Thermal Influence on Tetrahymena Growth: Comparative Analysis Across Optimal, Marginal, and Extreme Temperature

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Abstract

In this experiment, we studied the influence of temperature on the growth rate of Tetrahymena thermophila (T. thermophila), a unicellular organism with profound historical significance in biology and complex ties to ecosystem dynamics. The experiment entailed cultivating T. thermophila at three distinct temperatures (25°C, 33°C, and 40°C) in separate incubators, with two-hour sample collections over two days.

We predict that T. thermophila exhibits different growth rates under varying thermal conditions. The statistical analysis indicates no significant temperature differences (p-value = 0.7038 > 0.05) among different temperature treatments and thus denied our original hypothesis. We conclude that experimental errors, such as inaccurate sampling or statistical error, may lead to this result.

Introduction

As we navigate the complex ecosystem of single-celled organisms, the Tetrahymena thermophila as an ideal subject for investigation, offering a microcosmic perspective through which we can study the interplay between temperature and growth rates. Our study seeks T. thermophila's response to varying concentrations of temperature.

T. thermophila has been utilized as a model organism in biomedical discourse for detail, in part due to their rapid doubling time of 23 hours and their large size, which allows for easy viewing during microscopy (Ruehle et al., 2016). The thermal ecology of T. thermophila has been important in microbial ecology. with studies elucidating the optimal temperature range between 21 ℃ to 33 ℃ (Thormar,1962). However, within the broader spectrum of temperature fluctuations present in diverse aquatic habitats, uncertainties persist regarding Tetrahymena's adaptability to marginal and extreme temperatures. The population abundance of these planktons can also be influenced heavily by abiotic factors such as temperature and pH in the environment (Beaugrand & Reid, 2003).

In the pursuit of advancing our understanding of Tetrahymena thermophila's thermal plasticity, we hypothesize that T. thermophila will show different growth rates with varying treatments of temperature. Drawing inspiration from studies on microbial responses to temperature changes (Weber de Melo et al., 2020), we aim to identify the difference in T. thermophila’s growth rates, specifically focusing on the comparative analysis across the optimal (25℃), marginal (33℃), and extreme(40℃) temperatures. Based on the information we build up the hypothesis:

H0: Tetrahymena thermophila will not show different growth rates with varying treatments of temperature.

H1: Tetrahymena thermophila will show different growth rates with varying treatments of temperature.

The significance of the study depends on its potential to explore critical insights into the adaptability of T. thermophila to diverse thermal environments. By addressing the existing gap in knowledge regarding the growth rates of organisms under optimal, marginal and extreme temperatures, our research contributes to the fundamental understanding of microbial ecology. In the following sections, we will focus on the method employed to test our hypothesis, presenting a detailed framework that aligns with the objectives outlined in this introduction.

Methods

First, we began with the initial preparation of T. thermophila. For the purpose of examining the impact of temperature on the growth rate, T. thermophila strain B2086 was used for this study. The initial stock culture concentration was determined by direct cell counting and was found to be 2.5438×10^4 cells/mL. This stock culture was then diluted to 50 ml in the flask with a standard working concentration of 1.00×10^4 cells/mL, providing a consistent baseline for the commencement of the experiment.

Secondly, we designed the experimental group and set different treatments of temperature.

There were 9 culture tubes then divided into 3 experimental groups, each comprising 3 replicates, to assess the impact of varying temperatures on the growth rate. 5 mL of initial stock were dispensed into 9 sterile culture tubes. They were incubated at specified

temperatures as the following figures show: the optimal temperature of 25°C, the marginal temperature of 33°C, and the extreme temperature of 40°C.

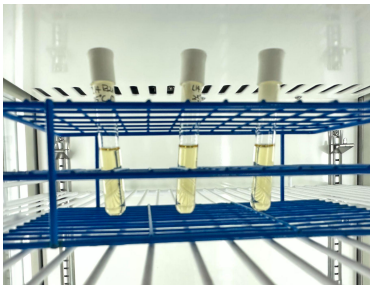

Figure1.Three T.thermophila replicate under 25°C.

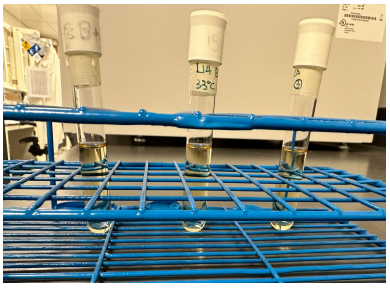

Figure2.Three T.thermophila replicate under 33°C.

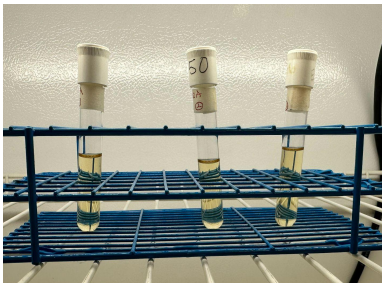

Figure3.Three T.thermophila replicate under 40°C.

Thirdly, we designed the sampling procedure and schedule of our experiment. Before each sampling, 10 μL of iodine potassium-iodide solution was pre-added to each designated plastic tube to ensure an immediate cessation of cell proliferation upon sample collection. Subsequently, 100 μL of the T. thermophila culture was carefully pipetted to each plastic tube, mixed thoroughly with the iodine potassium-iodide, and then stored in a refrigerator before cell counting. The investigation was conducted over two days due to the operational constraints of the laboratory facilities, which were accessible from 9 am to 5 pm. Sampling was synchronized to occur bi-hourly during the available hours, capturing growth data from the initiation point (0 hours) up to the 6-hour mark on the first day and then resuming at the 23-hour mark through to the 29-hour mark on the subsequent day. Finally, we did data collection and statistical analysis for our experiment. Before counting, each sample was homogenized to ensure an even distribution of cells and Iodine potassium-iodide (KI). For the cell counting process, a hemocytometer was employed. A 20 μL aliquot from each sample

was loaded onto the hemocytometer grid. Given the grid's design, we counted the entire grid when the number of T. thermophila was under 100 to avoid sampling error. Specifically, for each sample, three separate 20 μL aliquots were evaluated. The average cell count was then calculated from these nine counts (3 aliquots × 3 temperatures). Since there were three replicates for each of the three temperature conditions, this resulted in 27 individual counts per temperature group for each sampling instance (3 replicates × 3 aliquots × 3 temperatures = 27 data points).

The average cell concentrations from each tube were then calculated to get the mean concentration value for each temperature condition at each time point. This approach reduced variability and provided a precise, concise measurement of the T. thermophila population's response to the temperature.

To determine if the differences in mean concentrations across the temperature treatments were statistically significant, an Analysis of Variance (ANOVA) was conducted. Given our experiment's design, which investigated three distinct temperature conditions, ANOVA was the optimal method for identifying any statistically significant differences in growth rates due to temperature variance. In the event of significant ANOVA results, post hoc analyses would be implemented to explore the differences between specific temperature pairs further, providing a deeper understanding of the thermal influence on T. thermophila growth.

Results

The growth rate of T. thermophila did not exhibit a significant difference for the temperature factor using a one-way ANOVA test with a p-value of 0.7038. T. thermophila kept at 25°C displayed a mean growth rate of -0.007 ∓ 0.0315, T. thermophila kept at 33°C had a mean

growth rate of -0.0321 ∓ 0.0456, and T. thermophila kept at 40°C had a mean growth rate of -0.0823 ∓ 0.0308 (Figure 4). The dots represent the mean growth rate under each treatment temperature, and the error bars represent 95% confidence intervals.

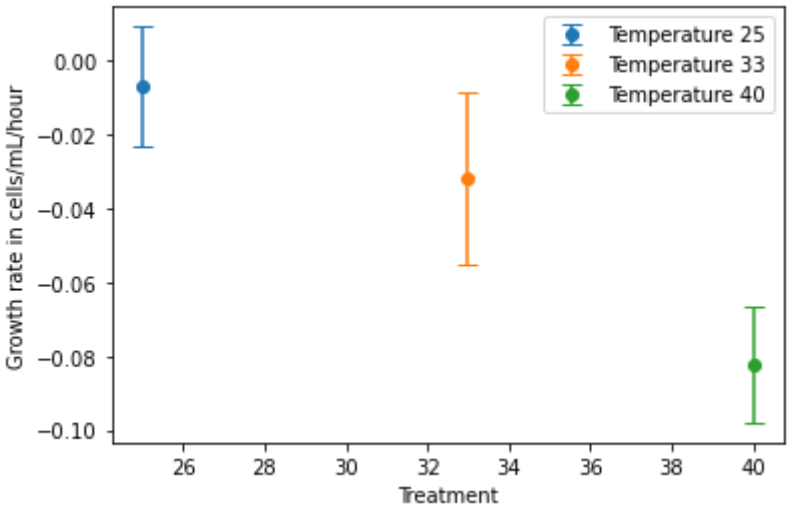

Figure 4. The growth rate of T. thermophila was measured in cells per mL per hour (n=3).

Discussion

Based on our statistical analysis, the results cause us to fail to reject your null hypothesis (H0) because the p-value (0.7038) of one-way ANOVA is greater than 0.05, indicating there is no significant difference in means between the treatments. Our statistics therefore failed to receive our alternate hypothesis (HA) that T.Tetra will show insignificant differences in growth rates under different temperatures. We did not expect that the growth rate was not statistically significantly different between the three temperatures and also didn't expect that all of those growth rates would show up negative.

We concluded three reasons that could contribute to this failure to reject null. Variability within groups: Knowing that the optimal temperature range of T. thermophila is 21 ℃ to 33 ℃ (Thormar, 1962), where our 33°C group lies on the boundary and the 40°C group is

beyond it. It is very possible that the boundary temperature (33°C) does not fit for T. thermophila to grow, which can lead to a similar growth rate to the extreme treatment (40°C), supported by the p-value ($0.9 > 0.05$) of 33°C and 40°C groups that show no significant difference between the means of growth rate by Tukey-Kramer test. Type II Error: This occurs when the statistical test fails to identify an actual effect or difference, and the null hypothesis is not rejected. This type of error is influenced by factors such as sample size, variability of the data, and the chosen significance level. Specifically, in our case, the temperature treatments may be too similar to one another and thus cannot evoke enough change in growing behavior. If the treatment changes to 15°C, 30°C, and 40°C, we predict the results will probably be enough to reject null. Also, the operations during sampling can lead to significant errors by not mixing up the sample enough, and the sampling can be uneven. To avoid these errors, we can set our working stock in a higher cell concentration to increase the sample size and set larger temperature gradients to be more statistically separated. Also, should adequately mix the cell solution before sampling to keep the cell evenly distributed.

For future research, there are multiple fields to explore using T. thermophila as a model organism. Expanding the scope of temperature ranges and the timeframe of exposure could provide deeper insights into the organism's adaptability and stress mechanisms. Studies could delve into the cellular and molecular biology of T. thermophila, such as stress response pathways and genetic adaptations to environmental changes. Additionally, investigating the effects of multiple simultaneous stressors, such as pollutant presence alongside temperature fluctuations, would give a more holistic view of environmental impacts.

As an environmental indicator, T. thermophila holds significant promise. Its sensitivity to temperature and chemical changes makes it an excellent bioindicator for water quality and the presence of pollutants. This can lead to direct applications in environmental monitoring, influencing industrial waste management practices and informing policy decisions. The responses of T. thermophila to environmental stressors can also provide valuable information for climate change research, offering a window into the resilience of microorganisms and their role in ecosystems under shifting global temperatures.

Conclusion

We failed to reject our null hypothesis and thus failed to provide support for our alternative hypothesis. We conclude that the growth rate of Tetrahymena thermophila does not change in the temperature range of 25°C to 40°C, which is opposite to the previous studies. Experimental and statistical errors may lead to this result. We hope that our study will aid future studies regarding Tetrahymena thermophila and its relationship with temperature.

Acknowledgments

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Appendix

Temperature	Replicates	Slope
25°C	1	-0.0305
25°C	2	-0.0079
25°C	3	0.0173
33°C	1	-0.0436
33°C	2	-0.0195
33°C	3	-0.0333
40°C	1	-0.1047
40°C	2	-0.0552
40°C	3	0.0869

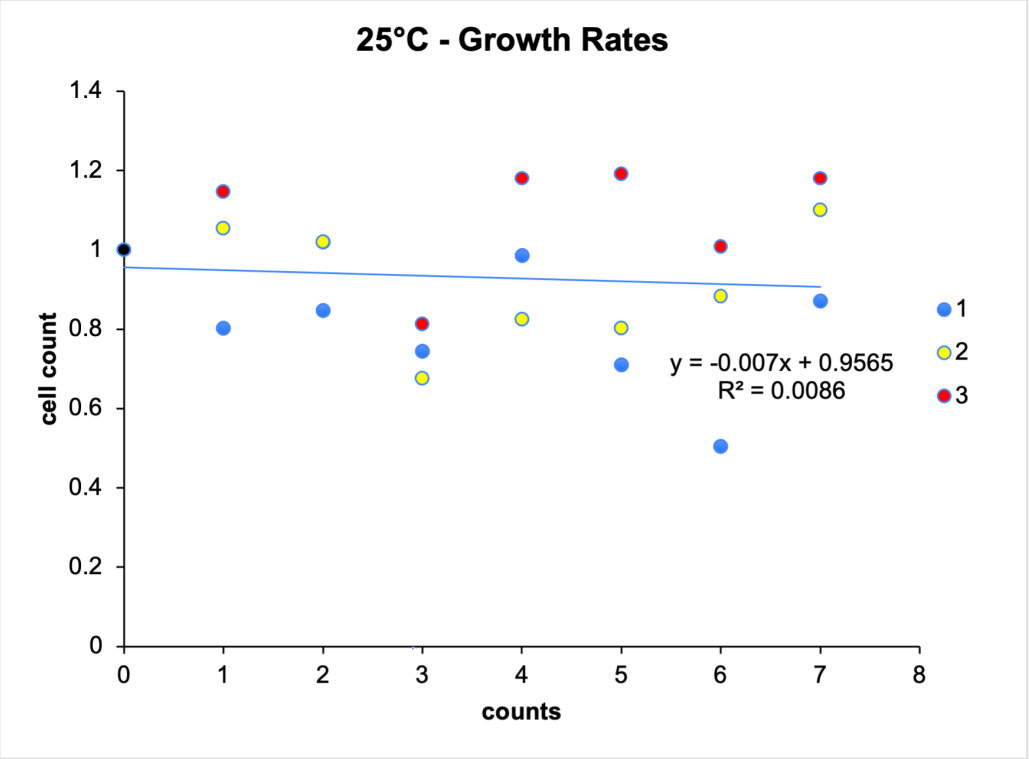

Figure A-1. Growth rates under 25 °C

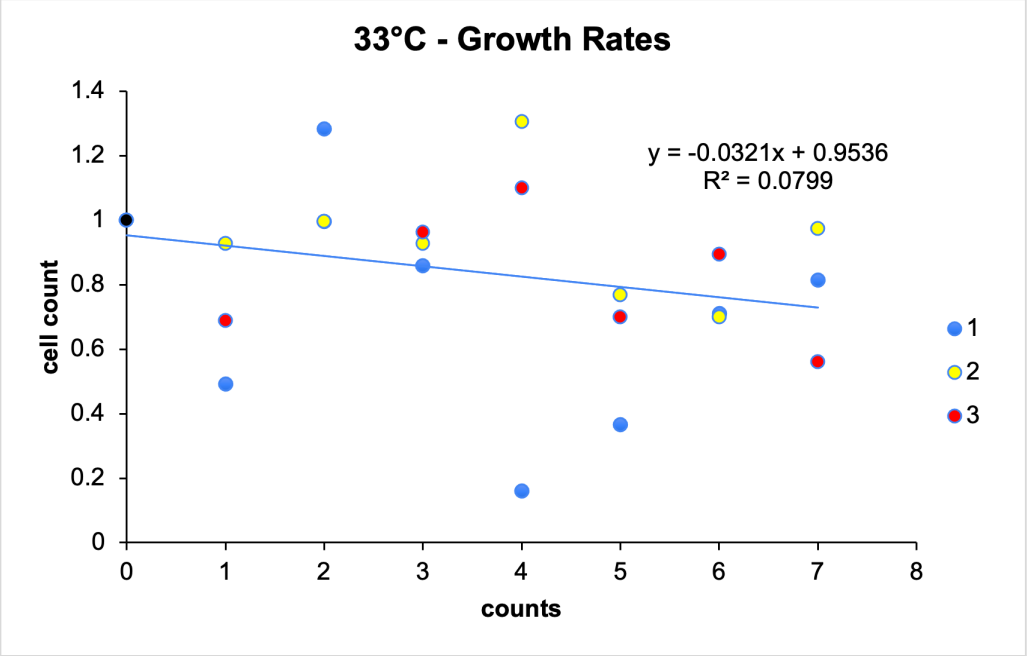

Figure A-2. Growth rates under 33 °C

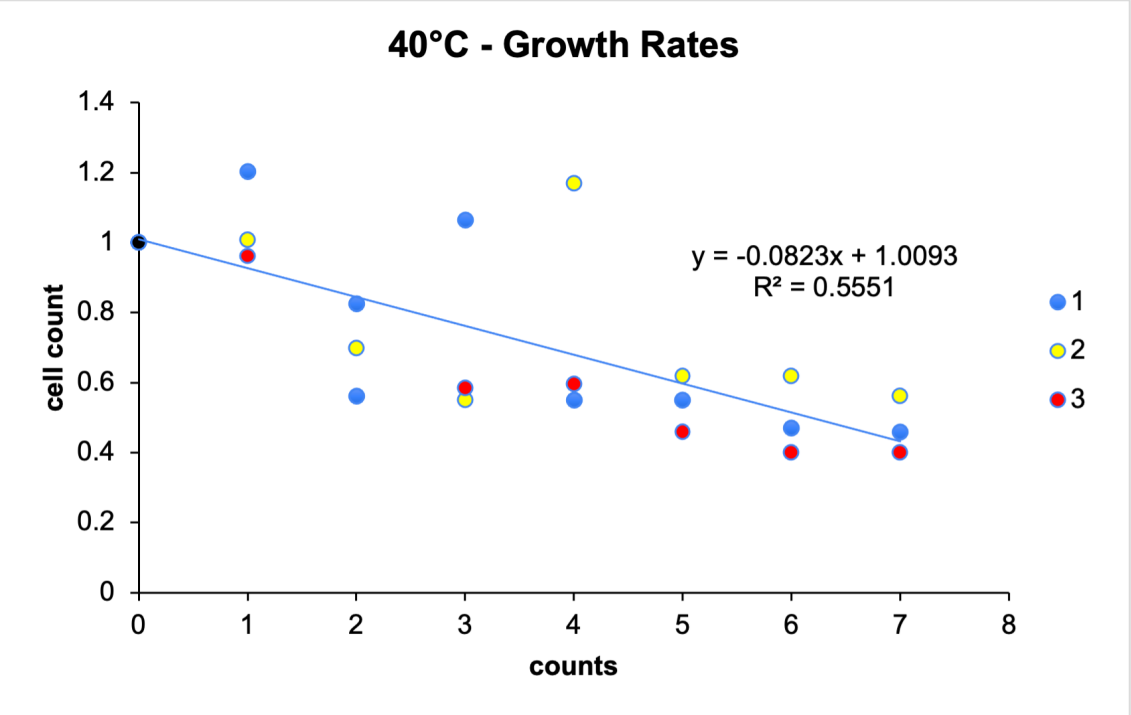

Figure A-3. Growth rates under 40 °C