Feel the Heat? An Experimental Study on Temperature and Food Vacuole Formation Rates in *Tetrahymena thermophila*

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Abstract

Tetrahymena thermophila are unicellular eukaryotes that take up nutrients, bacteria and other molecules through phagocytosis and the formation of food vacuoles. The purpose of this study was to examine the effect of temperature on the amount of food vacuoles formed by *T. thermophila*. In order to investigate this, *T. thermophila* cells were exposed to three temperature conditions: 25°C, 30°C and 35°C for 20, 85, 115 and 145 minutes, with 3 replicates of each. Food vacuoles were counted and recorded for 5 cells, effectively producing a rate of food vacuole formation at the observed treatment temperatures. Based on one-way ANOVA, our results showed no significant difference between temperature and the rate of food vacuoles formed by *T. thermophila.*

Introduction

Tetrahymena thermophila is a ciliated protozoan, measuring 30 x 50 μm (Chan et al., 2017). They are commonly found in freshwater habitats, and feed via phagocytosis (Chan et al., 2017). Phagocytosis not only allows cells to obtain nutrients from food, but it can also help dispose of waste and undigested material in the cell through the formation of food vacuoles (Luan et al., 2013). As food pushes against the cellular membrane, the membrane stretches inward until the food particle is completely in the cell, then the membrane pinches off, forming a vacuole. Once a food vacuole is formed, the nutrients inside can be taken up by the cell (Luan et al., 2013). Lysosomes in the cytoplasm fuses with the food vacuole and releases enzymes that can digest the food particle, and any wastes and undigested material left inside the vacuoles can leave the cell through exocytosis (Luan et al., 2013). *T. thermophila* can also ingest bacteria by phagocytosis (Stolfa et al. 2013), therefore, they play an important role in regulating bacterial

populations in marine environments. Understanding the metabolic rate of *T. thermophila* can increase water quality and prevent bacterial infections in aquatic species.

Environmental factors such as temperature, acidity and salinity can affect the process of phagocytosis and the formation of food vacuoles (Chan et al., 2017). As a result, we were interested in studying how temperature affects the food vacuole formation rate in *T. thermophila*. Temperatures between 25°C to 37°C have been shown to increase membrane fluidity, which in turn increases rate of phagocytosis (Chan et al., 2017).

Our objective is to study the effect of temperature and the rate of food vacuoles formed. Our alternate hypothesis states that temperature will have an effect on the rate of food vacuoles formation by *T. thermophila*, and our null hypothesis states that temperature will have no effect on the rate of food vacuoles formation by *T. thermophila*. Based on literature, we predict that an increase in temperature will increase the rate of food vacuoles formation by *T. thermophila* (Chan et al., 2017), therefore, we predict that cells at 35°C will have the highest rate of food vacuole formation.

Methods

First, we swirled a flask containing the *T. thermophila* culture to ensure even distribution. Then, we used sterile technique to pipette 3 ml of *T. thermophila* into three 6 ml test tubes. The samples were then allocated to each of the three temperature conditions: 25ºC, 30ºC, and 35ºC.

Beginning with the temperature treatment at 25ºC, we placed the test tube with 3 ml of *T. thermophila* into a 25ºC incubator for 20 mins. We then labeled four microcentrifuge tubes with the time at 20, 85, 115 and 145 minutes, and added 15 µl of fixative into each of them. After 20 minutes, we took the test tube out and added 0.75 ml of red dye into the test tube. We swirled the

solution until the colour was consistent. One hundred and fifty µl of *T. thermophila* was pipetted out of the test tube into the microcentrifuge tube with fixative labeled t= 20. We continued to pipette 150 µl out of the sample at 25ºC into its corresponding microcentrifuge tubes at 85, 115 and 145 minutes. These procedural steps were repeated three times to create three replicates.

We pipetted 25 µl of *T. Thermophila* out of the sample at 20 minutes and 25ºC onto a prepared slide. We placed the slide under an Axiostar compound microscope and counted the number of food vacuoles in 5 randomly chosen cells at 400X magnification, using bright field. This was repeated for samples at 85, 115, and 145 minutes, and their replicates. To minimize discrepancies in counts, we had two people count the number of food vacuoles in the same 5 cells. If 5 cells were not in the field of view, we shifted the slide until a total of 5 cells could be observed. Most vacuoles were red or pink and circular (see Figure 1). We counted the ones we thought were distinct. The number of food vacuoles were recorded and the average was taken between the two counts. The whole procedure was repeated at 30ºC and 35ºC. One-way ANOVA was conducted on the data by first converting

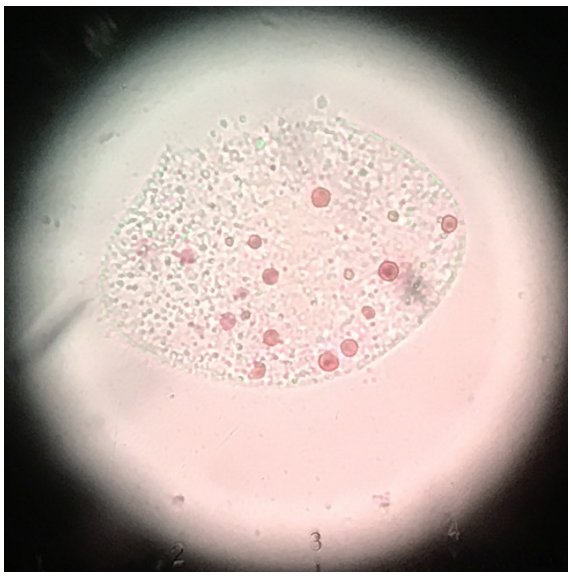

Figure 1. Stained food vacuoles observed within a *T. thermophila* cell.the raw count data into a rate by calculating the slope of the growth observed. The rates were then used to calculate the p-value and F-value.

Results

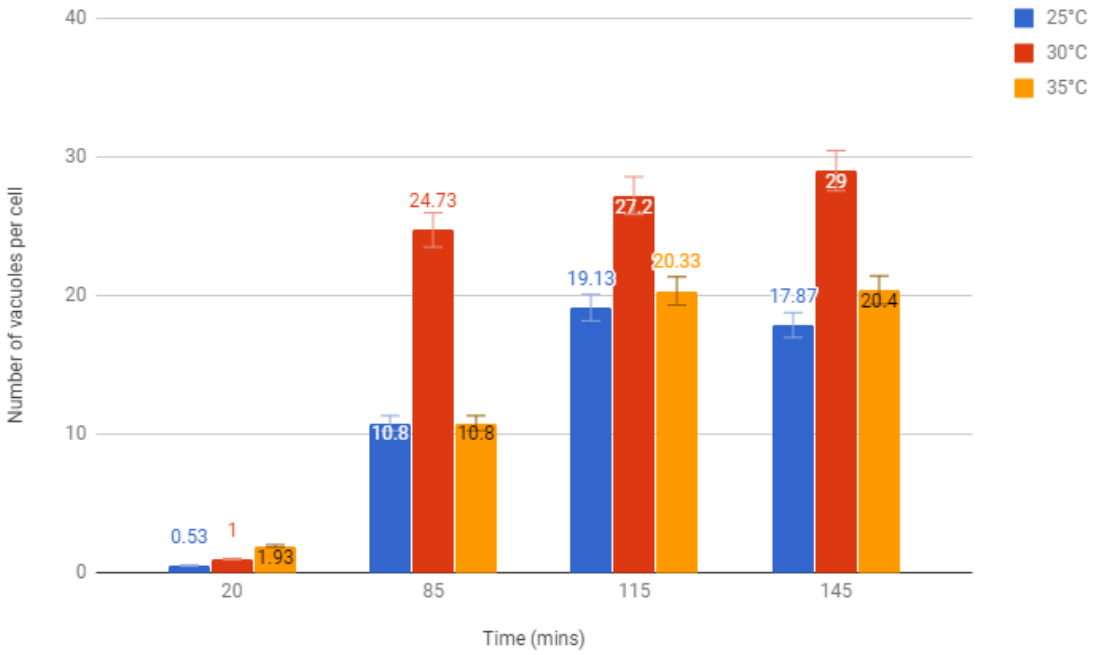

Figure 2. The average number of food vacuoles was measured at 20, 85, 115 and 145 mins in 15 cells from three replicates incubated at 25ºC, 30ºC, and 35ºC.

The average number of food vacuoles generally increased over time for all temperatures. At 20 minutes, cells incubated at 35ºC exhibited the highest number of food vacuoles. At 85 minutes, cells incubated at 30ºC showed the highest number of food vacuoles. At 115 minutes, cells incubated at 30ºC had the highest number of food vacuoles, while cells incubated at 35°C had a slightly higher number of food vacuoles than cells incubated at 25°C. At 145 minutes, the average number of food vacuoles at 25°C was slightly lower than that at 115 minutes. As observed in Figure 2, the average number of food vacuoles at 30°C appears to be the highest instead of at 35°C. The p-value was calculated to be 0.235 and the calculated F-value was 1.860. The 95% confidence intervals for the average number of food vacuoles across all temperatures were quite small, indicating higher certainty in the number of food vacuoles among the replicates.

Qualitative Observations

In the sample incubated at 25°C for 20 minutes, replicate 2, we observed no vacuoles in any of the cells. In the sample incubated at 30°C for 145 minutes, replicate 3, we found no cells present.

Sample Calculation

At 25°C and at t=20 minutes

1. Average number of food vacuoles present in three replicates of 5 cells
   = (4+0+4)/3 = 2.67

Discussion

After the analysis of our data, we were able to say that our results held no statistical significance. However, this does not mean that the experiment itself was futile. As mentioned earlier, we expected to observe an increase in the amount of food vacuoles formed as temperature increased, and our results showed some support for that trend, but it was not completely accurate. Based on our results, we observed that the most vacuole growth numbers occurred in the 30°C treatment for every time interval as opposed to the 35°C. The only exception to this observation was the 20 minute interval, which showed an increasing number of food vacuoles with each increasing temperature treatment.

Food vacuole formation is associated with high rates of phagocytosis, which in turn associates with faster nutrient uptake (Jacobs et al., 2006). Jacobs et al. (2006) found that the optimal temperature range for food vacuole growth was 28 - 30°C for *T. thermophila.* This result is further confirmed through a separate study conducted by Luan et. al (2013), which also noted that the highest number of food vacuoles formed was at 30°C and not 35°C. These two studies

give a biological explanation and coincide with the results of our experiment. Although not significant, the data trend does show the 30°C temperature treatment having the highest number of food vacuoles formed.

Unfortunately, the sample size was too small to offer statistical significance and thus, we were unable to reject the null hypothesis for this experiment. As the ANOVA test is a comparison between multiple means, it is necessary for the means of the treatments to have a large enough sample size to observe difference, but three data points per treatment did not meet this requirement. Given more time, we could have obtained additional data to mitigate the effects of a lack of data on the results of the ANOVA test.

Additionally, the error could have risen from the fact that we only took the counts of vacuoles formed within 5 cells per treatment, which led to a wide range of vacuoles counted for each treatment. These counts were not necessarily indicative of the total food vacuole growth at each treatment temperature as they varied so much between the three replicates. Ideally, we would have liked to take the counts across 10 cells of *T. thermophila,* but we were forced to reduce it to 5 due to not being able to observe enough cells in a single frame within many of the slides. This is important as we had to make sure that the cells we were counting the vacuoles for were different and thus, we could not shift the slide over to look for more cells without risking counting the same cells again and compromising the integrity of the data.

Furthermore, our time intervals were considerably long to observe food vacuole growth rates. We saw that the number of vacuoles began to plateau after 115 minutes. This suggests that after approximately two hours food vacuole growth in *T. thermophila* reaches its maximum amount. In other words, we may have just crossed the optimal growth threshold, meaning that at

any time interval past those tested would not have provided results any different from those already observed. These results concur with those found by Frankel et. al (1980) who saw that cell division in *T. thermophila* stalls after 1.5 hours, which falls below the 115 and 145 intervals where the plateau was observed. If cell division begins to stall, the cells do not require as many nutrients in order to provide energy for dividing processes and thus results in fewer additional food vacuoles being formed past this point. Furthermore, our second interval between 20 and 85 minutes must have been the interval where most food vacuoles were formed as it began to plateau shortly afterwards, which would explain the small increase observed in the 115 min interval compared to the 85 min interval. This being the case, follow up experiments would be advised to keep the time intervals between 20 and 120 minutes with intervals roughly ranging from 10-20 minutes; smaller intervals are advised if trying to create a growth rate curve instead of mean count numbers. Had this experiment been conducted in this manner we may have seen different results, but due to this not necessarily being a growth curve we had assumed that the excessive length of the time intervals would not be a significant factor as the raw number of food vacuoles should have been sufficient to determine a difference in means when analyzed.

For future studies of similar nature, we would recommend better regulation of the time intervals in order to get a better idea of the growth of food vacuoles in *T. thermophila.* Furthermore, the addition of a second factor such as acidity or salinity will help better understand the ideal growth environment of food vacuoles within *T. thermophila*.

Conclusion

Our experiment looked into the relationship between the formation of food vacuoles, used as a model for metabolism and growth, and temperature. We expected to see a change in the

number of food vacuoles found in the *T. thermophila* samples at 25°C, 30°C and 35°C and we predicted that as temperature increased, so too would the number of food vacuoles found in *T. thermophila*. However, our experiment did not produce any statistically significant results and thus, we failed to reject our null hypothesis, which states that temperature has no effect on the amount of food vacuoles formed by *T. thermophila*.

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Appendix

Table 1. Number of food vacuoles per five cells in each replicate.

Time (mins)	Replicates	25° C	30° C	35° C
20	1	4	2	3
	2	0	6	9
	3	4	7	17
85	1	36	77	75
	2	68	202	52
	3	58	92	35
115	1	69	79	43
	2	124	158	116
	3	94	171	146
145	1	58	92	73
	2	122	198	139
	3	88	**N/A***	94

* We did not find any cells in this replicate.

Table 2. Slope value/growth rates used in one-way ANOVA.

	Replicate 1	Replicate 2	Replicate 3
25° C	0.496	1.06	0.737
30° C	0.723	1.48	1.66
35° C	0.497	1.08	0.827

Table 3. One-way ANOVA analysis

Summary of Data						
	Treatments					
	1	2	3	4	5	Total
N	3	3	3			9
ΣX	2.293	3.863	2.404			8.56
Mean	0.7643	1.2877	0.8013			0.9511
ΣX^2	1.9128	5.4687	2.0973			9.4789
Std.Dev.	0.283	0.4972	0.2923			0.4089

Result Details				
Source	***SS***	***df***	***MS***	
Between-treatments	0.5118	2	0.2559	*F* = 1.85968
Within-treatments	0.8256	6	0.1376	
Total	1.3373	8		

The *f*-ratio value is 1.85968. The *p*-value is .235257. The result is *not* significant at $p < .05$.