Exploring the effects of temperature on the growth rate of Tetrahymena *thermophila*.

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Abstract

The objective of the study was to determine the effect of temperature on *T. thermophila* growth rate. *T. thermophila* are free-living unicellular eukaryotes that are found in freshwater lakes, ponds, and streams. We measured the growth rate of *T. thermophila* at temperatures of 20°C, 35℃ and 41℃. There were three replicates per temperature treatment. Cell counts were taken at 0, 3, 21, 24, 27, and 45 hours and were used to determine the growth rate. Our results showed that the 20°C treatment had the highest growth rate, followed by the 35°C treatment, and the 41°C had the lowest growth rate. One-way ANOVA and Tukey's multiple comparison test were performed to determine the significance of the results. The one-way ANOVA was run on samples, including hour 3, which showed no statistical significance between the groups ($p = 0.0575$). Hour 3 was then excluded from the data due to human error during cell counting, and another one-way ANOVA was run, which was found to be statistically significant ($p = 0.0157$). Based on Tukey's multiple comparisons test, there was statistical significance found between 20°C treatment and 35°C treatment ($p= 0.0407$) and 20°C treatment and 41°C treatment ($p=0.0169$). There was no statistical significance found between 35°C treatment and 41°C treatment ($p=0.7429$). Given these results, we can reject the null hypothesis that temperature will not affect the growth rate of *T. thermophilia* at various temperatures and accept the alternative hypothesis for the dataset excluding hour 3. Thus, we can conclude that temperature has an effect on *T. thermophilia* growth rate.

Introduction

T. thermophila is a free-living ciliated protozoan naturally found in freshwater lakes, ponds, and streams (Ruehle et al., 2016). *T. thermophila* is a widely studied model organism for molecular and cellular biology, having contributed to fundamental discoveries in biology, such as the existence of catalytic RNA and the function of histone acetylation (Eisen et al., 2006). The presence of *T. thermophila* in research is primarily due to its ability to grow quickly, with a doubling time of two to three hours, leading to a high density in a wide variety of culture media and conditions (Eisen et al, 2006). Using *T. thermophila* in research is also cost-effective, and its large size of 30 to 50 μm – larger than many mammalian cells is ideal for investigation by light microscopy (Ruehle et al., 2016). Furthermore, recent whole genomic analyses of *T. thermophila* have provided the path toward future genomic research (Eisen et al., 2006).

Despite the popularity of *T. thermophila* in research, it is not well-studied ecologically (Doerder et al., 1996). The unicellular eukaryote is known to be a crucial part of the ecosystem, feeding on bacteria

and being fed on by zooplankton, who are, in turn, food for salmon (Maltby et al., 2020). The phenomena of human-caused climate change raise the question of how *T. thermophila* and the salmon food chain will be affected as the average global sea levels rise by a predicted 2°C-4°C by the year 2100 (Maltby et al., 2020). Numerous studies investigating the effect of temperature on the growth rate of *T. thermophila* have been conducted; however, most focus on optimal and supraoptimal temperatures of 35°C-41°C (Carvalho et al., 2019, Diki et al., 2021, Caputo et al., 2014, Afshari et al., 2016). While this makes sense from the perspective of biochemical research, it is not applicable ecologically, as the freshwater temperature is typically 20°C (Maltby et al., 2020). This study investigates a large range of temperatures, including 20°C, where *T. thermophila* typically lives, 35°C, which is considered the optimal temperature for growth, and 41°C, which is considered the highest temperature at which the eukaryote will still exhibit population growth (Frankel & Nelson, 2001).

In conducting this investigation, the null hypothesis is that temperature does not have an effect on the growth rate, whereas the alternative hypothesis is that temperature does have an effect on the growth rate. Based on previous studies on *T. thermophila*, it is predicted that temperature will have a significant difference in growth rate, with 20°C resulting in the lowest growth rate and 35°C having the highest growth rate (Frankel & Nelson, 2001).

Method

Culture Preparation

The 50 mL stock culture of *T. thermophila* was prepared in a sterile environment and obtained in a 125 mL sterile Erlenmeyer flask. The flask top was covered with aluminum foil to ensure the sample was not contaminated. To maintain a sterile environment, the flask and test tubes were flamed each time they were exposed. The stock culture was resuspended multiple times using a micropipette, ensuring it was thoroughly mixed. Since the stock culture was of unknown concentration, a sample was obtained to determine the initial concentration. Two 100 µL samples of stock culture each were collected into sterile Eppendorf tubes. Then, 10 µL of fixative were micropipetted into each tube to freeze the *T. thermophila*

cells. The samples were once again thoroughly mixed using a micropipette. Then, 20 µL of fixed cells were pipetted beneath a coverslip onto a sterilized hemocytometer. The hemocytometer was placed onto the stage of the compound light microscope using the 10x objective lens. The average cell concentration of the stock solution was calculated as 360 000 cells/mL.

A dilution was performed to prepare a working culture with optimal cell concentration of ~100 000 cell/mL using stock culture and medium (Fig. 1). In a sterilized Erlenmeyer flask, 27.78 mL of stock solution and 72.22 mL of culture medium were mixed together. There were three replicates per temperature treatments of 20°C, 35°C, and 41°C. Nine test tubes with 10mL of working solution were labelled with temperature treatment and replicate number and placed into three test tube racks corresponding to their temperature treatment. Next, 54 Eppendorf tubes were prepared and labelled with the temperature treatment, replicate number and sampling time.

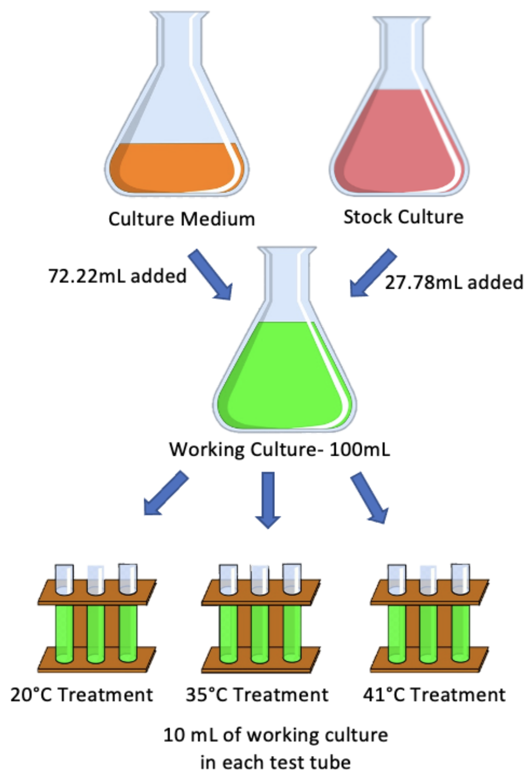

Figure 1: Procedure for producing working culture with the optimal cell concentration using serial dilution. The working culture was placed into three treatment test tubes with three replicates each. The test tubes were placed into their corresponding temperature incubators.

Sampling the Treatments

Based on prior research, *T. thermophila* have a doubling time of about two hours (Frankel & Nelson, 2001). Therefore, the treatments were sampled every three hours over a 45-hour period. On day 1, an initial count was taken at 12:00 pm, and a second count was taken at 3:00 pm. On day 2, three counts were taken at 9:30 am, 12:30 pm and 3:30 pm, respectively. Lastly, on day 3, a final count was taken. When opening the test tubes, each tube was inverted and flamed to ensure they were thoroughly mixed and there was no contamination. Then, 100 µL from each test tube and 10 µL of fixative were added to their corresponding Eppendorf tube. The tips of the micropipette were changed between each sample to ensure there was no contamination. The samples were thoroughly mixed by resuspending using the micropipette before they were placed onto the hemocytometer. There were a total of nine Eppendorf tubes used per counting time. There were a total of two counts taken per Eppendorf tube to ensure accuracy in counting. After each count, the test tubes were placed back into their corresponding incubators for the next round of counting.

Data Analysis

The number of cells counted was converted to represent cells/mL by using the dilution factors for the hemocytometer for *T. thermophilia.* The cells/mL for each time point were then put into the common logarithm. These values for each sample were plotted against the time point for each treatment group to determine the slope of the graph. The slope value was determined by the equation $y = mx + b$. The value of m represents the slope of the graph, which is the growth rate of the cells over time. The slope values for each sample and treatment were inputted into Graph Pad Prism 9 to run a one-way ANOVA and Tukey's multiple comparison test to determine the significance. The average of the slope values was calculated in Microsoft Excel for the samples in each of the three groups to give the average growth rate for each group. These steps were done for all the data and then repeated for data excluding the hour 3 time point due to experimental error.

Results

The number of cells was calculated per 1mm x 1mm for all 9 samples for each of the 6 time periods. All the graphs yielded exponential growth when the number of cells vs time was plotted, with all the slopes being positive in value.

A one-way ANOVA was run on the samples, including hour 3, and no statistical significance was found between the three groups ($p = 0.0575$) (Fig. 2). Hour 3 was then excluded from the data due to experimental error, and a one-way ANOVA was run for this dataset. It was found to be statistically significant ($p = 0.0157$) (Fig. 3). Tukey's multiple comparisons test found statistical significance between some of the groups. Statistical significance was found between Group 1 (20°C) and Group 2 (35°C) ($p = 0.0407$) and found between Group 1 (20°C) and Group 3 (41°C) ($p = 0.0169$). There was no statistical significance found between Group 2 (35°C) and Group 3 (41°C) ($p = 0.7429$). Group 1 (20°C) had the highest mean value (mean growth rate), followed by Group 2 (35°C) and then Group 3 (41°C).

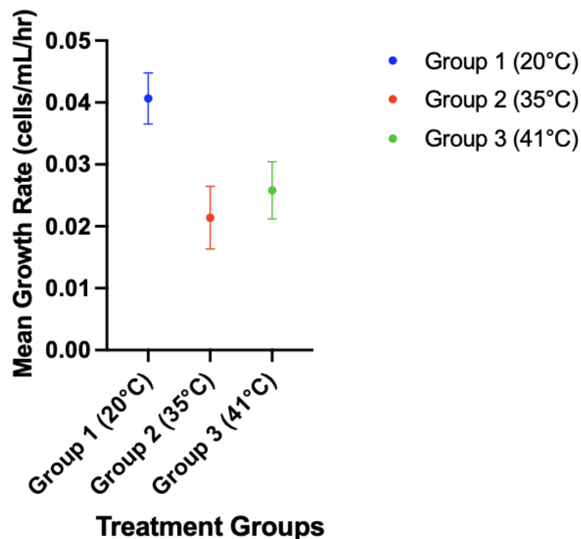

Figure 2. The graph plotted is the mean growth rate vs the treatment groups for the dataset, including hour 3. The sample for each group is n = 3. The arrow bars represent the standard error of the mean.

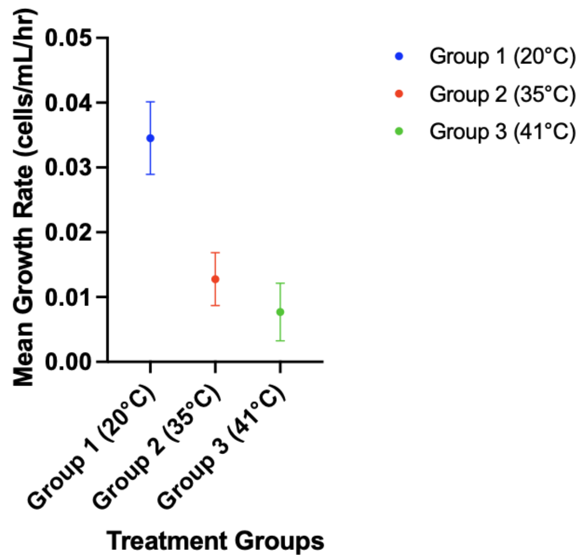

Figure 3. The graph plotted is the mean growth rate vs the treatment groups for the dataset, excluding hour 3. The sample for each group is n = 3. The arrow bars represent the standard error of the mean.

The average slope value was calculated for the groups with hour 3 time-point and without. Group 1 (20°C) had the highest growth rate for both with and without hour 3. For the dataset, including hour 3, Group 1 (20°C) had the highest ($\bar{x} = 0.0407$), followed by Group 2 (35°C) ($\bar{x} = 0.0214$), then Group 3 (41°C) ($\bar{x} = 0.0258$). For the dataset without hour 3, Group 1 had the highest mean ($\bar{x} = 0.0345$), followed by Group 2 (35°C) ($\bar{x} = 0.0128$), then Group 3 (41°C) ($\bar{x} = 0.00770$).

Discussion

Given the results of the one-way ANOVA, we can reject the null hypothesis that there will be no change in the growth rate of *T. thermophilia* at various temperatures and accept the alternative hypothesis for the dataset excluding hour 3. The cells experienced a significant growth rate when Group 1 (20°C) was compared with Group 2 (35°C) and Group 3 (41°C). However, there was no significant difference in growth rates between groups Group 2 (35°C) and Group 3 (41°C), which support the null hypothesis.

Our findings of the temperature affecting the growth rate of *T. thermophila* are supported by the findings in Frankel and Nelson's (2001) study. However, the specific temperatures at which *T. thermophila* exhibited the highest growth rate contradicted our findings. Frankel and Nelson (2001) found that *T. thermophila* exhibited the highest growth rate in the range of 30-39°C, which contradicts our study that found *T. thermophila* had the highest growth rate at 20°C followed by 35°C. This difference in results could be potentially due to the shift in temperatures from room temperature during sampling to the incubation temperature. These sudden temperature shifts could have caused "lags" in the increase in cell growth, which is known as the excess-day phenomenon (Frankel et al., 1980). The sudden change from room temperature to higher temperatures of 35°C and 41°C could put the cells in shock and delay division. This effect would be less for Group 1 at 20°C as it is similar to room temperature (Frankel et al., 1980). In our study, we did not return all our test tubes to the incubation fridges until after all samples were counted, taking approximately an hour each time and leaving more time for the cells to adjust to the room temperature. Whereas in Frankel and Nelson's (2001) study, they sampled directly from the shaking water baths, guaranteeing no sudden temperature changes and thorough mixing of the cells. Future studies should consider the excess-delay phenomenon and return the samples back to the incubation temperatures right away to reduce the shock and lag periods.

Salmon is a keystone species in the B.C. Coast which plays a significant part in the nitrogen cycle. On one hand, salmon are fed on by bears and wolves, which then carry the salmon carcasses into areas with lots of plant biomass. The breakdown of carcasses and the release of nitrogen into the soil, in turn, helps plant growth in that area (Walsh et al.,2020). Plants provide the planet with oxygen and remove CO_2. Therefore, salmon are crucial to the ecosystem and have an effect on the food chain, which means decreasing the salmon population would negatively impact many other species in the ecosystem by disrupting the whole food chain. *T. thermophila* plays a role in the salmon food web. *T. thermophila* eats bacterial microorganisms, and they are the primary food source for zooplankton. Zooplankton is, in turn, the main food source for salmon.

Based on Frankel and Nelson (2001), currently, *T. thermophila* are living below their optimal temperatures. However, based on studies, under optimal conditions, they have a rapid growth rate, with a doubling time of fewer than two hours (Frankel & Nelson, 2001). So, an increase in temperature would increase *T. thermophila* growth leading to decreases in the bacterial populations. This would lead to a decrease in zooplankton populations. Therefore, an increase in the *T. thermophila* growth rate could potentially negatively affect salmon populations. However, based on our results, *T. thermophila*, may be closer to their optimal temperature than originally seen in Frankel and Nelson's (2001) study. With our study finding that the highest growth rate was seen at 20°C, it demonstrates that more research is needed to determine the optimal growth of *T. thermophila*, to understand the risk to the salmon population better.

The context of our findings should be considered in terms of our limitations. There are some sources of error in our experiment. At time point hour 3, the test tubes were not inverted prior to sampling from the tubes. Therefore, all the cells were accumulated at the bottom of the test tubes, so the cell counts were significantly lower at this time point. It can be concluded that the experimental error that occurred in hour 3 did have a significant effect on the results as there was no significance found for the data, including hour 3. This error could have influenced our results as hour 3 is a critical time to see the growth of the cells, as it would be the first doubling time we observed. Without having adequate data from this time point, we could have missed a key element of our growth curve, reducing the validity and reliability of our results. Another source of experimental error was that two test tubes in Group 1 (20°C) were spilled, with over 50% of the solution being lost during hour 3. This error could have affected our cell counts for Group 1 and made these samples in Group 1 (20°C) more concentrated as the top portion was spilled from the test tube. This higher concentration in the samples compared to the other groups could potentially influence the growth rate of the cells in this group compared to the two other groups.

Conclusion

Based on our results, we can reject the null hypothesis that temperature will not affect the growth rate of *T. thermophilia* and accept the alternative hypothesis for the dataset excluding hour 3. The growth

rate was highest at 20°C, followed by 35°C, and lastly, 41°C. Our results oppose the initial prediction, as we predicted 35°C would have the highest growth rate and 20°C would have the lowest. However, we can still conclude that temperature has an effect on the growth rate of *T. thermophila.*

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References

Afshari, A., Noroozzadeh, K., Nowicki, J., Truong, K. "Hot or Cold: The effect of temperature on the growth rate of Tetrahymena thermophila". *The Expedition, vol. 6*, 2016. https://ojs.library.ubc.ca/index.php/expedition/article/view/189117

Caputo, T., Mrakovich, S., Sandhar, G., Sandhar, R. "The Effect of Changes in Temperature on the Doubling Time of Wild-Type Tetrahymena thermophila". *The Expedition, vol. 4,* 2014. https://ojs.library.ubc.ca/index.php/expedition/article/view/186420

Carvalho, C., Chow, C., Kraft, J., Strohan, M. "Growth Rate of Tetrahymena thermophila: Does progressive increases in incubation temperature result in a greater ability for T. Thermophila to adapt to temperatures outside literature ranges for tolerance". *The Expedition, vol. 9*, 2019. https://ojs.library.ubc.ca/index.php/expedition/article/view/193457

Diki, T., Gill, I., McPhail, S., Sun, C. "Effects of Increasing Temperature on the Growth Rate of Tetrahymena thermophila: the impact of climate change and adaptation for survival". *The Expedition, vol. 12*, 2021. https://ojs.library.ubc.ca/index.php/expedition/article/view/196825

Doerder, F. P., Arslanyolu, M., Saad, Y., Kaczmarek, M., Mendoza, M., & Mita, B. (1996). Ecological genetics of tetrahymena thermophila: Mating types, i-antigens, multiple alleles and epistasis. *The Journal of Eukaryotic Microbiology, 43(2)*, 95-100. https://doi.org/10.1111/j.1550-7408.1996.tb04487.x

Eisen, J. A., Coyne, R. S., Wu, M., Wu, D., Thiagarajan, M., Wortman, J. R., Badger, J. H., Ren, Q., Amedeo, P., Jones, K. M., Tallon, L. J., Delcher, A. L., Salzberg, S. L., Silva, J. C., Haas, B. J., Majoros, W. H., Farzad, M., Carlton, J. M., Smith, J., Roger K, . . . Orias, E. (2006). Macronuclear genome sequence of the ciliate tetrahymena thermophila, a model eukaryote. *PLoS Biology, 4(9)*, e286-e286. https://doi.org/10.1371/journal.pbio.0040286

Frankel, J., & Marlo Nelsen, E. (2001). The effects of supraoptimal temperatures on population growth and cortical patterning in tetrahymena pyriformis and tetrahymena thermophila: A comparison. *The Journal of Eukaryotic Microbiology, 48(2)*, 135-146. https://doi.org/10.1111/j.1550-7408.2001.tb00296.x

Frankel, J., Mohler, J., & Frankel, A. K. (1980). The relationship between the excess-delay phenomenon and temperature-sensitive periods in Tetrahymena thermophila. *Journal of Cell Science*, *43*(1), 75–91. https://doi.org/10.1242/jcs.43.1.75

Maltby, K. M., Rutterford, L. A., Tinker, J., Genner, M. J., Simpson, S. D., & Punt, A. (2020). Projected impacts of warming seas on commercially fished species at a biogeographic boundary of the european continental shelf. *The Journal of Applied Ecology, 57(11)*, 2222-2233. https://doi.org/10.1111/1365-2664.13724

Ruehle, M. D., Orias, E., & Pearson, C. G. (2016). Tetrahymena as a unicellular model eukaryote: Genetic and genomic tools. *Genetics (Austin), 203(2)*, 649-665. https://doi.org/10.1534/genetics.114.169748

Walsh, J. C., Pendray, J. E., Godwin, S. C., Artelle, K. A., Kindsvater, H. K., Field, R. D., Harding, J. N., Swain, N. R., & Reynolds, J. D. (2020). Relationships between Pacific salmon and aquatic and terrestrial ecosystems: Implications for ecosystem‐based management. *Ecology, 101(9).* https://doi.org/10.1002/ecy.3060