The Effect of Temperature and Salinity on the Growth Rate of *Tetrahymena thermophila*

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Abstract

Tetrahymena thermophila are ciliated unicellular eukaryotes that inhabit freshwater habitats and use the same food supply as salmon species. Its simple cellular structure, ability to adapt to different environments, and availability of genetic information make it an ideal organism for studying a wide range of biological phenomena. *T. thermophila* feed on bacteria which in turn are eaten by zooplankton, a primary food source to salmon species. The objective of our study was to determine the effects of temperature and sodium chloride (NaCl) on *T. thermophila* while keeping these interactions in mind. There is a global increase in temperature and freshwater salt content driven by human activities over the years. This study measured the growth rate of *T. thermophila* in 0mM and 50mM of NaCl that were incubated at 25°C and 35°C. For each temperature and salinity treatment, there were three replicates. Cell counts were taken at 0, 3, 6, 22.5, 25, 30 hours after incubation and cell concentrations were calculated from those counts. The results of this experiment showed that in the 0mM salinity treatments, the growth rate of *T. thermophila* increased from 25°C to 35°C. For the 50mM salinity treatments, the growth rate of *T. thermophila* decreased from 25°C to 35°C. Based on a two-way ANOVA, we can infer that temperature has little to no effect on growth rate of *T. thermophila*, salinity has a significant effect on growth rate of *T. thermophila*, and the interaction between temperature and salinity has a significant effect on the growth rate of *T. thermophila.*

Introduction

T. thermophila are members of the ciliated Protozoa which are an ecologically successful monophyletic group of unicellular eukaryotes (Orias et. al, 2011). They are free-living organisms in freshwater habitats around the world (Orias et. al, 2011). It is a common and widely-studied organism that is known for its ability to adapt to different environments and ability to carry out a wide range of functions, including photosynthesis, herbivory, and predation. *T. thermophila* are considered one of the fastest growing eukaryotes with a doubling time of less than two hours (Frankel & Marlo, 2001). The specific doubling time for *T. thermophila* can vary depending on a number of factors, such as the growth conditions, the presence of other organisms, and the availability of food and other resources.

The usefulness of *T. thermophila* as a model organism for biological research is influenced by its rapid growth rate. Various salmon species inhabit the same freshwater ecosystem during juvenile and spawning periods and also acquire the same resources for food as *T. thermophila* (Bardonnet & Baglinière, 2000). *T. thermophila* feed on zooplankton, which is the primary source of food for salmon species. (Eggers, 1978). Salmon eat zooplankton, which also prevents infections from infecting salmon and other animals higher up the food chain by phagocytosing contaminated germs (Eggers, 1978). Keeping these relationships in mind and knowing global temperatures are rising and humans are globally increasing the salt concentration of freshwaters, we investigated the effect temperature and salinity would have on the growth rate of *T. thermophila.*

Abiotic factors which include temperature and salinity play an important role in the variation of growth rate amongst these organisms. Temperature affects the cell function, metabolism, and growth rate of many aquatic organisms (Afshari et. al, 2017). In general, higher temperatures can increase the growth rate of *T. thermophila*, while lower temperatures can slow it down. This is because higher temperatures can speed up the chemical reactions that the organism needs to carry out in order to grow and reproduce. However, there is a limit to how high the temperature can be before it becomes harmful to the organism. At very high temperatures, the enzymes that are essential for the organism's survival can become denatured, leading to a decrease in growth rate or even death. *T. thermophila* can grow from anywhere between 20°C to 42°C and the optimal temperature is 35°C (Juren et. al, 2012). Salts are naturally found in soil and water, which contribute to water salinization and influence chemical processes in aquatic organisms. Considering that *T. thermophila* live in freshwater environments that contain little to no salt concentration, they grow best when exposed to 0.05% of NaCl (St Denis et. al, 2009). Salinity

above 20mM, reduces the metabolism and overall growth rate of *T. thermophila* (St Denis et. al, 2009). In general lower salinity can increase the growth rate of T. thermophila, while lower salinity can slow it down and potentially become lethal for the organism to grow.

The purpose of this experiment is to observe the impact of temperature and salinity on the growth rate of *T. thermophila*. We selected two different temperatures: 25°C and 35°C, as well as two salinity conditions: 0mM and 50mM of sodium chloride (NaCl). We hypothesized that temperature has an effect on the growth rate of *T. thermophila*, salinity has an effect on the growth rate of *T. thermophila*, and the interaction between temperature and salinity on the growth rate of *T. thermophila* will have an effect. As we are altering the organism's optimal temperature and optimal salinity in its natural habitat, we predict that increased temperature and salinity will have a significant impact on the growth rate of *T. thermophila.*

Methods

Preparation of Media and Samples

Media was prepared the day before the experiment for both salinity conditions. 5 mL of Tetrahymena medium was used for conditions under no salinity. For conditions under 50 mM salinity, Tetrahymena medium was mixed with NaCl under 1:1 ratio (2.5 mL each). Then, concentration of the cell stock was calculated for each of the three samples to determine the amount of stock to put into each of the test tubes. 12 test tubes were prepared as we had 3 replicates for each of the conditions. The starting concentration of the cell stock was 2.5 x 10^4 mM, and 3.31 mL of the cell stock was added to the prepared test tubes. Labels were created for each of the four conditions, then the calculated amount of stock was put into each of the test tubes. Our data at 0 hour was calculated from the cell stock. After the first 3 hours, tubes were taken out of the

incubator for sampling. Each tubes were sterilized by flaming the top opening of the test tube with an alcohol lamp before sampling. Cells in each of the test tubes were resuspended, as the cells were concentrated in the bottom of the test tube forming a white, foggy texture. 100 μL of cells were ejected into Eppendorf tubes for sampling. 10 μL a yellowish-brown Iodine Potassium Iodine (IKI) fixative was gently added to each of the samples, and made sure it was mixed well with the sample.

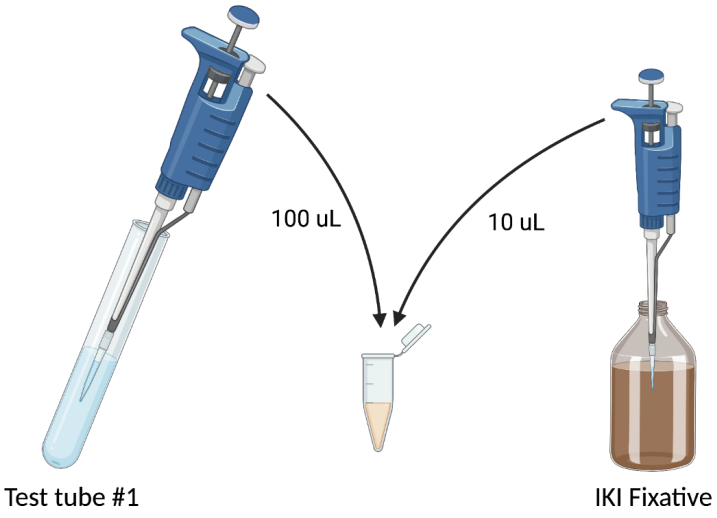

Figure 1. Preparing samples for hemocytometer. Created with BioRender.com

Counting cells

20 μL of the sample was pipetted between the cover glass and hemocytometer, then were counted approximately up to 150 cells. Cells were counted in 1 mm x 1 mm boxes. The boxes were counted from the top left to the right, with 4 boxes in one row until we reached the rightmost box with closed ends. Then the box right underneath was counted, moving in the opposite direction to reach the leftmost box with closed ends. The process was repeated until it reached 150 cells, or 16 boxes maximum. After counting a sample, the cover glass was sprayed with 70 % alcohol then

dried using a kimwipe. The aforementioned was repeated 4 times, with the unit of time(t) being hours, t=3, 6, 22.5, 25, 30.

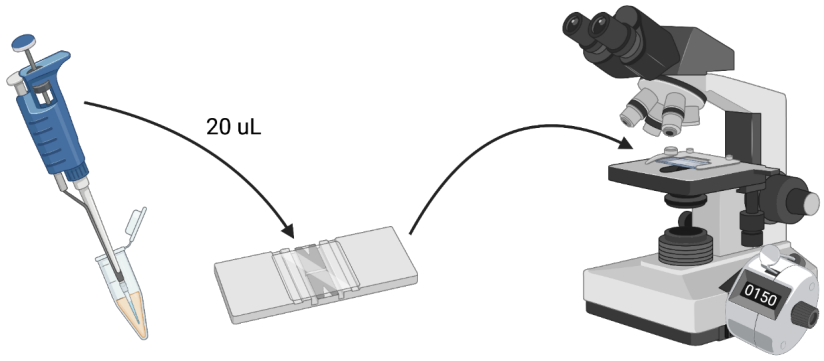

Figure 2. Pipetting between hemocytometer and the cover glass, then counting the number of cells using a tally counter. Created with BioRender.com

Statistical analysis

Cell counts were converted to cells/mL using excel spreadsheet. Then, the growth rate of the cells from each of the samples were graphed, with the number of cells plotted over time as the x-axis.

Values were put into Prism 9 to statistically determine the mean cell growth of each variable group using the 2-way ANOVA test with a confidence interval of 95%, to determine the significance of each of the variables and the growth rate of cells. Post-hoc analysis was done to determine significant interaction between different variable groups.

Results

Sample Calculations Concentration/Dilutions:

Sample Calculation for T. thermophila Concentration:

$$Concentration\ (cells/mL) = \frac{\#Cells}{\#\ Grids} \times \left(5 \times 10^3\right) \times 1.1$$

$\left(5 \times 10^3\right)$: dilution factor of 1mm x1mm hemocytometer grid
1.1 : dilution factor from using tetrahymena fixative

Sample Calculation for T. thermophila Stock:

$$Concentration\ (cells/mL) = \frac{155}{11} \times \left(5 \times 10^3\right) \times 1.1 = 77500$$

Sample Calculation for Starting Dilutions:

$$C1V1 = C2V2$$
$$C1 = average\ concentration\ T.thermophila\ stock$$
$$V1 = volume\ to\ achieve\ desired\ starting\ concentration$$
$$C2 = desired\ starting\ concentration\ (2.5 \times\ 10^4)$$
$$V2 = Total\ volume\ (10mL)$$

$$75544 \times V1 = \left(2.5 \times 10^4\right) \times 10$$
$$V1 = 3.31mL$$

2-WAY ANOVA TEST:

The 2-way ANOVA test results of the effect of temperature (°C) and salinity (mM) on *T. thermophila* growth rate (cells/hr). All data represented as means with ± s.e.m, alpha value of 0.05. 2-way ANOVA results for variables show salinity (P=0.0006), temperature (P= 0.0765), interaction between salinity and temperature (P=0.0020). Illustrated in Figure 1, significant interactions between groups found using post-hoc analysis denoted by asterisks (P<0.05). 0mM:25°C vs. 0mM:35°C

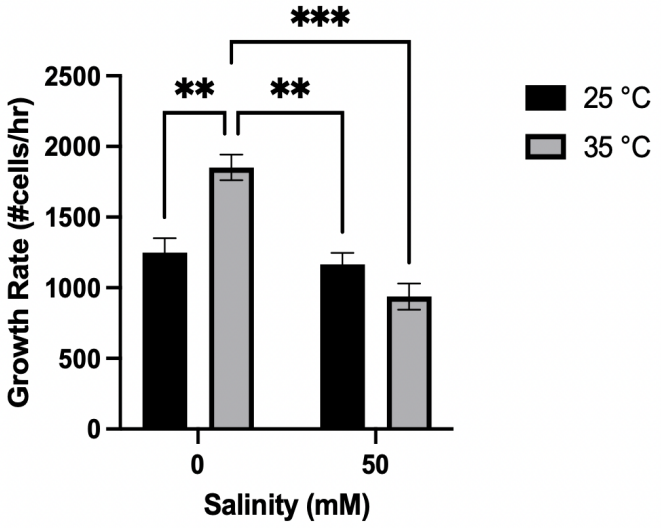

Figure 1. Interactive effects of temperature (°C) and salinity (mM) on growth rate of *T. thermophila* (cells/hr). All data reported as means ± s.e.m. Sample size: N=3 for each group. An asterisk denotes significant differences between groups from the significant interaction (P<0.05).

(P=0.0100); 0mM:35°C vs 50mM:25°C (P=0.0045); and 0mM:35°C vs. 50mM: 35°C (P=0.0007). Furthermore, s.e.m error bars only overlap for 0mM:25°C and 0mM:35°C experimental groups.

Discussion

The purpose of this study was to measure the impact of salinity and temperature on *T. thermophila*. A two-way Anova test revealed that we were able to reject the null hypothesis for salinity indicating that NaCl concentration had a significant effect on the growth rate of *T. thermophila*. We also found from the two way Anova test conducted that we fail to reject the null hypothesis for temperature. Although we could not reject our null hypothesis regarding temperature, we discovered that the interaction of salinity and temperature appears to have a significant impact on the overall growth rate. Therefore, we can reject our third null hypothesis proposed. This result is what we expected based on previous research conducted such as Juren et al (2012), El-Ashry, Mohamed T., et al. (1985),Jacobs et al., (2006).

Temperature effects phagocytic efficiency of the membrane of *T. thermophila* impacts its growth rate (Rasmussen, 1973). We hypothesized that the two temperatures selected 25°C and 35°C would have a significant effect. However, our results show that two temperatures tested do not have a significant effect on the growth rate of *T. thermophila* on its own. Therefore, we fail to reject our null hypothesis stating that temperature has no effect on the growth rate of *T. thermophila.* In Figure 1, the growth rate of *T. thermophila* increased significantly from 25°C to 35°C for the experimental group of 0mM salinity. This result aligns with the optimal temperature value of 35°C for *T. thermophila* (Frankel & Nelsen, 2001). However, for the 50mM salinity group it appears that increasing temperature from 25°C to 35°C decreased the growth rate. This could be due to possible sources of error as well as 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 by increasing the growth rate. Furthermore, phagocytosis serves as the defense mechanism against pathogens according to (Jacobs et al., 2006). Therefore, lower rate of phagocytosis could not only be due to temperature but the changes in food vacuole formation might have increased the possibility of *T. thermophila's* exposure to external bacteria exposure.

Salinity is an essential abiotic factor that affects all aquatic organisms. Since salt occurs naturally in soil and water, associated cations can change the chemical distribution of water molecules within aquatic organisms. This happens because increasing the cation size diminishes the electrostatic force of the ion on the water, leading to increased water–water hydrogen bonding, as would be seen around nonpolar solutes (El-Ashry et al.,1985). Our findings showed that increased salinity affected the growth of *T. thermophila* by decreasing the growth rate of *T. thermophila* significantly in the 35°C temperature group whilst there was no significant impact for the 25°C group. This is supported by other literature sources such as Juren et al (2012) that discovered around 20mM of NaCl slows down the food vacuole formation. This affects the overall metabolism and the growth rate of *T. thermophila* (Juren et al. ,2012).

NaCl is one of the most common salts in the ocean (Taylor and Kuwairi ,1978) and the relationship between sodium cation and *T. thermophila* has been well studied. Additionally, although there have been studies on the effect of salinity along with temperature for close relatives of *T. thermophila* such as microalgae; *Chlorella capsulata* and *Skeletonema costatum* which showed significant results in Ebrahim and Salarzadeh (2016) research , it has not been investigated for *T. thermophila*. Salinity is influenced by temperature through the rate of evaporation. As water

temperatures increase, so too does the evaporative rate which results in salt being left behind, making the remaining waters more saline (Walker, Richard H et al. ,2020). This aligns with our findings since there is a significant effect seen in the 35°C group where NaCl decreased the growth rate of *T. thermophila* whilst there was no significant impact for the 25°C group.

Additionally, sources of possible errors during the experiment include the caps of the test tubes not being closed right away after, which can lead to bacterias being built as it is exposed to the airborne contaminants. Improper or missing the step of sterilization could have been another source of contamination regarding the test tubes. Another source of discrepancy might have occurred due to insufficient resuspension when collecting the samples. Since the cells are mostly submerged on the bottom of the test tubes, the data collected would be highly insignificant without resuspension, as it wouldn't represent an even portion of the sample. For future studies, we recommend preparing the cell culture two days prior to the main experimental day. This will allow the experimenters to observe the growth of *T . thermophila* under optimal conditions and observe if the doubling times differ from the literature values expected. We also recommend future experiments to determine the concentration of each *T. thermophila* cell in each replicate for all experimenters at the same time, to ensure that a uniform concentration is present in all the samples tested and to minimize variation in our data.

Conclusion

Our results provided partial support for our hypotheses, two hypotheses were rejected and one failed to reject. Temperature has little to no effect on the growth rate of *T. thermophila*.. Salinity has a significant effect on the growth rate of *T. thermophila.* The interaction between temperature and salinity have a significant effect on the growth rate of *T. thermophila*. At 0mM salinity treatments, the growth rate of *T. thermophila* increased from 25°C to 35°C. The opposite was found in the 50mM salinity treatments. At 50mM salinity, there was a decrease in the growth rate amongst organisms from 25°C to 35°C that resulted in the least amount of cells reproducing at 35°C.

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