Investigation of How Temperature Impacts the Population Growth Rate of *Tetrahymena thermophila*

Saurav Acharya, Jocelyn Cheng, Maddy Haines, Sung Ju Lee

Abstract

Global temperatures are increasing as a result of climate change (Hansen et al., 2010). Alterations in temperature have the potential to significantly impact all ecosystems and the species that inhabit them. *Tetrahymena thermophila* (*T. thermophila*), a freshwater phagocytic cilate, is one of many species that may be impacted by rising water temperatures. The objective of this study was to measure the differences in the population growth rate of wild-type *T. thermophila* at 11˚C, 20˚C, 30˚C, and 40˚C, respectively. This experiment was executed by initially diluting the stock solution of *T. thermophila* with *Tetrahymena* media, SSP growth medium. This step was performed to provide optimal conditions and avoided restricting growth rates of *T. thermophila* by limiting space and nutrients. A sample was taken to be counted at each respective temperature every 2 hours for a total of 8 hours and lastly, at the 26 hour mark to complete the growth curve. A one-way ANOVA analysis was performed and a p-value of 0.04706 was obtained. This provided moderate evidence to reject the null hypothesis at a 5% significance level that temperature has no effect on *T. thermophila* population growth rate. The rejection of the null hypothesis lent support for the alternative hypothesis that temperature does in fact have an effect on population growth rate. Overall, it was noted that as temperature increases, growth rate increases as well. Using a post-hoc Tukey-Kramer HSD test on the one-way ANOVA results, it was determined that there was a significant difference in the population growth rate for the 11˚C and 40˚C treatment groups.

Introduction

In 2016, the Canadian seafood industry generated more than $6.6 billion profit with salmon sales contributing largely to this economic success (Fletcher, 2017). Not only do salmon species play an integral role in the Canadian economy, but they are also a keystone species within North American aquatic ecosystems. Salmon play an ecologically significant role in maintaining the circulation of nutrients and organic matter within both aquatic ecosystems and surrounding terrestrial environments (Garibaldi & Turner, 2004). However, salmon return rates

have been declining in recent years throughout the British Columbia and the western coast of North America (Naiman et al., 2002). This decline in salmon population threatens the health of ecosystem and national economy.

Tetrahymena thermophila (*T. thermophila*) are eukaryotic heterotrophic unicellular organisms (Figure 1). These teardrop-shaped organisms are approximately 20 μm in width and 50μm in length and are covered in cilia (Wloga & Frankel, 2012 and Collins & Gorovsky, 2005). These ciliates are extremely motile and inhabit temperate freshwater environments throughout North America (Eisen et al, 2006). Some ciliates are opportunistic pathogens that have the capacity to consume fish tissues when the fish is distressed or wounded (Pinheiro & Bols, 2014). Also, Pinheiro and Bols noted on another paper that when some species of *Tetrahymena* have large enough population sizes, they may be pathogenic and necrotizing to fish populations (2014). A study by Pinheiro and Bols cultured specifically *T. thermophila* with different animal cells, which included salmon tissues such as steelhead and chinook (2014). It was found that with incubation time, ciliates continued to flourish and swam around increasing contact with monolayers of fish epithelial cells. When in contact, *T. thermophila* were able initiate monolayer destruction and over few days, cells were completely consumed. The result also showed that at low temperature, 4˚C and 14˚C, the swimming of ciliates slowed, decreasing the contact made between ciliates and epithelial cells.

Another research study conducted by Stolfa and Koudelka (2013) indicates that the *T. thermophila*, in particular, consume bacteria through phagocytosis. By limiting the growth of

bacterial populations, *T. thermophila* may impact food-web interactions within aquatic ecosystems. Bacteria serve as a food source for zooplankton populations who in turn are a main food source for juvenile salmon. Therefore, *T. thermophila* population size may influence the availability of food sources for salmon through *T. thermophila's* predatory interactions with bacteria (Werlin et al., 2011). By understanding temperature's influence on the population growth rate of *T. thermophila*, it may be possible to gain insight into how temperature changes in aquatic ecosystems may impact salmon populations in the future.

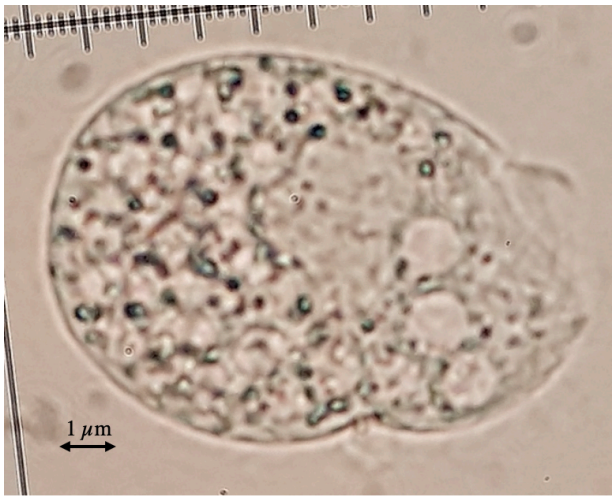

Figure 1. *T. thermophila* under an Axiostar compound light microscope at 100x magnification.

As a result of these findings, it is imperative that factors that influence the population size of *T. thermophila* should be better understood in order to gain insight into how these populations may impact key stone species, such as salmon. *T. thermophila* growth rates demonstrate a direct relationship with changes in their environmental temperature (Doerder et al., 1995).

T. thermophila subjected to replicate, in aquatic environments with a temperature of 30°C, were observed by Cole and Sugai on average every 150 minutes (2012). Frankel and Nelsen recorded that *T. thermophila*'s highest population growth rate was at 37.5˚C and a decline in population growth rate was observed at 39.5˚C (2001). The maximum temperature at which *T. thermophila* were able to grow was observed to be 41˚C (Frankel and Nelsen, 2001). In fact, after being subjected to 41˚C for a period of 6 hours, the number of *T. thermophila* cells in the solution were observed to decrease.

Following analysis of this literature, we examined how temperature affects the population growth rate of *T. thermophila.* As a result of our primary research, we developed our null hypotheses that states that temperature has no effect on the growth rate of *T. thermophila* population size. However, our alternative hypothesis states that temperature does have an effect on the growth rate of *T. thermophila.* We predict that greatest growth rate of *T. thermophila* will occur at our control temperature treatment of 40˚C.

Methods

Sample size:

The population growth rate of *T. thermophila* were measured at four different temperature treatments within the tolerable range for *T. thermophila* (11°C, 20°C, 30°C, and 40°C, respectively). The control temperature was set at 40˚C, as it is the optimal temperature for *T. thermophila* population growth (Franklen & Nelsen, 2001). Each temperature had three replicates, therefore, the total sample size for all temperature treatments were twelve. The growth

rate of each temperature was determined by averaging the growth rate of the three respective replicates.

Data Collection:

1. *Sample Preparation:*

The concentration of the stock solution of wild-type *T. thermophila,* grown by Mindy Chow in UBC's Biology 342 Integrative Biology Laboratory, with *Tetrahymena* media, SSP growth medium, was determined using a *Tetrahymena* haemocytometer. Then, the given stock solution was then diluted to 20,000 cells/mL to produce the sample solution. This sample solution, in turn, was divided into twelve respective test tubes. Each temperature condition required three replicates and therefore, three test tubes were subjected to one of the specified temperature conditions (11°C, 20°C, 30°C, and 40°C).

To begin, the stock solution's initial concentration of *T. thermophila* was determined (Figure 2). The stock solution was mixed well using a Fischer Vortex Genie 2™. Next, 200 µL of the solution and 20 µL of glutaraldehyde fixative were micropipetted into a sterile Eppendorf tube. The solution in this Eppendorf tube was vortexed to fully fix the *T. thermophila* and to ensure that their initial population size could be determined. This vortexing was achieved using a micropipette by pushing the micropipette button up and down only to the first stop.

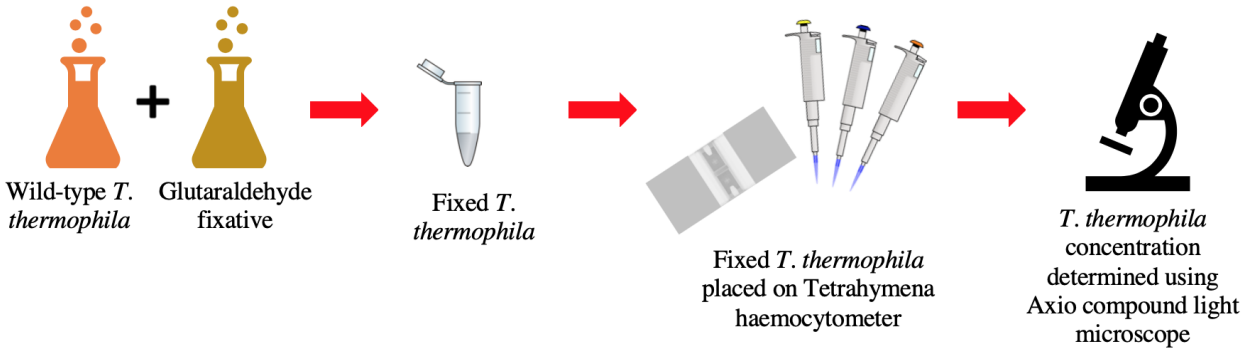

Figure 2. Steps for determining the concentration of given stock solution wild-type *T. thermophila.*

A Fisherbrand® micro-coverslip was placed over the haemocytometer grid (Figure 3) and the 20µL of fixed solution was micropipetted under the micro-coverslip. Then, the haemocytometer was placed on the stage of the 10175 Axio compound light microscope under 10x objective lens. On a 1 mm x 1 mm grid, 86 cells were counted, and due to this a dilution factor of 5 x 10^3 was used. Equation (1) was used to calculate the concentration of the stock solution with a value of 1.1 set for the correction of fixative value. The stock solution of *T. thermophila* was determined to have a concentration of 473,000 cells/mL.

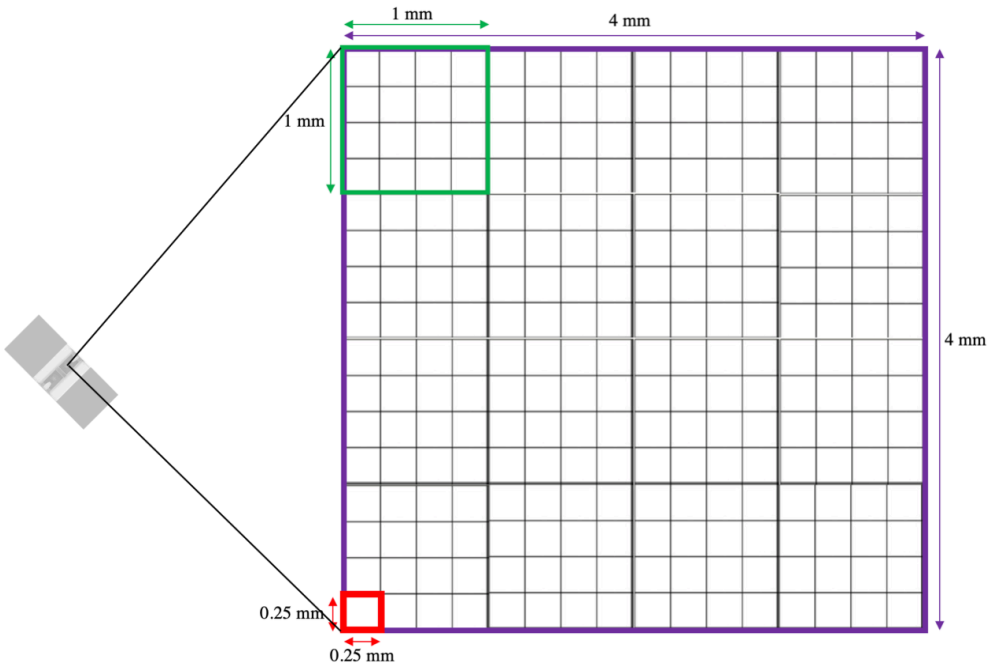

Figure 3. *Tetrahymena* haemocytometer grid: Red box has a dimension of 0.25 mm by 0.25 mm with dilution factor of 8 x 10^4, green box is 1 mm by 1 mm with dilution factor of 5 x 10^3 and the purple box is 4 mm by 4 mm corresponds with a dilution factor of 3.125 x 10^2.

$$Final\ Cell\ Count\ \left(\frac{cells}{mL}\right) = Numbers\ of\ cells\ x\ Dilution\ factor\ of\ haemocytometer\ square\ x\ Correction\ for\ fixative \quad \textbf{(1)}$$

Afterwards, the stock solution was diluted to ensure that researchers could accurately observe the growth rate. Equation (2), was used to determine the volume of the initial stock solution (V_1) with an initial concentration (C_1) of 473,000 cells/mL, which was required to make a final concentration (C_2) of 20,000 cells/mL along with final volume (V_2) of 80 mL. In a new sterile Erlenmeyer flask, 3.38 mL of stock solution and 76.62 mL of SSP growth medium was added to make a final volume of 80 mL of diluted *T. thermophila* solution (Figure 4A). To make 80 mL of SSP growth medium, the following materials were combined: 1.6 g of 2% proteose peptone, 0.08 g of 0.1% yeast extract, 0.16 g of 0.2% glucose and 0.058 mL of 33 µM $FeCl_3$(chlamy stock). However, if

the common chlamy stock is unavailable for use in lab, it is possible to have it substituted with approximately 0.029 mL of [25g/L] $FeCl_3*6H_2O$ instead.

$$C_1V_1 = C_2V_2 \quad \textbf{(2)}$$

From the Erlenmeyer flask, 6 mL of diluted solution was taken out using a pipette and placed into 12 separate test tubes. It was crucial at this step that the diluted solution was thoroughly mixed to ensure that an even number of cells were divided into each test tube. Test tubes with a fitted cap were opened and sterilized over an open flame prior to the transfer of diluted solution. Each test tube was labelled with the temperature and the replicate (A, B or C) (Figure 4B). Three test tubes sealed with caps were then placed into a test tube rack, with four test tube racks being used in total.

Due to lab time constraints (lab access was only permitted between 9:00 am to 5:00 pm daily) the research team performed stock dilutions one day prior to monitoring population growth rates. As a result, the test tube racks and samples were stored in the fridge at 4˚C overnight on Nov. 6, 2018 to minimize population growth. The following morning, on Nov. 7, 2018, researchers placed one test tube rack into each incubator at its respective temperature (Figure 4C). The temperatures of each of the incubators were 11˚C, 20˚C, 30˚C and 40˚C.

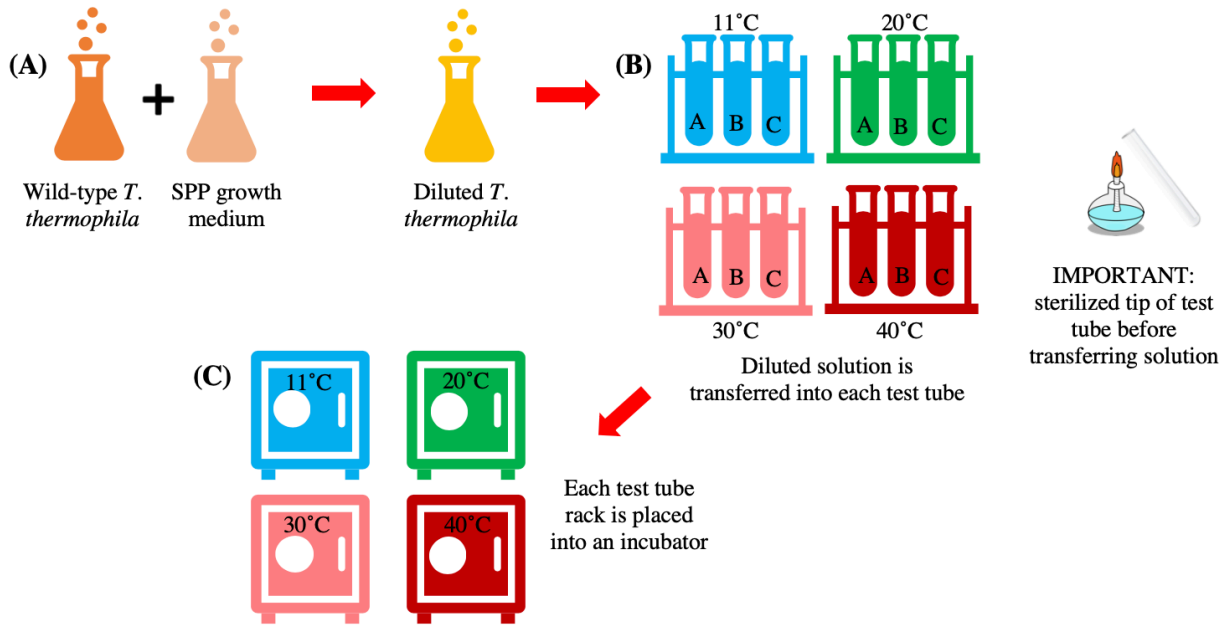

Figure 4. Preparation steps to generate the diluted solution for incubation at the four respective temperatures. **4A.** dilution of wild-type *T. thermophila*. **4B.** preparation of four test tube racks for different temperature, each with three replicates with diluted solution of *T. thermophila*. **4C.** four incubators were preheated to desired temperature before test tube racks were placed in.

2. *Measuring Population Growth:*

T. thermophila were incubated at different temperatures for a total 26 hours. For the first 8 hours, a sample was collected every 2 hours and the last sample collection took place at the 26th hour (Figure 5). In advance, all 72 Eppendorf tubes were filled with 20 µL of glutaraldehyde fixative and labeled with the temperature, replicate, and the time collected (0, 2, 4, 6, 8, or 26 hours). The sample time started at 0 hours (Figure 5) due to the fact that the test tubes were left in the fridge overnight because of lab time constraints.

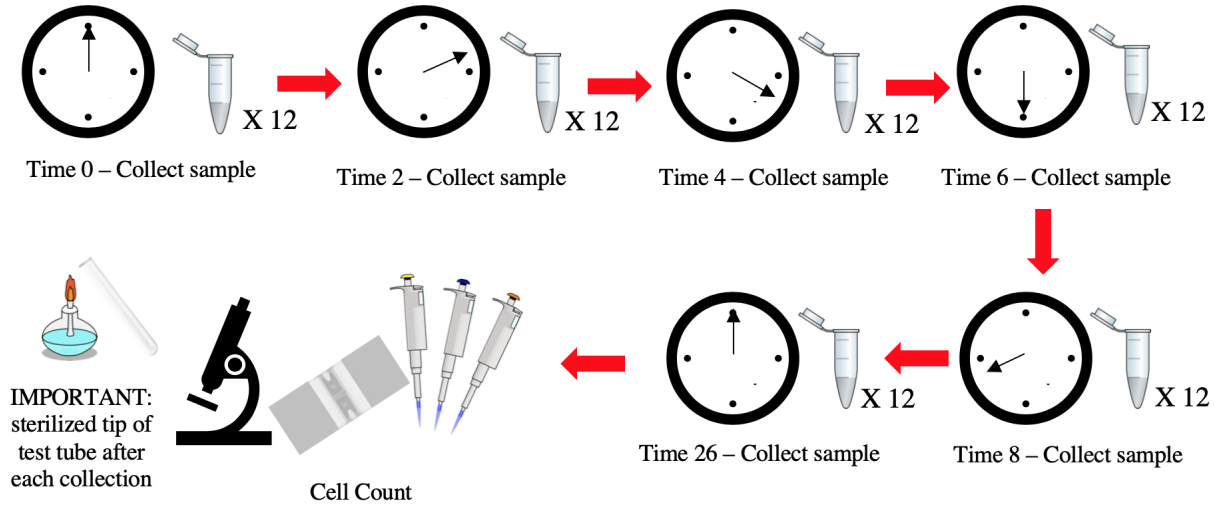

Figure 5. Step by step illustration on our method collecting 12 samples every 2 hours over a 26 hour period.

For our sample collection, 200 µL of *T. thermophila* was taken out from each of the 12 cultures, respectively, and placed into its correctly labelled Eppendorf tube. It was crucial that after each sample collection, the test tube tips were again sterilized over an open flame to reduce contamination of sample from growth of other microorganisms other than *T. thermophila*. Cell counts using the haemocytometer occurred after all samples were collected on Nov. 9, 2018. Researchers aimed to perform at least two pseudo-replicates on each Eppendorf tube within the allotted time and averaged the cell number afterwards. The process of determining cell counts was the same as the process used when determining the stock solution's concentration. The solution inside the Eppendorf tube was first vortexed, then 20µL solution was micropipetted out and placed on the haemocytometer to observe. The same Axio compound light microscope (10175) and 10174 were used to count the number of cells. Equation (1) was used to calculate the cell density of each temperature by averaging the number of cells of the three replicates and a value of 1.1 was used for the correction of the fixative value.

Data Analysis:

With the collected cell counts, two data analyses were performed. First, we studied the growth curve over time for different temperature treatments. To do so, the average cell density at each time interval and temperature treatment was calculated and plotted on a multiple line plot using R Studio (Figure 6). The log of the average cell density was taken and plotted with the semi-logarithmic graph to observe the relationships and trends. Secondly, from the collected data on cell counts, the mean growth rate (cell density/day) of *T. thermophila* was calculated at teach temperature. A one-way ANOVA test was conducted on the data. If the null hypothesis was rejected, a Tukey-Kramer HSD test was used for multiple pairwise comparisons.

Results

From the observed cell counts of *T. thermophila*, growth trends for the four treatments (11˚C, 20˚C, 30˚C and 40˚C) were observed by plotting the log average cell density (cells/mL) for each treatment over a period of 26 hours at 2-hour increments for the first 8 hours (Figure 6). There appeared to be a general positive trend that displayed an increase in average cell density over time for each temperature treatment. However, it should be noted that average cell density decreased slightly from the 3 to 4 time interval at 11˚C, 5 to 6 time interval at 20˚C, as well as from the 5 to 6 time interval at 40˚C. It should be noted that at time 0, the log of average cell density at all temperatures were different. To test the null hypothesis that temperature has no effect of the population size of *T. thermophila*, a one-way ANOVA test was performed on the

results. A one-way ANOVA was conducted, rather than a two-way ANOVA because we only considered one variable of interest, that is, temperature.

To compare the growth rate of *T. thermophila* at different temperature treatments, a bar chart was plotted using R Studio (Figure 7). From the one-way ANOVA, the F statistic was compared to the $F_{3, 8}$ distribution. The 95% point of this distribution is 2.700409, and as the F statistic of 4.1755 is greater than this value, it lies in the critical region. A p-value of 0.04706 was also calculated. At a 5% significance level, the null hypothesis stating that temperature has no effect of the population size of *T. thermophila* was rejected. Since the p-value was only slightly less than the significance level of 0.05, there is moderate evidence to reject the claim that the mean growth rates under the four temperature treatments are the same.

Furthermore, since the underlying means of the growth rates of *T. thermophila* were not equal between the treatment groups, a post-hoc Tukey HSD test was conducted on the one-way ANOVA results. The Tukey HSD multiple pairwise comparisons test showed that growth rates were significantly different at a significance level of 0.05 between the 11˚C-40˚C groups. However, there were no statistical differences measured in growth rates between the 11˚C-20˚C, 11˚C-30˚C, 20˚C-40˚C, 20˚C-30˚C, and 30˚C-40˚C groups.

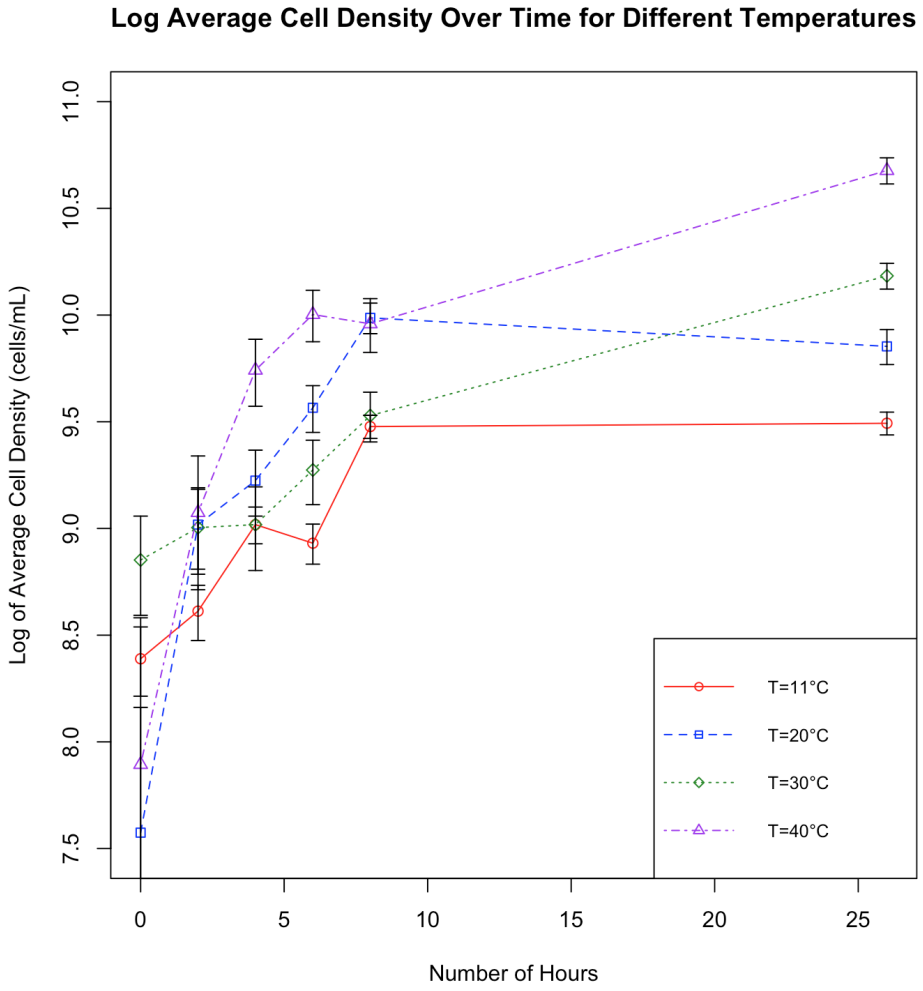

Figure 6. Log of average cell density (cells/mL) of *T. thermophila* for the four treatments (11˚C, 20˚C, 30˚C, and 40˚C) over a period 26 hours. The red line represents the 11˚C treatment, the blue line represents the 20˚C treatment, the green line represents the 30˚C treatment, and the purple line represents the 40˚C treatment. Error bars indicate the 95% confidence intervals.

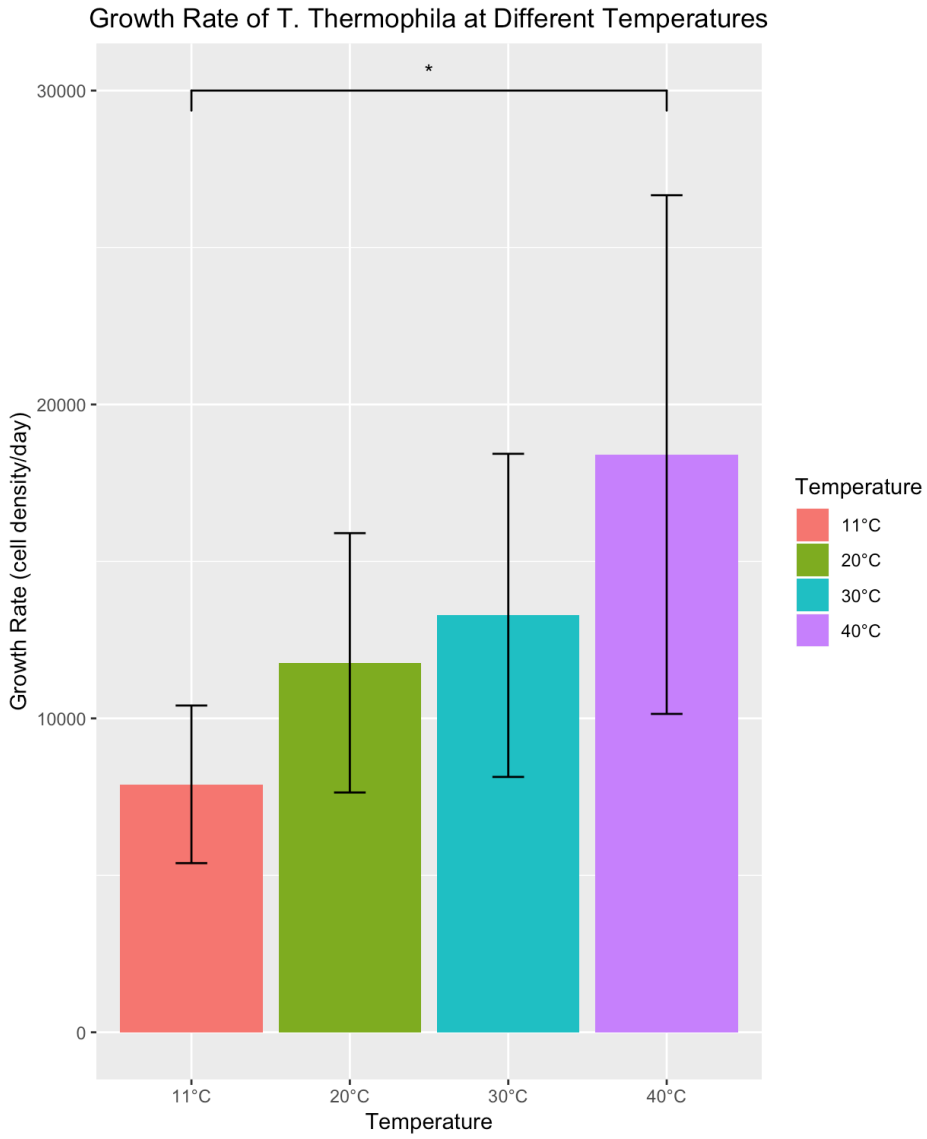

Figure 7. Comparison of *T. thermophila* growth rate (cell density/day) between 11˚C (n=3), 20˚C (n=3), 30˚C (n=3), and 40˚C (n=3) treatments. Error bars represent 95% confidence intervals. The asterisk bar at the top between the 11˚C and 40˚C represent that these two data were significantly different at a 0.05 significance level.

Discussion

The results from the one-way ANOVA gave a p-value of 0.04706. At a 5% significance level, the null hypothesis was rejected, providing support for the alternative hypothesis. The alternative hypothesis stated that temperature does have an effect on the growth rate of *T.*

thermophila. From the results of the ANOVA test, the Tukey-Kramer HSD multiple pairwise comparisons test was used to determine which pairs within the four treatment groups were statistically different. Of the treatment groups (Figure 7), it was determined that the growth rates are significantly different between the 11˚C and 40˚C treatment groups at a 5% significance level. This significant difference between the lowest and highest temperatures was anticipated as the optimal growth rate of *T. thermophila* occurs at 37.5˚C (Franklen and Nelsen, 2001), which explains the higher growth rate of *T. thermophila* of 18,404 cells/mL/day at 40˚C. According to Cassidy-Hanley, *T. thermophila* are usually cultured at room temperature (2012). This accounts for the low growth rate of *T. thermophila* of only 7,651 cells/mL/day at 11˚C, which is 9˚C to 11˚C below room temperature.

The greatest growth rate of *T. thermophila* is observed at 40˚C (Figure 7). While Franklen and Nelson's study (2001) found that *T. thermophila* have optimal population growth rates occurring at 37.5˚C, our control temperature of 40˚C also observed the greatest growth rates. The 40˚C treatment group is slightly greater than the aforementioned optimal growth temperature but illustrates a very similar growth rate and can be explained by having the greatest growth rate observed out of four temperature treatments. Therefore, our results at 40˚C are in accordance with what Franklen and Nelsen (2001) displayed.

The obtained experimental results were consistent with literature, such as that of Franklen and Nelsen (2001) which states that *T. thermophila* have a temperature dependent population growth rate. To account for this reproduction at such high temperatures, it is known that *T. thermophila* have a key temperature specific regulatory surface protein $SerH_3$ (Stargell et. al, 1990). $SerH_3$ is an mRNA expression protein, whose stability impacts the reproduction rate of *T.*

Thermophila. Stargell and colleagues' work displayed that above 40˚C, $SerH_3$ expression decreased, supporting the evidence of Frankel and Nelsen's (2001) work where at 41˚C the number of *T. thermophila* cells decreased after six hours (1990). However, at 30˚C, Stargell et al., found a greater expression of $SerH_3$ relative to its expression levels at 41˚C (1990). This could mean that, at temperatures higher than 40˚C, *T. thermophila* may not be able to survive and further studies would be required to investigate the growth rate past 40˚C. Regardless, these findings relate to our data as they support the fact that *T. thermophila* have mechanisms to support their growth at high temperatures. Such temperature dependent regulatory proteins support why *T. thermophila* are able to tolerate a higher temperature. Lastly, Hallberg et al., conducted an experiment examining the induction of acquired thermotolerance in *T. thermophila* exploring its effects on protein synthesis (1985). In that study, it was found that *T. thermophila* utilize heat shock proteins, which are proteins produced by cells under stressful conditions such as high heat, for their survival at higher temperatures, where at 40˚C, growth was observed.

Sources of Uncertainty and Variation:

A source of uncertainty or variation was the sterile technique in which we conducted the experiment. The sterile technique was not consistent throughout our experiment as test tubes were not flamed each time the fitted cap came off. According to Cassidy-Hanley (2012), *Tetrahymena* species may demonstrate sensitivity to even the slightest levels of impurities within their cultures. This inconsistency enabled the possibility to overestimate *T. thermophila* cell abundance through the potential misidentification of cells. Cells that were not *T. thermophila* due to contamination in its growth medium may have been included in *T. thermophila* counts.

However, microscopically contamination was not observed in solution as no bacterial contamination at 10x magnification under the Axiostar microscope was noted. Researchers also ensured to count *T. thermophila* cells that had similar appearance as Figure 1. It is imperative that researchers flame the top of the test tube each time as it minimizes the contamination from free-floating microorganisms in the air to the growth medium.

In addition, our team was expecting to count roughly 100-300 cells after micropipetting our solution to our hemocytometer, however, we counted only 20-50 cells. A plausible reason for the differences in expected and actual cell count could be that we refrigerated the *T. thermophila* culture a day prior to the cell culturation in the incubators. Placing it in a fridge at 4˚C impacted our cell counts by drastically decreasing the number of cells in each sample. Cassidy-Hanley (2012) highlights the fact that *T. thermophila* are usually placed in cell cultures that are maintained at room temperature. Placing the *T. thermophila,* in the fridge could have hindered the culture and ultimately *T. thermophila*. We noticed this in our data as the initial cell density concentrations illustrated in Figure 6 vary, which could have had an impact on our data collection.

Insufficient mixing of samples, as a result of failing to vortex the samples for a long enough period of time, may have also produced error. This may have caused unequal distribution of *T. thermophila* culture into each test tubes. In addition, only two members of our team counted the number of cells, which may have led to human error. However, a more accurate cell count could have been obtained if sample mixing was more thorough and if the research team performed more pseudo-replicates for each Eppendorf tube. This would have enabled the cell

count for each tube to be averaged to increase accuracy. Due to time constraints that were present in this investigation these factors were not able to be minimized as effectively as would be ideal.

As mentioned previously, *T. thermophila* ingest bacteria through phagocytic means. These bacteria, in turn, serve as a primary food source for zooplankton. Salmon species rely on zooplankton for food. With increases in aquatic temperatures being anticipated in the future, *T. thermophila* have exhibited elevated population growth rates at these higher temperatures. This may result in decreases in bacterial abundance. Therefore, decreasing the amount of food available to zooplankton and in turn salmon species. This may significantly impact salmon population sizes and may potentially lead to their decline.

Future studies may aim to minimize the amount of contamination *T. thermophila* are exposed to through following more stringent sterile techniques and ensuring that test tubes are thoroughly flamed. What's more, these studies may also store *T. thermophila* at a temperature higher than 4˚C prior to examination to limit growth not initiate cell death.

Furthermore, future research may examine how various pH conditions may influence the population growth rate of *T. thermophila*. This research may prove significant as increases in anthropogenic CO_2 emissions have been linked to increases in the acidification of aquatic environments (Hoegh-Guldberg et al., 2007). In addition, it would be interesting for researchers to navigate the impact of various abiotic variables on the growth rate of *T. thermophila,* either independently or dependently. Lastly, temperatures such as 5˚C and as well 50˚C and 60˚C ought to be investigated to understand the extreme temperature tolerance that *T. thermophila* might exhibit.

Conclusion

To conclude, our experiment found that *T. thermophila* has a faster population growth rate at a higher temperature of 40˚C than at lower temperatures (11˚C-30˚C). Our null hypothesis, which states that temperature has no effect on the growth rate of *T. thermophila* was rejected, lending support to the alternative hypothesis. Upon further statistical analysis, we also found that there is a significant difference between the 11˚C and 40˚C treatment groups. The findings agree with similar research experiments found in the literature, but limitations in our study warrant further research.

Acknowledgements

We would like to sincerely thank Dr. Leander for her continued support and encouragement throughout the course of this investigative research project. Dr. Leander's guidance enabled us to create a clear proposal and helped to minimize error in order to strengthen our project in ways we may have been unable to do without her expertise. As well, Jordan Hamden continually assisted us with our statistics inquiries and provided us with reassurance. He also helped to strengthen our note taking skills throughout the course of this project. Lastly, we would like to thank Mindy Chow for her dedication. Without her assistance in growing our *Tetrahymena thermophila* cultures, educating us on proper experimental techniques, and her positive perspective our project would not have been what it is today. Lastly, we would like to thank the Dr. Pam Kalas from the Combined Major in Science (CMS) Department for providing us the opportunity to take Biology 342 by offering it to CMS students

References

Cassidy-Hanley, D. M. (2012). Tetrahymena in the laboratory: strain resources, methods for culture, maintenance, and storage. In *Methods in cell biology* (Vol. 109, pp. 237-276). Academic Press.

Cole, E., & Sugai, T. (2012). Developmental progression of Tetrahymena through the cell cycle and conjugation. In *Methods in cell biology* (Vol. 109, pp. 177-236). Academic Press.

Collins, K., & Gorovsky, M. A. (2005). Tetrahymena thermophila. *Current Biology*, *15*(9), R317-R318.

Doerder, F. P., Gates, M. A., Eberhardt, F. P., & Arslanyolu, M. (1995). High frequency of sex and equal frequencies of mating types in natural populations of the ciliate Tetrahymena thermophila. *Proceedings of the National Academy of Sciences*, *92*(19), 8715-8718.

Eisen, J. A., Coyne, R. S., Wu, M., Wu, D., Thiagarajan, M., Wortman, J. R., ... & Tallon, L. J. (2006). Macronuclear genome sequence of the ciliate Tetrahymena thermophila, a model eukaryote. *PLoS biology*, *4*(9), e286.

Fletcher, M. (2017, Oct. 13). B.C. salmon farm value growing rapidly. Merritt Herald. Retrieved from http://www.merrittherald.com/b-c-salmon-farm-value-growing-rapidly/

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.*Journal of Eukaryotic Microbiology, 48*(2), 135-146.

Garibaldi, A., & Turner, N. (2004). Cultural keystone species: Implications for ecological conservation and restoration.*Ecology and Society, 9*(3), 1. doi:10.5751/ES-00669-090301

Hallberg, R. L., Kraus, K. W., & Hallberg, E. M. (1985). Induction of acquired thermotolerance in tetrahymena thermophila: Effects of protein synthesis inhibitors. *Molecular and Cellular Biology, 5*(8), 2061-2069.

Hansen, J., Ruedy, R., Sato, M., & Lo, K. (2010). Global surface temperature change. *Reviews of Geophysics, 48*(4) doi:10.1029/2010RG000345

Hoegh-Guldberg, O., Mumby, P. J., Hooten, A. J., Steneck, R. S., Greenfield, P., Gomez, E., ... & Knowlton, N. (2007). Coral reefs under rapid climate change and ocean acidification. *science*, *318*(5857), 1737-1742.

Pinheiro, M. D., & Bols, N. C. (2015). Some but not all Tetrahymena species destroy monolayer cultures of cells from a wide range of tissues and species. *Journal of Eukaryotic Microbiology*, *62*(5), 605-613.

Pinheiro, M. D. O., & Bols, N. C. (2014). Delineating cellular interactions between ciliates and fish by co−culturing tetrahymena thermophila with fish cells. Cell Biology International, 38(10), 1138-1147. doi:10.1002/cbin.10310

Stargell, L. A., Karrer, K. M., & Gorovsky, M. A. (1990). Transcriptional regulation of gene expression in tetrahymena thermophila. *Nucleic Acids Research, 18*(22), 6637-6639.

Stolfa, G., & Koudelka, G. B. (2013). Entry and killing of Tetrahymena thermophila by bacterially produced Shiga toxin. *MBio*, *4*(1), e00416-12.

Werlin, R., Priester, J. H., Mielke, R. E., Krämer, S., Jackson, S., Stoimenov, P. K., ... & Holden, P. A. (2011). Biomagnification of cadmium selenide quantum dots in a simple experimental microbial food chain. *Nature nanotechnology*, *6*(1), 65.

Appendix

Equipment List: *Tetrahymena* haemocytometer (No. 3720), micro-coverslips (Fisherbrand®), micropipettes (different volumes of Thermo Scientific) and tips (Fisherbrand® SureOne), Axio compound light microscope, click-counters (Fisher® Scientific), KIMTECH Kimwipes*, Eppendorf tubes, test tubes and cap, test tube racks, incubators, Erlenmeyer flask, graduated cylinder, alcohol lamp

Chemical List: glutaraldehyde fixative, wild-type *T. thermophila* stock solution, SSP growth medium, distilled sterile water (dH_2O)

Table 1. One-way ANOVA Table

Source	SS	d.o.f.	MS	F	Pr (>F)
Between	209644953	3	69881651	4.1755	0.04706
Within	133889695	8	16736212		
Total	343534648	11			

Table 2. Tukey HSD Test

Groups	p-value
11˚C - 40˚C	0.0364956
11˚C - 30˚C	0.7472754
11˚C - 20˚C	0.6492817
30˚C - 40˚C	0.1512803
20˚C - 40˚C	0.1928788
20˚C - 30˚C	0.9978401

T = 11°C		
Time	**A Count 1**	**A Count 2**
0	8	10
2	14	12
4	16	15
6	14	18
8	38	27
26	50	46
Time	**B Count 1**	**B Count 2**
0	13	5
2	21	16
4	28	35
6	21	
8	44	
26	8	
Time	**C Count 1**	**C Count 2**
0	28	
2	17	

4	26	
6	35	
8	43	
26	43	46

T = 20°C		
Time	**A Count 1**	**A Count 2**
0	6	
2	20	
4	41	
6	66	
8	44	
26	50	
Time	**B Count 1**	**B Count 2**
0	8	
2	32	
4	33	

6	39	
8	90	
26	74	
Time	**C Count 1**	**C Count 2**
0	3	
2	17	27
4	20	24
6	35	26
8	62	57
26	42	

T = 30°C		
Time	**A Count 1**	**A Count 2**
0	20	
2	22	
4	27	
6	24	
8	37	

26	42	58
Time	**B Count 1**	**B Count 2**
0	18	
2	30	
4	27	
6	38	
8	44	
26	111	115
Time	**C Count 1**	**C Count 2**
0	23	
2	19	
4	18	
6	31	
8	39	
26	62	74

T = 40°C		
Time	**A Count 1**	**A Count 2**

0	9	14
2	15	14
4	38	21
6	54	46
8	32	41
26	108	105
Time	**B Count 1**	**B Count 2**
0	3	2
2	29	30
4	58	
6	66	
8	70	
26	126	140
Time	**C Count 1**	**C Count 2**
0	11	
2	39	
4	81	
6	91	
8	103	

26	155	123