Kimiya Majidi, Kaylee Nguyen, Sumi Liu

Effects of Temperature on Tetrahymena Growth

Abstract

This study was conducted to determine the effects of temperature on the growth of *Tetrahymena thermophilia* and at which temperatures do we see *T. Thermophilia* experience the most and least growth. We chose 3 sets of temperatures for this study, 25 °C, 30 °C, 40 °C, and carried out multiple replicates per set of temperature. Over a span of 48 hours, we collected samples and did cell counts to determine how each temperature category was affecting the cell growth. Over the two days, we took cell counts at 3-hour time intervals starting from 9:30am to 5:00pm, counting 3 times per day. Our results didn’s show any particular optimal growth at the temperatures specified, however we did notice that there was a wider distribution range for the cell count box plot at 30 °C. We had initially expected T. thermophilia to exhibit the strongest growth at the 30 °C, which is something we saw, however growth in the other two temperatures were relatively similar. Overall this study was used to confirm or contradict past experiments and showed strong correlations to past results seen in some capacity.

Introduction

Tetrahymena thermophila is a free-living, single-celled eukaryotic organism commonly found in freshwater environments such as ponds, lakes, and streams. Previous study shows that temperature plays a vital role in determining the metabolic rates, reproductive capacity, and overall physiological activities of these organisms (Thormar, 1962). Generally, within a specific range, higher temperatures tend to accelerate metabolic processes which leads to a faster growth rate. Conversely, lower temperatures might slow down metabolic process and growth rate. According to the previous study, the optimal doubling time for *T. thermophila* occurs at 35 °C with a generation time of two hours and the growth rate starts to drop at approximately 39°C and 39.5°C (Frankel et al. 2001).

The study of tetrahymena is crucial and any results, whether significant or not, are helpful in contributing to scientific literature and furthering knowledge on *T. thermophilia*. Contributing to a large component of the marine food web and as a major source of food for salmon, the growth of *T. thermophilia* is something that needs to be continually studied while we are experiencing significant changes in the environment (Beaugrand et al, 2003). As temperatures continue to rise, including the temperature of the waters that *T. thermophilia* reside in, we must see how this may affect the long term growth of these organisms.

The purpose of our study is to determine the optimal temperature for *T. thermophila*, hypothesizing that there exists a difference in growth rates of *T. thermophila* across various temperatures. Our null hypothesis, conversely, posits no significant variance in T. thermophila's growth rates at different temperatures.

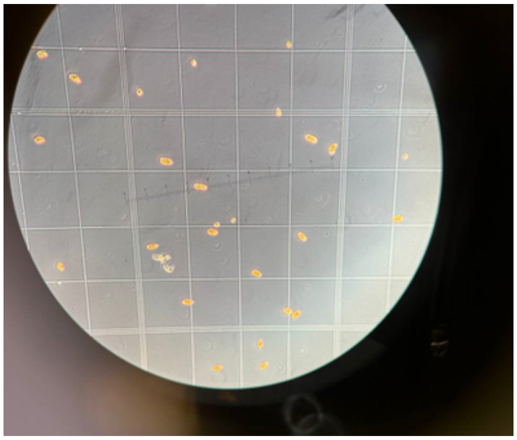
Methods

We started by determining the initial cell concentration of an unknown stock culture of *T. thermophila* First, 10 uL of glutaraldehyde fixative was added to an Eppendorf tube using a micropipette. Subsequently, 100 uL of the initial stock of *T. thermophila* was added and mixed with fixative. The Erlenmeyer flask containing the stock culture was shielded with aluminum foil. Every time the foil was removed, we applied flame to the flask to ensure the prevention of contamination.

After mixing the stock and fixative, 20 uL of the fixed *T. thermophila* were loaded onto a haemocytometer using a micropipette. The haemocytometer was then placed under a compound microscope with a 10x objective lens to count the initial cell concentration of the stock culture. The cell count was performed three times using a click counter for accuracy, and the average concentration was calculated to be approximately 12,719 cells/mL. With this optimal cell concentration, we determined that we needed to dilute 78 mL of *T. thermophila* stock culture in 22 mL of medium culture to make a 100 mL working culture.

Next, 10 mL of the working culture was added to nine test tubes. Three test tubes were designated for each desired temperature condition (25 °C, 30 °C, 40 °C). Each set of three test tubes was placed in a rack and then incubated at the specified temperature. For each round of sampling, three replications were conducted under the specified temperatures. Sampling was performed every two hours over a two-day period from 9:30 AM to 3:30 PM, resulting in a total of 54 replications. For each round, a 100 uL sample was taken from each of the nine cultured test tubes, mixed properly, and placed into a labeled Eppendorf tube with the addition of 10 uL fixative. Then, 20 uL of the solution was loaded onto the haemocytometer to count the cell number under the compound microscope with a 10x objective lens. The test tube neck was flamed and sterilized each time the caps were removed.

To compare the mean growth rates of *T. thermophila* at varying temperatures, we employed a one-way ANOVA test. Subsequently, we visualized the distribution of growth rates across different temperatures using box plots generated in RStudio. Additionally, we constructed line graphs using Excel to observe the growth trends of Tetrahymena over time across different temperatures.



Figur 1. T.thermophila cell under compound light microscope at 10x Magnification.

Figure2. Three replication test tubes for each temperature.

Results

The examination of *T. thermophila* growth rates at 25 °C, 30 °C, and 40 °C showed distinct trends over the experimental period. Initial cell densities were 4812.5 cells/mL at 25 °C, 7906.25 cells/mL at 30 °C, and 4354.17 cells/mL at 40 °C. At 25 °C, a consistent and gradual increase in cell density was observed throughout the experiment. At 30 °C, initial cell density at

0 hours was notably higher than at 25°C. However, over time, the cell density shows a fluctuating pattern. Contrasting with the previous two, Tetrahymena at 40°C presented a distinct trend. While starting with a moderate cell density at 0 hours, rapid increase, reaching its peak at 2 hours was recorded. The largest growth rate was observed at 30 °C, while the smallest growth rate was observed at 25 °C.

There were two types of variances in cell density, within-group and between-group variances. The sum of squares for the between-group variance was found to be 29,624,155.25, yielding a mean square value of 14,812,077.63. The within-group variance, 469271030.5 with 15 degrees of freedom was computed to represent the variability within each temperature condition. This resulted in a mean square value of 31,284,735.37. The ratio of within-group variation to between-group variance is measured by the F-ratio, which was 0.473460218. The associated p-value was 0.632, which is greater than the conventional significance level of 0.05.

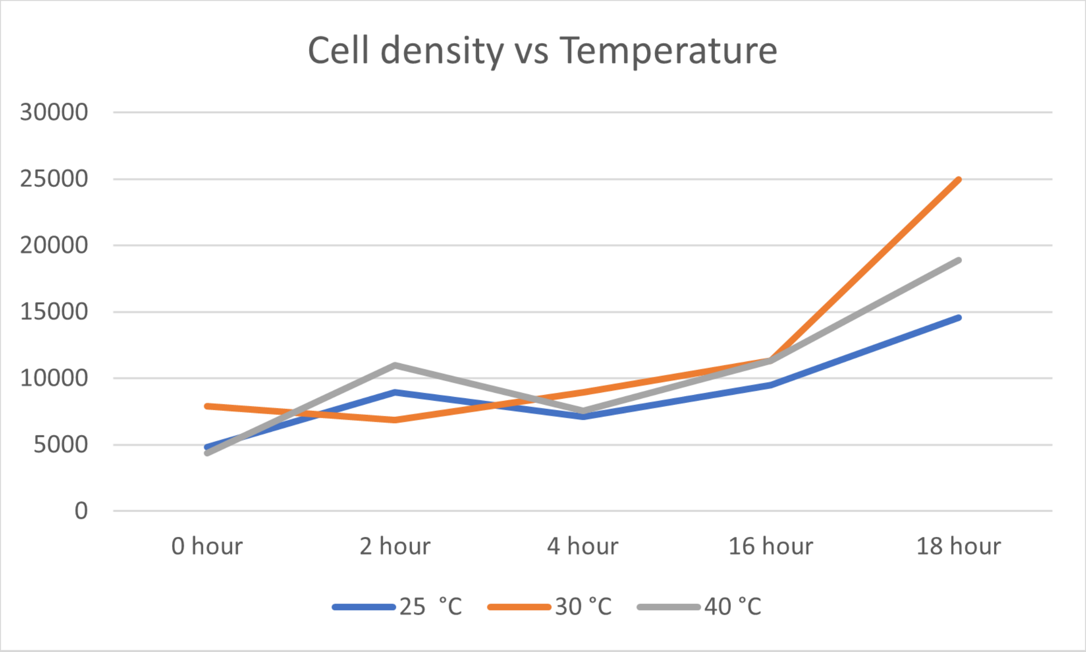


Figure 4. Cell density over time at 25 °C, 30 °C, and 40 °C

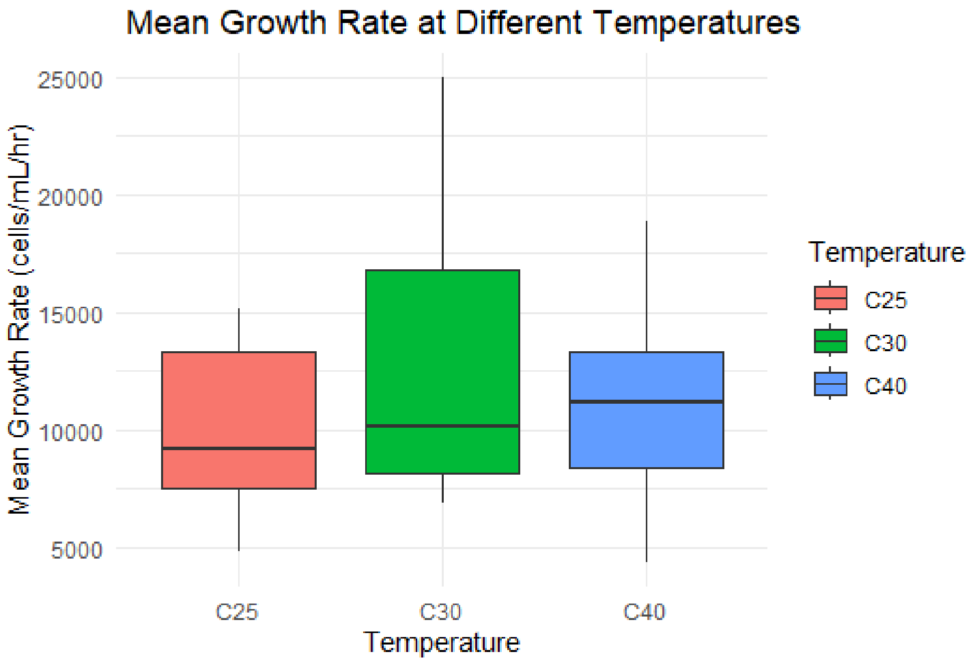


Figure 5. Mean growth rate at 25 °C, 30 °C, and 40 °C

Discussion

The one-way ANOVA test results led to the acceptance of the null hypothesis, indicating no significant difference in *T. thermophila's* mean growth rates across temperatures of 25, 30, and 40 degrees Celsius. The obtained p-value of 0.632 exceeded the 0.005 significance level. Despite this statistical outcome, notably the wider distribution of cell concentrations at 30 degrees compared to 25 and 40 degrees, This broader dispersion might be attributed to outliers within the cell density. Furthermore, the upward trend in cell density across time for all temperatures didn't result in a statistically significant difference in mean growth rates among them.

Interestingly, these findings present a contradiction to previous research indicating that higher temperatures generally expedite metabolic processes, accelerating growth rates, while lower temperatures impede such processes. This prior study by Frankel et al. (2001) suggested an optimal doubling time for *T. thermophila* at 35°C, with a subsequent decline in growth rate around 39°C and 39.5°C. However, our results fail to align with this established understanding, as no significant variance in T. thermophila's growth rates was observed across the temperature range tested, contradicting the anticipated temperature-dependent growth patterns proposed in earlier research. This might be because the selected temperature range might fall within an optimal growth zone for the *T. thermophila*, minimizing significant growth differences, while *T. thermophila* adaptation mechanisms could maintain relatively consistent growth rates despite varying temperatures (Weber de Melo et al., 2020).

The absence of significant differences, a broader range of temperatures needs to be explored to establish a more definitive correlation between temperature and the growth of *T. thermophila.* Understanding the temperature preferences of this organism is crucial, especially considering its role in the marine food web and its significance as a primary food source for salmon. With environmental changes, including rising water temperatures, continually affecting the habitats of *T. thermophila*, further investigation is imperative to assess the long-term implications on their growth dynamics and subsequently on the ecosystem they influence (Beaugrand et al, 2003).

Throughout this study, numerous sources of uncertainty were identified, notably concerning cell counting using a hemocytometer as cell numbers increased. The presence of tiny or clustered cells posed challenges in determining whether to include them in the count. Therefore, a secondary counter was required to ensure accuracy. Maintaining a sterile environment emerged as another critical challenge to prevent potential contamination risks. Sterilization techniques are essential when working with Tetrahymena cultures, including sterile glass and plasticware, and monitoring media for contamination as bacteria are sensitive to contaminations (Cassidy-Hanley, 2012). Moreover, inadequate mixing before pipetting the bacterial culture into tubes or the hemocytometer resulted in uneven cell distribution, potentially impacting cell density under the microscope. The growth trends were found to be intriguing under a variety of temperature situations. Although there was a noticeable rise in cell density at 30 °C, the ANOVA findings indicate that these differences are not statistically significant. Future studies can emphasize stringent contamination prevention measures and explore broader environmental factors beyond temperature (e.g., pH, salinity). Expanding the team involved in cell counting and implementing validation checks could improve the reliability and accuracy of experimental outcomes.

Conclusion

This study aimed to determine how the temperature effect on *Tetrahymena thermophila* growth rates in three different temperature settings: 25 °C, 30 °C, and 40 °C. The findings revealed distinct growth tendencies, including a significant peak at 30 °C. However, the ANOVA analysis failed to establish statistical significance. Our study acknowledged the difficulties in accurately counting cells and the necessity of using strict sterile procedures. Further studies could take on such challenges and investigate additional environmental factors, providing a deeper understanding of the details of *T. thermophila* growth behaviour.

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References

Beaugrand, G., & Reid, P. C. (2003). Long-term changes in phytoplankton, zooplankton and salmon related to climate. Global Change Biology, 9, 801817. doi:10.1046/j.13652486.2003.00632.x

Cassidy-Hanley, D. M. (2012). Tetrahymena in the laboratory: Strain resources, methods for culture, maintenance, and storage. *Methods in cell biology* (pp. 237-276). Elsevier Science & Technology. https://doi.org/10.1016/B978-0-12-385967-9.00008-6

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

Thormar, H. (1962). Effect of temperature on the reproduction rate of Tetrahymena pyriformis. *Experimental Cell Research*, *28*(2), 269–279. https://doi.org/10.1016/0014-4827(62)90283-5

Weber de Melo, V., Lowe, R., Hurd, P. J., & Petchey, O. L. (2020). Phenotypic responses to temperature in the ciliate tetrahymena thermophila. *Ecology and Evolution, 10*(14), 7616-7626. https://doi.org/10.1002/ece3.6486