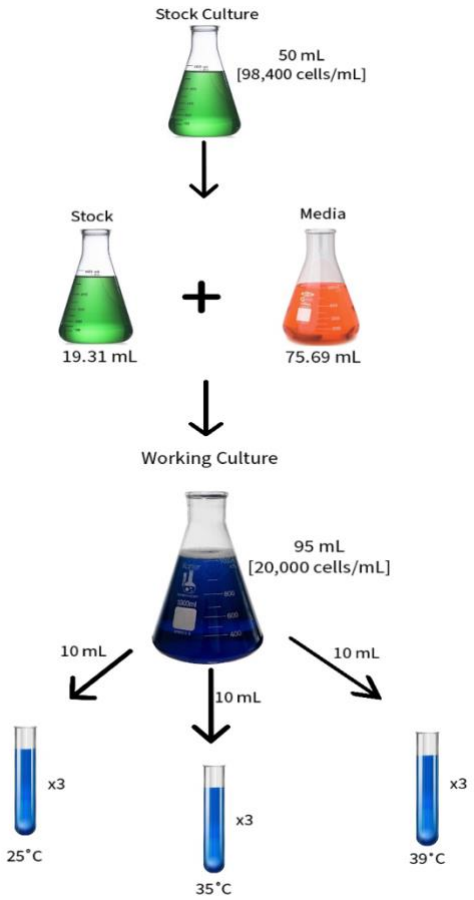

Figure 1. Previously prepared stock culture had been grown for use and was diluted according to serial dilution procedure to get to the optimal cell concentration. The final working stock was separated into the three treatments, with three replicates each then incubated at their corresponding temperatures.

Sampling the Treatments

Since *Tetrahymena thermophila* is able to double in about two hours (Frankel & Nelsen, 2001), the treatments were sampled every two hours in one single day, at 9AM, 11AM, 1PM, 3PM and 5PM. Upon opening the test tubes with the working stock, each tube was flamed and the cultures were re-suspended using micropipettes. For each replicate, 20 microlitres of fixative was already added to each Eppendorf tube during our preparation, and so then 100 microlitres was removed from the working stock and added to its corresponding Eppendorf tube and was thoroughly mixed with the previously added fixative. For each temperature treatment, there were a total of nine

Eppendorf tubes prepared for counting at a later date. All treatments were placed back in their appropriate incubators for another two hours until the next round of sampling.

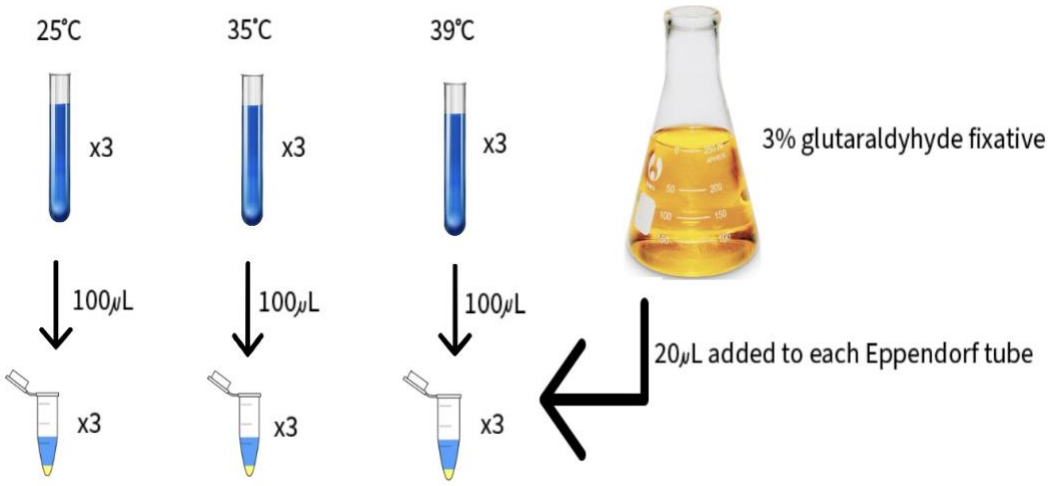

Figure 2. Procedure for sampling each replicate. Fixative was added and samples were placed into Eppendorf tubes for counting at a later date. All samples were re-suspended several times to ensure complete mixing.

Counting the Cells

There were nine Eppendorf tubes per sampling time, three replicates per temperature, for a total of 45 tubes to be counted. Using the Fuchs-Rosenthal Scientific Haemocytometer, 20 microlitres were loaded and counted using a compound microscope (Fig. 3). There were a total of two counts per tube and the average was later taken using an Excel spreadsheet. The counting was completed by all members of the group and each *T.thermophila* cell was recorded in our laboratory notebooks and compiled at the end of day into the same Excel spreadsheet.

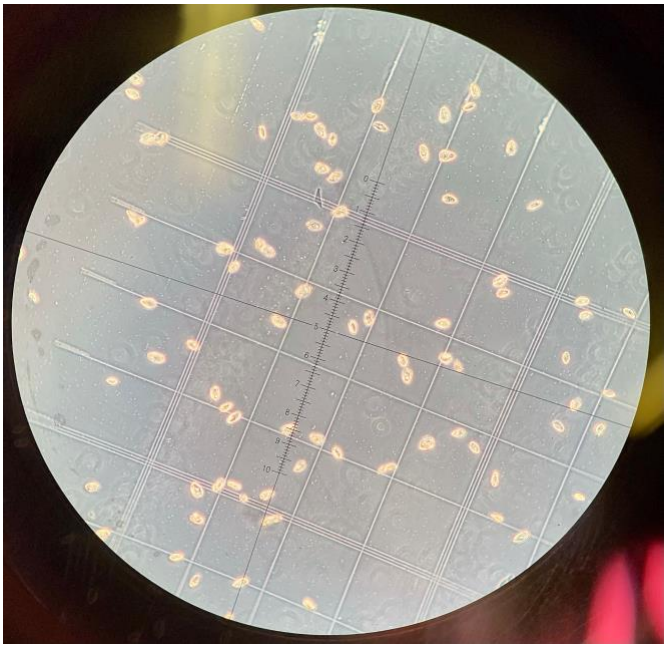

Figure 3. Image of *Tetrahymena thermophila* cells on a haemocytometer, from lens of compound microscope AxioLab R1517 C6, while using the 10X objective lens. Each count consisted of counting the cells present within the triple-lined squares until reaching around 150 for optimal cell volume.

Data Analysis

Once all the cell counting data was compiled into Excel, the data was then analyzed. Data analysis began with plotting the average number of cells/box ($1mm^2$) of the two counts against the time unit, hours. Three different graphs were plotted for the three different temperature treatments and within each graph, three data sets were plotted for the three replicates.

After the data was plotted, a linear regression line was used to obtain the slope which, in this case, would be the growth rate: number of cells/box ($1mm^2$) per hour. With those growth rates, the significance in the mean values between each different temperature treatment was determined using a one-way ANOVA test with the software, GraphPad.

Results

The growth rate of *T. thermophila* is known as the number of cells grown in a $1mm^2$ Haemocytometer square per hour. The growth rate values for replicates 1, 2 and 3 incubated at 25°C were -0.654, -0.612 and -0.546 respectively. The growth rate values for replicates 1, 2, and 3 incubated at 35°C were 0.340, 0.539 and -0.452 respectively. Finally, the growth rate values for replicates 1, 2 and 3 incubated at 39°C were -0.151, -0.224 and 0.231 respectively. Overall, the average growth rate values for *T. thermophila* incubated at 25°C, 35°C and 39°C were calculated to be -0.604, 0.142 and -0.048 respectively. The data indicates little to no growth in *T. thermophila* for all three temperature treatments. The average growth rate values were negative for 25°C and 39°C, while the average growth rate values for all three temperature treatments were near zero.

The growth rate for each replicate was obtained in Excel by plotting a linear regression line against the data, with the slopes representing the replicates' growth rate. The p-value was calculated to be 0.079; because this value is greater than the significance level 0.05, the ANOVA test concluded that there is no statistically significant difference between the average growth rates of *T. thermophila* incubated at 25°C, 35°C and 39°C.

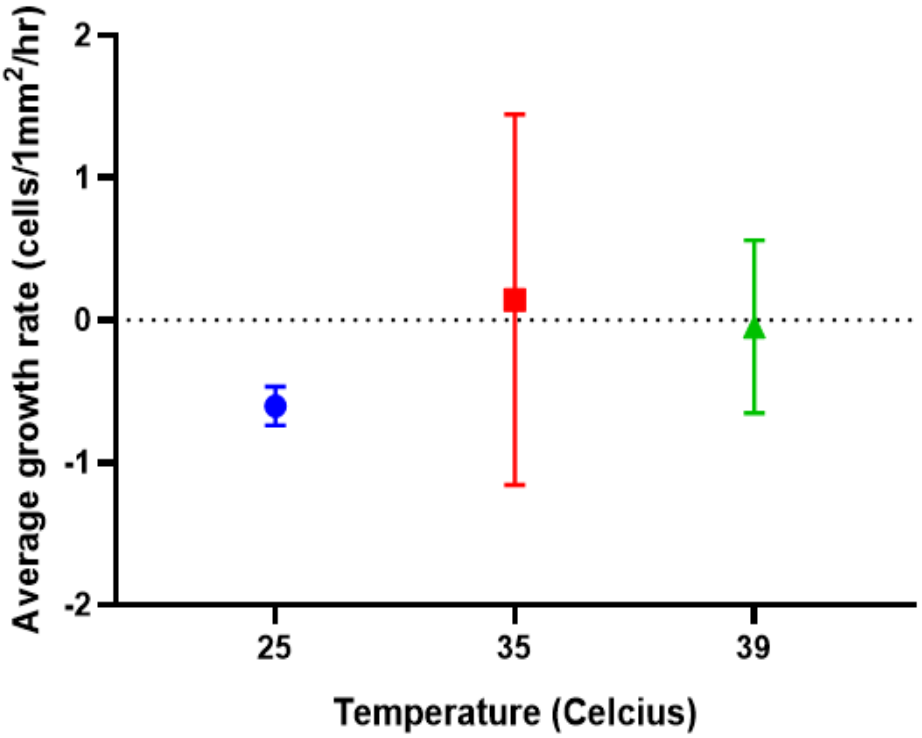

Figure 4. Average growth rate of *Tetrahymena thermophila* incubated for 24 hours at three different temperatures (25°C, 35°C and 39°C). Error bars represent 95% Confidence Interval. (n=3) for each temperature treatment.

Discussion

After a one-way ANOVA test, p-value was determined to be 0.079, which is greater than 5% significance value. Hence, the data failed to reject the null hypothesis, which states that the growth rate of *T. thermophila* will not be affected by temperature over time. Furthermore, the findings, in general, were inconsistent with various literature findings, however, the optimal temperature for *T. thermophila* growth at 35°C was in accordance with the number of studies (Holz et al., 1959, Frankel et al., 1976), which showed the highest growth rate among three temperature levels. Nonetheless, the findings were not in accordance with our prediction (Fig.4) that states — as the temperature increases the growth rate will also increase.

The difference between growth rate at 35°C and 39°C was fairly small, given that there was a strong overlap of 95% confidence intervals. The confidence intervals of the average growth rate of *T. thermophila* incubated at the three temperatures are all overlapping; thus, there was unlikely a large difference between the growth rate values of each temperature. This observation is further supported after analysing the replicate growth rates from Table 1 with a one-way ANOVA test. Likewise, a similar pattern has been observed in a study conducted by Frankel and Nelson (2001). One of the possible explanations from a biology standpoint is the changes in the formation of food vacuole in *T.thermophila.* Food vacuole and phagocytosis are proportional to each other in a way that the increase in food vacuole will increase the rate of phagocytosis (Jacobs et al., 2006), higher intake of nutrients which would in turn help in faster reproduction — increase growth rate. Furthermore, phagocytosis serves as the defence mechanism against pathogens according to Jacobs et al., (2006). Therefore, lower rate of phagocytosis due to changes in food vacuole formation might have increased the possibility of *T.thermophila's* exposure to bacterias from outside.

Uncertainties of the study

The results may have been influenced by some of the uncertainties presented in the study. A primary source of uncertainty was the lack of proper sterilization of the glassware used during sampling. *T. thermophila* is susceptible to contaminants (Cassidy-Handley, 2012), potentially leading to their destruction. Although careful, members might have forgotten to flambé glassware such as test tubes in between samples given that four members were sampling at the same time. Flambé is one of the

viable sterile techniques to avoid contaminations, such as exposure to microorganisms from the environment. Moreover, before pipetting the samples to the Haematocytometer for cell counting, it is essential to mix the samples properly for the possibility of equal distribution of cells. Although cell counting was averaged to a minimum of 150 cells, lack of proper distribution of cells could have resulted in a difference in the number of boxes used to reach an average minimum, at least.

To further improve this study, the consistency of sterility should be maintained throughout the study. By doing so would reduce the exposure of *T. thermophila* to the bacterias from the outside environment. Another factor that would help to improve this study is by increasing the number of temperature measurements and increasing the replicates during sampling because we had only 3 replicates per sample at a time, in order to increase the accuracy. According to a number of studies, it seems that the cell growing medium plays a vital role in understanding the cell growth rate of *T. thermophila* (Cassidy-Handley, 2012; Pinheiro et al., 2013; Frankel & Nelson, 2001). Thus, we could compare cell growth rate of *T. thermophila* in different mediums to observe if that influences our findings.

Conclusion

From the results of the data analysis, we fail to reject the null hypothesis. The results of this study oppose the initial prediction, as well as the past studies that were researched prior to our experiment which suggested *T. thermophila*'s growth rate increases with temperature (up to a certain degree). With that said, it is hoped that this

study will be useful for any future studies involving *T. thermophila*'s average population growth rate and its relation to temperature.

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