The Effect of Temperature on Food Vacuole Formation in *Tetrahymena thermophila*

Cindy Pham, Kattereya Kennedy, Nicola Popper, and Ravneet Ghotra

Abstract

Through the process of phagocytosis for food vacuole formation, *Tetrahymena thermophila* can fulfill its nutritional needs for growth. This study aims to determine the effect of temperature on the number of vacuoles produced in *T. thermophila* over time as they possess a relationship with zooplankton, an important food source for salmon. Our null hypothesis states that there is no change in vacuole production due to temperature change. Next, our first alternative hypothesis states that vacuole production will be highest at 30°C. Lastly, our second alternative hypothesis states that food vacuole production will be highest at 10°C. *T. thermophila* was observed every 20 minutes over a 2-hour interval at temperature groups 10 °C, 23 °C, and 30°C. Three replicates were made at every 20-minute interval consisting of counting the number of vacuoles in 10 randomly selected cells. Our research showed that *T. thermophila* formed more food vacuoles at lower temperatures, 10°C and 23°C, than at 30°C. This contradicts our initial prediction, but supports the second alternative hypothesis. Statistical analysis was performed using two-way ANOVA and 95% confidence intervals. The P-value is consistently lower than our confidence level of 0.05, thus our results are statistically significant.

Introduction

Tetrahymena thermophila is a unicellular eukaryote (Orias *et al.* 2011). It maintains homeostasis and fulfills its nutritional requirements by performing a metabolic process called phagocytosis. To initiate phagocytosis, *T. thermophila* uses its outer pseudopods to sweep food particles nearby into an oral groove (Gronlien *et al.* 2002). *T. thermophila* then forms a sac around the engulfed food particles within its cytoplasm, which then produces food vacuoles (Braun 2006). Lysosomes containing digestive enzymes then merge with the food vacuole to break food particles down (Braun *et al.* 2006). Thus, nutrients are made available for *T. thermophila's* cells to absorb, while its indigestible materials are excreted out (see Figure 1) (Braun *et al.* 2006).

T. thermophila are found in freshwater aquatic ecosystems such as ponds, lakes and rivers around the world that have a temperature range between 8°C to 35°C (Bamdad *et al.* 1999, Elliott *et al.* 1955). Past studies have shown that the organism population excels at specific temperatures. Thus, the objective of the experiment is to determine the effect of

vacuole production via phagocytosis in *T. thermophila* due to temperature over time. This investigation is important because *T. thermophila* creates a link between bacteria and zooplankton, an essential food source for juvenile sockeye salmon (Eggers 1978). Thus, investigating vacuole formation of *T. thermophila* at different temperatures can give information about the food chain cycle of salmon. A previous study performed by Thormar (1962) examined the effect of temperature on the reproduction rate of *Tetrahymena pyriformis* at temperatures between 10.5°C to 33.5°C. The experiment concluded that the maximum reproduction rate occurs at 27.5°C, whereas temperatures above 30°C and below 20°C produced a delay in cell division. Since both phagocytosis and cell division involves the process of sub-cellular formations, we believe temperature will have a similar effect on vacuole formation rates (Deschamps *et al.* 2006).

Thus, to determine the effect of temperature on vacuole production, we examined food vacuole formation at 10°C, room temperature 23°C, and 30°C. For our first alternative hypothesis, we predict food vacuole production will be highest at 30°C. Our second alternative hypothesis states that food vacuole production will be highest at 10°C, while our null hypothesis predicts no change in food vacuole production due to temperature change. We predict that *T. thermophila* will have the highest vacuole formation at 30°C.

Null Hypothesis (H_0): Temperature has no effect on *T. thermophila* vacuole formation.

Alternative Hypothesis 1 (H_A): *T. thermophila* vacuole formation is highest at 30°C.

Alternative Hypothesis 2 (H_A): *T. thermophila* vacuole formation is highest at 10°C.

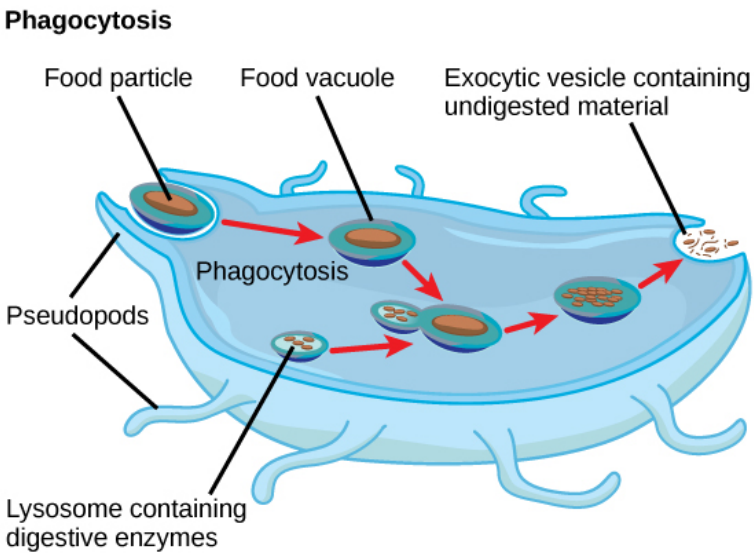

Figure 1. The formation of food vacuole through the process of phagocytosis in Macrophage. *Retrieved online from Wikimedia Commons, the free media repository.*

Methods

The experiment was carried out with *T. thermophila* strain B 2086 that was prepared on October 24th, 2017 in a 500 mL Erlenmeyer flask. The flask was swirled to ensure thorough mixing of the cells prior to sample preparation. Sterile heating was performed on three 6 mL test tubes to avoid contamination. With our experiment testing three different temperatures at 10°C, 23°C and 30°C for vacuole formation over time, each sample was tested for 2 hours. Over the course of 2 hours, we tested 3 replicates of the sample every 20 minutes, resulting in 7 time intervals with 3 replicates each. We chose 20 minute intervals because that gives the cells sufficient time to digest the dye for adequate viewing. The intervals were at 0, 20, 40, 60, 80, 100, and 120 minutes. Thus, since there are 63 samples total for the three treatment groups, we then prepared 63 Eppendorf tubes with 10 uL of glutaraldehyde fixative. Glutaraldehyde fixative is used to ensure that the cells are fixed for viewing and vacuole counting. We prepared the the glutaraldehyde fixative prior to adding the *T. thermophila* to ensure consistency that the cells are fixed at the exact time intervals.

To begin our control temperature sample at 23 °C, 5 mL of *T. thermophila* was pipetted into a 6 mL test tube followed by the addition of 1 mL Carmine red dye and re-suspended for thorough mixing. A timer was then set for 20 minutes while the observations for the first sample at time 0 minutes began. For the first sample at time 0, we pipetted 100 uL of the Tetrahymena-dye into three Eppendorf tubes containing glutaraldehyde fixative for three replicates. Once the cells become fixed, they migrate to the bottom of the Eppendorf tube, so we pipetted 30 uL from the bottom of the tube onto three slides for vacuole counting. Each slide was viewed at total magnification of 400X under a compound microscope. One member was dedicated to randomly select 10 cells and count the number of pink-red vacuoles in each. Another member entered the observed data onto an Excel table. The process of fixing and vacuole-counting was repeated every 20 minutes over a 2-hour period with three replicates for each fixed sample, resulting in 21 total samples at each temperature. The control temperature group was held constant in a test tube rack next to a thermometer for regulating.

Upon completion of the control group, two new test tubes of 6 mL *T. thermophila* with 1 mL red dye were prepared for temperature treatment groups 10°C and 30°C. The same procedural steps performed for the control group was done over the course of 2 hours, resulting in 42 samples. We had two group members monitor and fix the 10°C and 30°C sample groups of *T. thermophila*, a third member count the vacuoles, and the fourth member enter data. The members responsible for temperature treatment groups 10°C and 30°C were also in charge of maintaining their temperatures in the ice bath and hot water bath mediums. Each member performed the same duties for each temperature group to ensure consistency and to reduce the possibility of error.

Observations of the number of vacuoles seen in 10 randomly chosen cells were recorded. To randomly pick 10 cells, the slide was moved several times. This technique had to be done because the cells were dispersed, making it difficult to have 10 cells within the field of view. Although this technique's randomness is biased, the same group member was

responsible for all the vacuole counting to maintain consistency. The dye was extremely faint and difficult to see. We tried to reduce discrepancies by having one member do the task identifying the vacuoles for consistency. The classifications of our identification of the vacuoles included whole cells with intact membranes that must be free floating cells with no clustering. Statistical analysis was performed using two-way ANOVA and 95% confidence intervals.

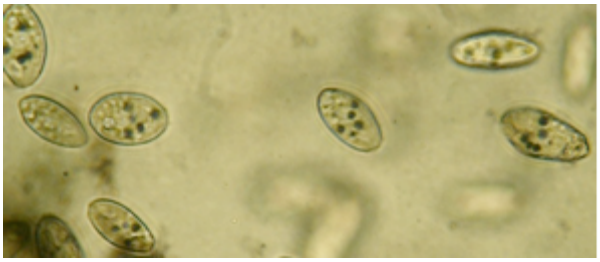

Figure 2. What the *T. thermophila* cells looked comparable to but with very faint pink/red outlines around vacuole. The darker circles in the cells of this figure have ingested a sufficient amount of dye to clearly show distinct vacuoles. We did not use a camera to capture experimental slides of the cells, but this is very similar to what was seen in relation to cell and vacuole size and dispersion. The vacuoles, however, did not appear as dark as in this figure, instead, they were much more faint in red/pink colour. *Retrieved online from ASSET: Advancing Secondary Science Education thru Tetrahymena with Cornell University [October 23, 2017]*

Results

The number of food vacuoles formed was highest at the lower temperatures 10°C and 23°C. Over time, there were more vacuoles per *T. thermophila* cell at 10°C, with a maximum of 2.67 vacuoles per cell at 80 minutes. Similarly, the number vacuoles at 23°C possessed steady growth finishing with the same number of vacuoles at the end of the two hours. The fewest observed number of vacuoles formed was at 30°C, with the exception of a spike at 60 minutes. Its value of 2.13 vacuoles per cell was uncharacteristically high, ergo we determine it to be an anomaly. Please refer to **Figure 3** for the graphical results.

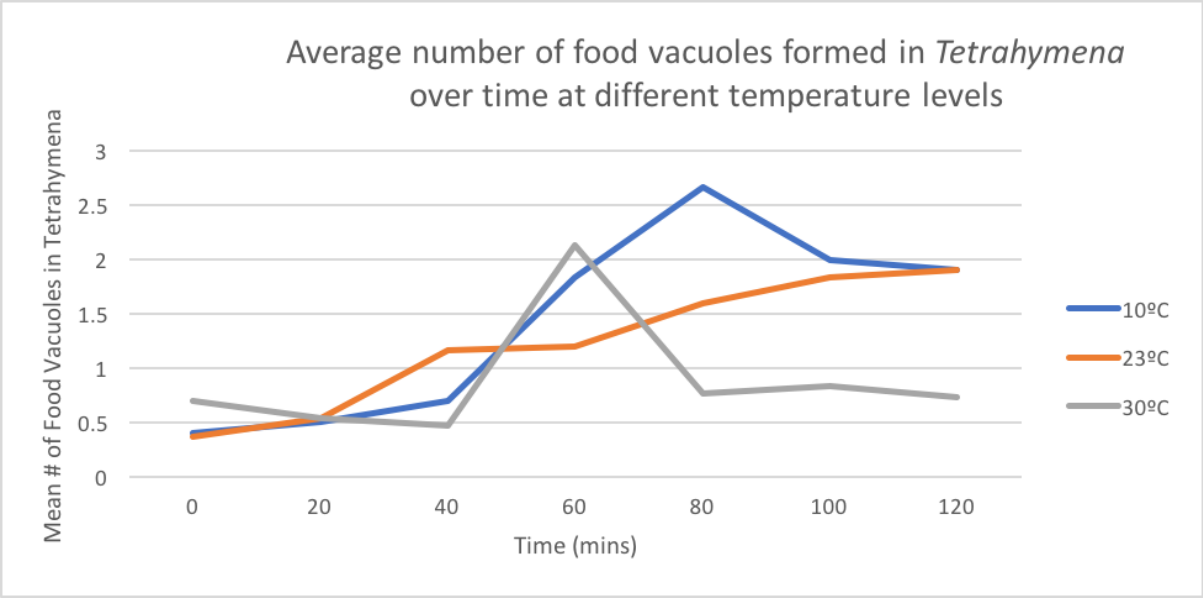

Figure 3: Line graph comparing the mean number of food vacuoles formed in Tetrahymena at three different temperatures. The lower temperatures of 10°C and 23°C saw higher numbers of food vacuoles being formed while 30°C had the lowest.

Using Excel (Version 14.0; Redmond, Washington), we conducted a two-way analysis of variance table with our results. Please refer to Table 1 in Appendix for the relevant statistical data analysis. Our F-values were consistently larger than F-critical, allowing us to reject the null hypothesis while our P-values were consistently smaller than our significance level of 0.05, which makes our results statistically significant. To determine which alternate hypothesis our results suited we created a bar chart to compare the rates of food vacuole growth for each temperature (Figure 4). This confirmed that the rate of vacuole formation decreases as temperature increases between the temperatures 10°C to 30°C.

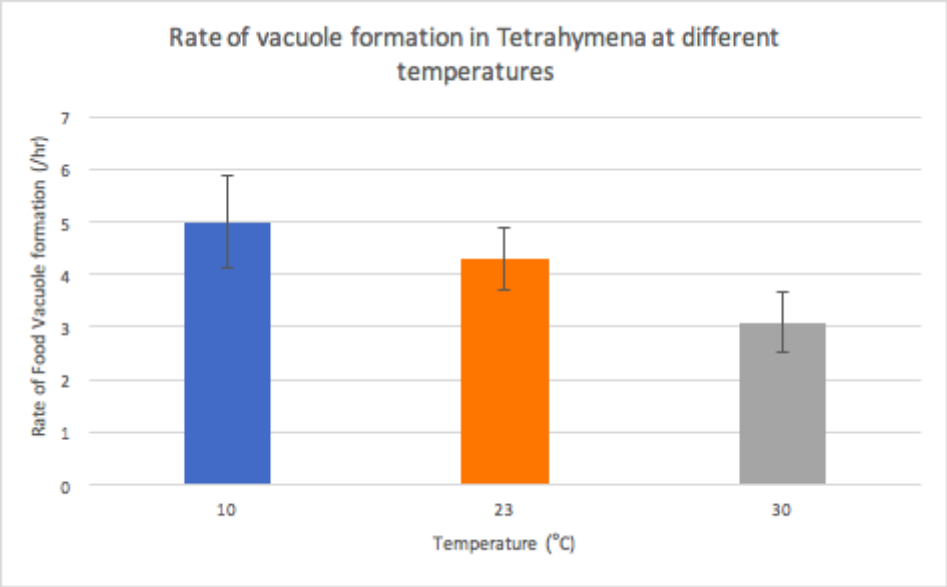

Figure 4: Bar chart comparing the rate of food vacuole formation in T. thermophila at each temperature interval. The error bars represent the standard deviation of the rate.

While counting the food vacuoles within the *T. thermophila* cells, we observed two recurring patterns. While *T. thermophila* formed the most food vacuoles at lower temperatures, the colour of the dye was the weakest at 10°C, making it harder to distinguish and count food vacuoles. The colour of the dye was most vibrant at 23°C. With reference to Figure 5, this can be seen with well-defined food vacuoles on the right and the less definitive vacuoles on the left. Secondly, the cells were much more dispersed at 30°C. Despite collecting our samples with the same methods, there were fewer cells and vacuoles at this temperature.

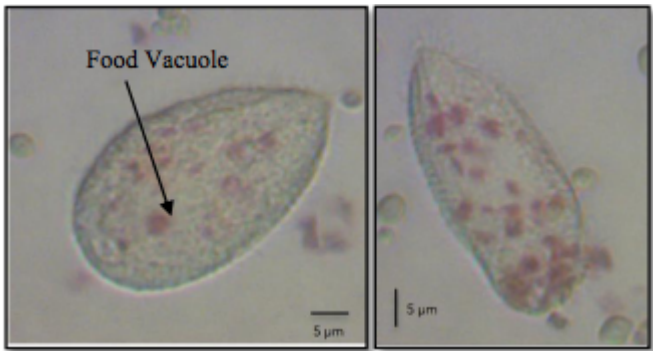

Figure 5: *T. thermophila* with red-dyed food vacuoles viewed under 400x magnification (Chan 2016)

Discussion

With respect to our two-way ANOVA analysis ($P<0.05$), we reject the null hypothesis, indicating that vacuole formation of *T. thermophila* is affected by temperature and time. However, our prediction of the first alternative hypothesis is rejected, while the second alternative hypothesis is supported. The second alternate hypothesis predicted that *T. thermophila* vacuole formation would increase as temperature decreases, which was seen in our experiment. According to **Figure 3,** although there were periods where the number of vacuoles increased rapidly between 40 to 60 minutes and slowly from 80 to 100 minutes at 30°C, the remaining time intervals showed decreases in the number of vacuoles. Conversely, the number of vacuoles increased more steadily at 10°C relative to the 23°C and 30°C groups throughout the 2 hours.

With regards to time, an extensive study focusing on the digestive cycles of *T. thermophila* determined that the processing period was approximately 45 minutes long and the defecation period was approximately 2 hours, resulting in a total of 3 hours (Fok and Shockley, 1985). This information can be related to our results as all three temperatures for the first 40 minute exhibited low number of food vacuoles followed by a sudden increase in number of vacuole starting at approximately 45 minutes. It is possible that oral ciliary membranelles of *T. thermophila* cells were sweeping nearby food particles into the oral groove, undergoing phagocytosis, and other metabolic processes leading up to vacuole formation for digestion of nutrients during this delay-period.

With regards to temperature, *T. thermophila* cells were much more dispersed with low counts and very faint-stained vacuoles at 30ºC compared to 10ºC and 23ºC. An experimental error may have contributed to this by not swirling the flask vigorously enough prior to transferring the organism into the test tube. This would affect the number of cells being transferred. A possible biological explanation for this is *T. thermophila* having to adjust to higher

temperatures by changing their vacuole formation rate. *T. thermophila* possess an oral region that undergoes four stages of phagocytosis for vacuole formation (Nillson,1977). The stages include opening of the cell's membrane to open the vacuole, followed by filling of the vacuole. The vacuole then closes and begins to detach from the cytostome (Nillson,1977). It is possible that at higher temperatures, *T. thermophila's* adjustment to this condition resulted in the delay of vacuole formation during these phagocytic stages. This could explain why there were fewer vacuoles, as phagocytosis is critical for *T. thermophila's* cell division and growth (Orias and Pollock, 1974).

Another study conducted on *T.thermophila* suggests that an increase in temperature causes additional stress, leading to changes of the lipid composition (Diranghi and Parangi, 2013). A specific lipid found in *T. thermophila* ciliary membranes, pentacyclic triterpenoid, commonly known as tetrahymanol, could have been compromised at high temperatures and lost its function (Williams, 1990). Thus, this affects the overall stability of the cellular membrane and alternately, processes such as phagocytosis. This could be a potential factor contributing to the low number of food vacuoles found in cells fixated at 30°C compared to 10°C and 23°C.

Lastly, there are several other external factors that contribute *T. thermophila* cell growth and vacuole formation that were not taken into account during the experiment. Such factors include cell oxygen supply, pH and nutrient availability (Hoffmann and Cleffmann, 1981). Measurements were not made for these extraneous factors, which could have been different between the different temperature treatment levels. Higher temperatures may have affected the amount of nutrients available at 30°C. *T. thermophila* requires several essential amino acids and vitamins for growth (Wilson, L *et. al.* 1999). At higher temperatures, it is possible that different amino acids and proteins denature, preventing further growth to occur. Since cell density is roughly proportional to nutrient availability, this may account for fewer cell formation (Hoffmann and Cleffman, 1981).

Although there are several factors that could account for rejection of the first alternative hypothesis, there is still insufficient information to explain the increase in vacuole formation in *T. thermophila* at 10°C. This is an area for further investigation for how lower temperatures affect vacuole formation in *T. thermophila*.

Conclusion

In conclusion, our research showed that *T. thermophila* forms more food vacuoles at lower temperatures (10-23°C) than at a higher temperature of 30°C. This is the opposite of what we initially predicted. However, upon analysis, we can confirm that the results are statistically significant. We reject our null hypothesis, which stated that food vacuole formation is not influenced by temperature and time. Conversely, our prediction of the first alternative hypothesis was not accepted as well, as our data supported the second alternative hypothesis stating that vacuole formation is greatest at 10°C. These results may be related to salmon, as they spawn during the fall (October to November) when water temperatures drop. This is a possible relationship where *T. thermophila* growth increases at lower temperatures, resulting in more available zooplankton for salmon to feed upon when spawning.

Acknowledgements

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APPENDIX

Table 1. Two-way ANOVA table for the difference in food vacuole formation in Tetrahymena over time at three different temperatures. The F value is consistently higher than Fcrit so we can reject our null hypothesis and the P value is consistently lower than our confidence level of 0.05 so our results are statistically significant.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*Fcrit*
Sample	167.8095238	6	27.96825397	30.19440997	9.148E-32	2.113450281
Columns	32.25079365	2	16.12539683	17.40891095	4.443E-08	3.010517027
Interaction	96.57142857	12	8.047619048	8.688175855	2.135E-15	1.768072951
Within	564.1	609	0.926272578			
Total	860.731746	629				

Ravneet - Abstract, Discussion, Citations
Cindy - Methods, Citations, Discussion, Figure 2, Acknowledgment, corresponding author
Kat - Introduction, Citations, Figure 1
Nicola - Title, Results, Figure 3&4, Table 1, Appendix, Conclusion