Effect of temperature on the growth rate of Tetrahymena thermophila

Rachael Callaghan, Petra Catsi, Pravnit Kooner and Nevrose Mangat

Abstract

In this experiment we studied the effect of temperature on the growth rate of Tetrahymena thermophila. T. thermophila are unicellular organisms that have been used in biology for many years and have a complex link with many key organisms in the ecosystem. We allowed the T. thermophila to grow in incubators at 3 separate temperatures, 13°C, 20°C, and 30°C. Samples were collected every hour on the first day and then our last sample was collected the next morning. Once all of our samples were collected we counted the number of cells in each. We predicted that T. thermophila will have the greatest growth at the highest temperature and through analysis of our results we were able to say that the temperatures were significantly different with a p-value of less than 0.05. The T. thermophila that grew at 13°C had the slowest growth compared to the growth at 30°C ($P < 0.031$). There was not a significant difference in growth between the 20°C and either of the 13°C or 30°C conditions ($P < 0.115$ and $P < 0.574$, respectively). Higher temperatures may result in a greater abundance of T. thermophila which could prove be to beneficial for other key organisms in the ecosystem that reply on these species.

Introduction

Tetrahymena thermophila is a unicellular ciliated protist that is commonly found in freshwater habitats around the world. T. thermophila has been utilized as a model organism in the field of biology for years, in part due to their rapid doubling time of 2-3 hours and their large size which allows for easy viewing during microscopy (Ruehle et al., 2016). In addition to these features, the organism also possesses two nuclei with separate genomes which has proven to be incredibly useful in understanding a variety of genomic processes (Orias et al., 2011).

Typically, ciliates such as T. thermophila obtain nutrition from bacteria, algae, and organic macromolecules found in their freshwater environments (Laakso et al., 2003). It has been suggested that T. thermophila and other ciliated protists may be a crucial link in freshwater food-chains between bacteria and larger organisms such as zooplankton that prey on them

(Wickham, 1995). Zooplankton in turn serve as a major food source for juvenile salmon in the early phases of their life cycle (Clark & Levy, 1988). The relationships between primary producers, protists, zooplankton, and fish are linked; therefore the abundance of one organism has widespread effects on the levels of the food-chain. The abundance of these organisms is also influenced heavily by abiotic variables such as temperature and pH in the environment (Beaugrand & Reid, 2003).

In this experiment, we investigated the effects of temperature on the growth rate of Tetrahymena thermophila culture grown in the laboratory in order to determine whether differences in temperature had a significant impact on their growth rate. Temperature is linked to the efficiency, rate of enzyme function, and protein synthesis in cells. Exposure to higher temperatures allows for increased protein synthesis required for cell division (Farewell & Neidhardt, 1998). Our hypotheses for the experiment were as follows:

H_0: Temperature will not have a significant effect on the rate of growth of Tetrahymena thermophila.

H_A: Temperature will have a significant effect on the rate of growth of Tetrahymena thermophila.

We predicted that growing replicates of T. thermophila at three separate temperatures: 13°C, 20°C, and 30°C would result in different growth rates for each temperature. Furthermore, we predicted that the replicates kept at 30°C would have the highest rate of growth while the replicates at 13°C would have the slowest growth rate.

The results of this experiment could prove useful in the continued understanding and use of T. thermophila as a model organism in the field of biology. Change in the growth rate of T. thermophila in freshwater streams due to temperature could influence both zooplankton and salmon abundance through food chain interaction. Salmon provide a range of ecosystem services to to other organisms and to humans in a given region. Therefore, the abundance of salmon greatly affects the proper functioning of the ecosystems that rely on them (Holmlund & Hammer, 1999). The rate of population growth of T. thermophila may play a part in maintaining the overall health of an ecosystem due to the potential effect on salmon populations.

Methods

We began by performing a dilution so that our T. thermophila stock solution would contain 20,000 cells/mL. We mixed the initial solution of T. thermophila that was given and then measured out 100 uL into an Eppendorf tube using a micropipette. Then we added 10 uL of glutaraldehyde fixative into the same tube and thoroughly mixed the sample. We loaded 20 uL onto a haemocytometer and counted the number of cells under an Axiostar Plus compound microscope. After obtaining the initial number of cells in our sample we calculated that we needed to add 14.5 mL of the T. thermophila sample and 85.5 mL of the media to make a 100 mL stock solution with a concentration of 20,000 cells/mL.

Once our stock solution was prepared we measured out 10 mL from the stock solution for each of the 9 test tubes (Figure 1). We had 3 replicates for each of our 3 temperature conditions (13°C, 20°C, 30°C). The test tubes were all stored in incubators at the specific

temperatures and every hour we collected samples from each of the 9 test tubes. At each time interval, we took out 100 uL from each of the test tubes and added it to an Eppendorf tube, in which we had previously added 10 uL of glutaraldehyde fixative. Using a micropipette, we thoroughly mixed the samples each time so that the fixative was well mixed in our samples. We took samples out from each of the 9 test tubes every hour on the first day, and then came in the next morning to obtain the last sample for a total of 63 samples (Figure 2).

After collecting all of our samples we began to count the number of cells in each of the samples using a haemocytometer and compound microscope (Figure 3). We mixed each of the samples thoroughly to have an even distribution of cells before loading 20 uL onto the haemocytometer, which was then placed under the compound microscope. We used a click counter to keep track of the number of cells observed under the microscope and recorded these values in a table. We used a dilution factor for the haemocytometer in conjunction with the dilution factor from adding fixative to calculate the average number of cells per mL for each sample.

We performed a one-way ANOVA to determine whether there was a significant difference in growth rates of Tetrahymena thermophila at the three different temperatures. We first calculated the growth rate of T. thermophila by subtracting the initial count from the final cell count, and then dividing by total time elapsed. For the one-way ANOVA we used this calculated growth rate as the response variable and the treatment temperature as the categorical variable. In this way we were able to test if the growth rate at the three different temperatures were significantly different from each other. After finding a significant difference between the means, we performed a Tukey-Kramer test to differentiate which

means where significantly different from each other, because this information is not conveyed in the one-way ANOVA. The statistical tests were performed with the statistical package R.

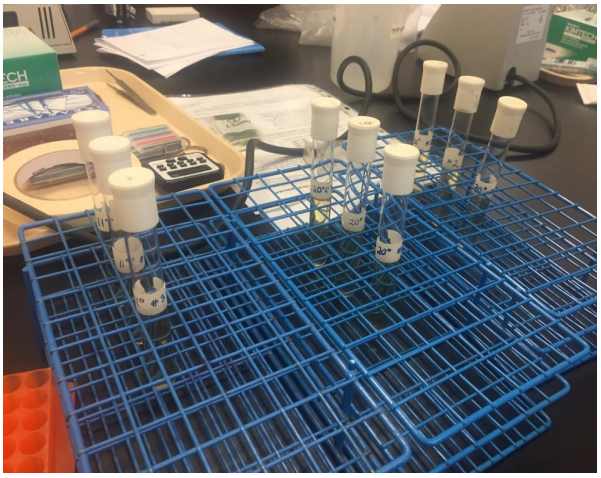

Figure 1. 9 test tubes with 3 replicates at each temperature (13°C, 20°C, 30°C).

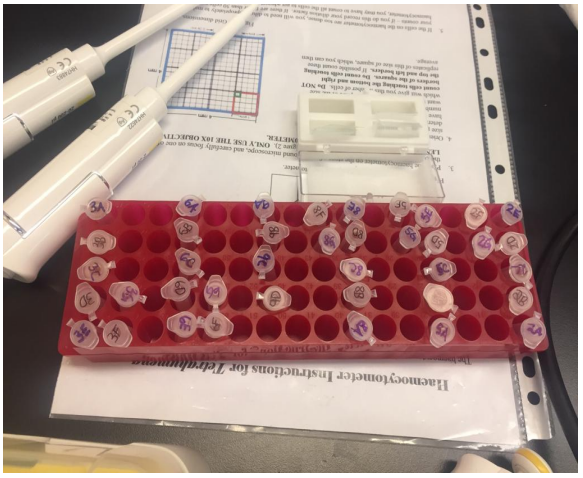

Figure 2. 100 uL samples collected every hour using micropipette and put in Eppendorf tubes.

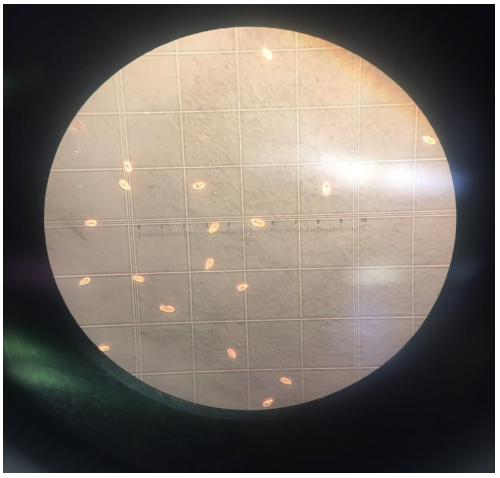

Figure 3. Tetrahymena thermophila cells under a compound microscope.

Results

The growth rate of T. thermophila was found to be significantly different between temperatures ($P < 0.0337$). T. thermophila kept at 13°C had a mean growth rate of -109 ∓ 182 cells/mL/hour, whereas cells kept at 20°C had a mean growth rate of 667 ∓ 695

cells/mL/hour. T. thermophila cells kept at 30°C had a mean growth rate of 1003 ∓ 268 cells/mL/hour (Figure 4).

It was found that the mean growth rate of T. thermophila at the lowest temperature, 13°C, was significantly different from those kept at the higher temperature, 30°C ($P < 0.031$). The mean growth rates of T. thermophila kept at 20°C was not significantly different from cells kept at 13°C or 30°C ($P < 0.115$ and $P < 0.574$, respectively).

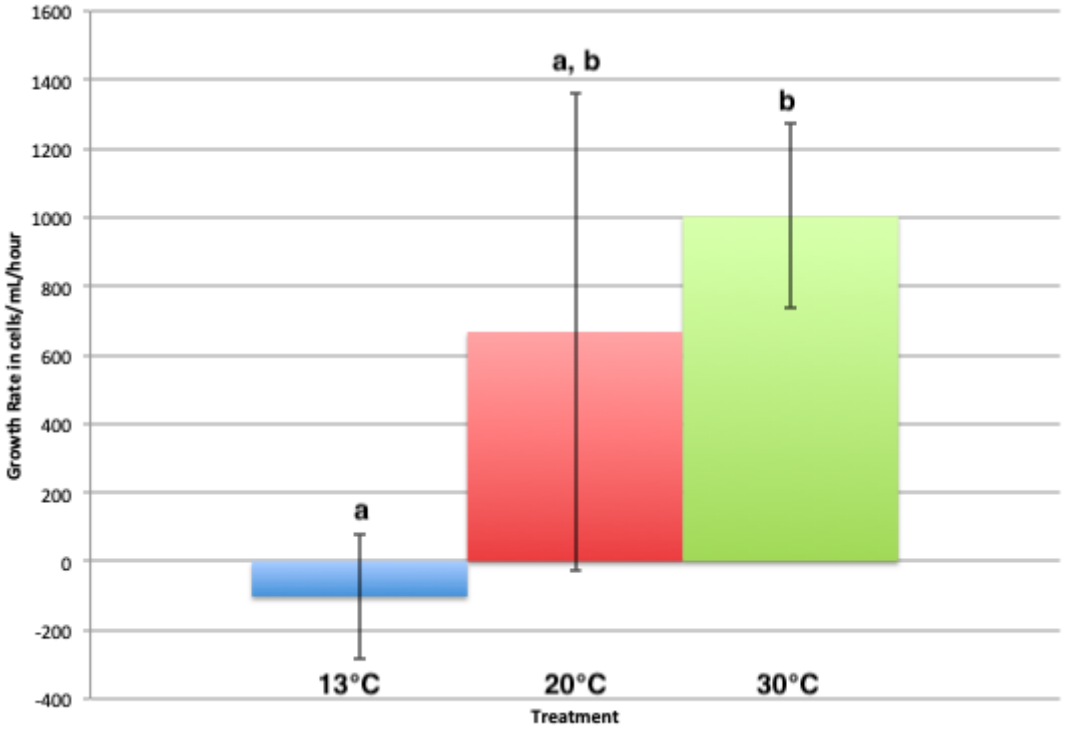

Figure 4: Growth rate of Tetrahymena thermophila **measured in cells per mL per hour (n=3).** Bars represent mean growth rate at each treatment temperature, and error bars represent 95% confidence intervals. Letter differences represent significant differences between treatments ($P < 0.031$).

Discussion

Based on our statistical analysis, we found that the growth rate at 13°C was significantly slower than 30°C. The difference between 20°C and either 13°C or 30°C, however, was not found to be statistically significant. Thus, we can reject our null hypothesis because the

growth rate was significantly different ($P < 0.05$) between the lowest and highest temperatures (13°C and 30°C), and thus provide support for our alternative hypothesis. Our prediction, which stated that growth rate would be highest at 30°C and lowest at 13°C, was also correct. Although the difference between 20°C and 30°C was not statistically significant, the rate at 30°C was greater. The trends overall were as follows: at 30°C the microorganism had a positive upward trend over time, at 20°C the number of cells fluctuated, but remained relatively the same over time, and at 13°C there was no trend in the growth of T. thermophila.

Our findings are consistent with most studies exploring the effect of temperature on Tetrahymena thermophila. Although the exact optimal temperature varies across studies, overall the trend observed is that an increase in temperature results in an increase in growth rate. One study exploring temperature's effect on T. thermophila growth is conducted by Frankel and Nelson (2001). They observed that T. thermophila grew faster when exposed to higher temperatures. In this study, however, they observed the optimal temperature to be 40°C (Frankel & Nelson, 2001). Although the optimal temperature was not explored in our study, our results align with those of Frankel and Nelson (2001).

One biological reason for the low growth rate at 13°C could be due to the findings in a study by Nägel and Wunderlich (1977). They found that at low temperatures the transport of RNA and ribosomes in cells is impaired in a different Tetrahymena species (Nägel & Wunderlich, 1977). RNA and ribosomes are required for the formation of proteins, which in turn are required for the growth of organisms. These results imply that the growth of Tetrahymena thermophila was slow at 13°C due to the lack of productivity in cell processes. Further, Jacobs et.al (2006) and Chan et. al (2016) found that increasing temperature increases the rate

of phagocytosis and vacuole formation, respectively. Phagocytosis is the process by which microorganisms engulf nutrients, which are needed for growth. Vacuole formation allows for storage of these nutrients, and their growth is positively correlated with the amount an organism can phagocytize (Jacobs et. al, 2006). Since both processes increase with temperature, the low growth rate we observed at 13°C compared to the higher temperatures can be explained.

The limitations of our methodology included the restricted lab hours where we collected our samples. We minimized the impact of this limitation by utilizing the entire window of time available to us for one day. Since we could not access the lab overnight we collected an additional sample the following morning. The impact this has on our results is that we could not monitor the growth of T. thermophila overnight. Another limitation of our study includes the small sample size. A larger number of replicates would aid in removing uncertainty or variation in our results, making our results more robust. A source of human error includes the variation in individuals using the haemocytometer to count cells. In order to limit the error posed by this, we attempted to calibrate our methods by practicing together before individually counting cells.

The results from our study can be extrapolated to environments where salmon species spawn and hatch (ie, freshwater environments). Since Tetrahymena thermophila is a microorganism that feeds the salmon population (Clark & Levy, 1988), higher temperatures imply that there may be an abundance in the nutrition gained by salmon through their food source. Although the effects for the environment cannot be concluded, it can be assumed that the increase in

nutrient abundance may impact the growth of salmon species with the increase in temperature.

Although more research is suggested to test the results of our study, they are nonetheless important because higher temperatures could mean a greater abundance of Tetrahymena thermophila in freshwater environments (Farewell & Neidhardt, 1998) . This implies an increase in abundance in organisms of higher food chain levels, such as zooplankton and ultimately freshwater fish, such as salmon (Wickham, 1995).

Conclusions

We rejected our null hypothesis, and thus provide support for our alternative hypothesis, which states that temperature has an effect on the growth rate of Tetrahymena thermophila. We hope that our results will aid future studies regarding Tetrahymena thermophila and its relationship with temperature.

Acknowledgments

We would like to thank UBC for giving us the opportunity to take this course. We would also like to thank Celeste Leander for giving us feedback and direction in the preparation, execution, and data analysis. We would like to thank Tessa Blanchard for her help and direction as well. Last but not least, we would like to thank Mindy Chow for providing us with the materials needed to perform the experiment.

Literature Cited

Beaugrand, G., & Reid, P. C. (2003). Long−term changes in phytoplankton, zooplankton and salmon related to climate. Global Change Biology, 9, 801-817. doi:10.1046/j.1365-2486.2003.00632.x

Chan, M., Pinter, A., & Soh, I. (2016). The effect of temperature on the rate of food vacuole formation in Tetrahymena thermophila [online]. The Expedition, vol. 6.

Clark, C., & Levy, D. (1988). Diel Vertical Migrations by Juvenile Sockeye Salmon and the Antipredation Window. The American Naturalist, 131(2), 271-290. Retrieved from http://www.jstor.org/stable/2461848

Farewell, A., & Neidhardt, F. C. (1998). Effect of temperature on in vivo protein synthetic capacity in Escherichia coli. Journal of bacteriology, 180(17), 4704-10.

Frankel, J., & Nelsen, E. M. (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. doi:10.1111/j.1550-7408.2001.tb00296.x

Holmlund, C. M., & Hammer, M. (1999). Ecosystem services generated by fish populations. Ecological Economics, 29(2), 253–268. https://doi.org/https://doi.org/10.1016/S0921-8009(99)00015-4

Jacobs, M. E., DeSouza, L. V., Samaranayake, H., Pearlman, R. E., Siu, K. W., & Klobutcher, L. A. (2006). The Tetrahymena thermophila phagosome proteome [online]. Eukaryotic Cell, 4 (12), 1990-2000. doi:10.1128/EC.00195-06

Laakso, J., Löytynoja, K., & Kaitala, V. (2003). Environmental noise and population dynamics of the ciliated protozoa Tetrahymena thermophila in aquatic microcosms. Oikos, 102, 663-671. doi:10.1034/j.1600-0706.2003.12319.x

Nagel, W., & Wunderlich, F. (1977). Effect of temperature on nuclear-membranes and nucleocytoplasmic RNA-transport in Tetrahymena grown at different temperatures. Journal of Membrane Biology, 32(2), 151-164.

Orias, E., Cervantes, M. D., & Hamilton, E. P. (2011). Tetrahymena thermophila, a unicellular eukaryote with separate germline and somatic genomes. Research in microbiology, 162(6), 578-86.

Ruehle, M. D., Orias, E., & Pearson, C. G. (2016). Tetrahymena as a Unicellular Model Eukaryote: Genetic and Genomic Tools. Genetics, 203(2), 649-65.

Wickham Stephen A., (1995). Trophic relations between cyclopoid copepods and ciliated protists: Complex interactions link the microbial and classic food webs. Limnology and Oceanography, 40, doi: 10.4319/lo.1995.40.6.1173.

Appendix

Time Point (in hours after start)	**13℃**	**20℃**	**30℃**
1	9281.25 ∓ 5730.07	8364.58 ∓ 9.29	11458.33 ∓ 14.50
2	8708.33 ∓ 1996.10	28760.42 ∓ 19.21	17989.58 ∓ 17.04
3	6645.83 ∓ 594.18	14666.67 ∓ 18.04	15697.91 ∓ 16.01
4	8937.50 ∓ 5487.27	23604.17 ∓ 24.01	18562.50 ∓ 6.93
5	8135.42 ∓ 5627.94	13750.00 ∓ 25.53	33572.92 ∓ 25.43
6	11114.58 ∓ 4284.70	22000.00 ∓ 22.91	23947.92 ∓ 36.35
16.5	6989.58 ∓ 5694.75	23375.00 ∓ 35.00	34031.25 ∓ 29.46

Figure A: Number of cells per mL at each time point (n=3). 95% confidence intervals are included. Numbers are calculated by averaging the number of cells counted in each haemocytometer, and then multiplied by the dilution factor of adding fixative and the dilution factor of the haemocytometer. This gives a final answer in cells per mL.