Don't be salty! The effects of salinity on vacuole formation of *Tetrahymena thermophila.*

Jullia Ayre, Florence Ng, David (SungJin) Suh

Abstract

The rate of vacuole formation of *Tetrahymena thermophila* was tested under different salinity conditions. *Tetrahymena thermophila* are heterotrophic organisms that synthesize food vacuoles in freshwater conditions. We incubated *T. thermophila* in 2 mM NaCl and 200 mM NaCl solutions to make comparisons to *T. thermophila* in plain media, which is the optimal growing conditions for the cells. To determine if the rate of vacuole formation, dye was added to the treatments and samples were taken out every five minutes until the 45th minute. Little to no vacuoles were formed in high salinity conditions (200 mM NaCl) throughout the experiment. There was a statistically significant difference between groups as determined by one-way ANOVA (p = 9.89 x 10 ^-5). *T. thermophila* vacuole formation was found to be highest in low salinity (2 mM NaCl) treatments.

Introduction

Tetrahymena thermophila is a large, ciliate protozoan living in any temperate freshwater environments (Elliot 1974). It is a heterotroph, ingesting organic molecules from its environment by the process called phagocytosis. Engulfed particles are trapped into an internal compartment, known as 'food vacuole' (Aijaz 2017).

Beyer et al. (2001) conducted a test on *T. thermophila* to observe their population growth under different salinities. They found that under the high salinity condition, *T. thermophila* becomes stressed enough to follow programmed cell death. This suggests that the population of *T. thermophila* will decrease with increasing salinity, and the number of food vacuoles observed will be much less than those under ideal conditions.

Since it inhabits freshwater ponds, there must be a maximum toxicity level threshold of salinity for *T. thermophila*. This is supported by the work of Broadfoot et al. (2009). They found that high salinity condition (1250 ppm NaCl) was toxic enough to cause abnormalities in the organism. This also may affect the number of food vacuoles due to abnormalities.

Considering the ideal habitat of *T. thermophila*, we hypothesized that T. thermophila would form higher number of food vacuoles under ideal condition (that is 'normal') than low or high salinities. The hypotheses we decided to test were:

Null Hypothesis (H_o): The number of food vacuoles formed by *T. thermophila* will not be affected by different salinity conditions.

Alternative Hypothesis (H_a): *T. thermophila* will form more food vacuoles under 'normal' salinity conditions (unknown) than those under low (2 mM NaCl) or high salinities (200 mM NaCl)

Methods

First, we prepared and labeled nine test tubes: three replicates of three differing salinities. In addition, we labelled 81 Eppendorf tubes (3 sets of Eppendorf tubes labeled 1-9 for 3 replicates). In order to make the experimental groups, 2 mM and 200 mM of NaCl, we mixed equal volumes of *T. thermophila* culture with 4 mM and 400 mM NaCl solutions, respectively. Since we were provided with 400 mM NaCl, we diluted 0.15 mL 400 mM with 14.85 mL of plain media to make 15 mL of 4mM NaCl. The first test tube contained 2 mL of 4 mM NaCl to represent low salinity conditions, the second had 2 mL of plain media for our control group, and the third test tube contained 2 mL of 400 mM NaCl to represent high salinity conditions. In addition to all test tubes, we added 2 mL of *T. thermophila* culture. All solutions will be kept at room temperature. Next we made dilutions to our black dye by adding 6 mL of 4 mM NaCl with a few drops of dye for our low salinity treatments. For the high salinity treatments, we mixed 6 mL of 400 mM NaCl with a few drops of dye. We did not need to make any changes to the dye for the plain media test tubes. After all mixtures are made, we pipetted 10μL of fixative into all Eppendorf tubes. Next, we pipetted 1 mL of the dye mixtures and pure dye into the corresponding test tubes. We then set a timer for 10 minutes. After the 10 minutes passes, we extract 100μL of the sample from each test tube into the Eppendorf tube labelled with the correct replicate and salinity that is labelled with a 1, which represents the time at 0 minutes. After the first time interval of 5 minutes we pipetted another sample and placed it in the corresponding Eppendorf tubes labelled with a 2. This process was repeated until a total of 45 minutes had passed and the tubes labelled with a 9 are collected. Once all the samples were collected, we pipetted 10 μL of each sample onto a slide, placed a coverslip over the sample, and placed the slide on a compound microscope set to 40X magnification. In each sample, we counted the number of vacuoles of 10 cells that contained stained vacuoles and determined the average for each sample. After the average numbers were calculated, we plotted three separate graphs, each representing the average number of

vacuoles vs. time interval under different salinities. Also, we analyzed our calculated means through one-way ANOVA testing to verify our hypothesis.

Results

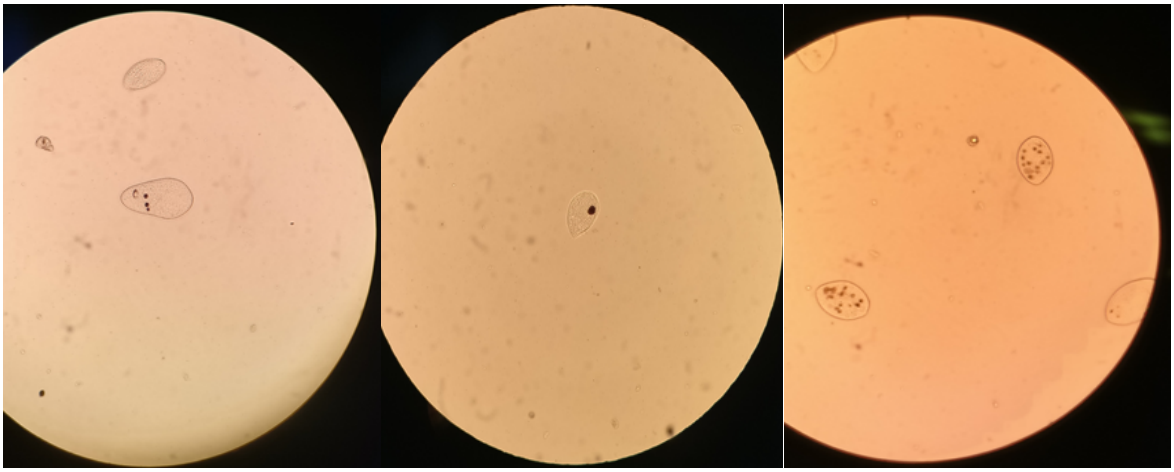

Figure 1. *T. thermophila* under 100X magnification within the first replicate. From left to right, (1) control at 15 min., (2) high salinity at 10 min., and (3) low salinity at 40 min. Imaged using the OnePlus 3 phone camera positioned over the objective lens.

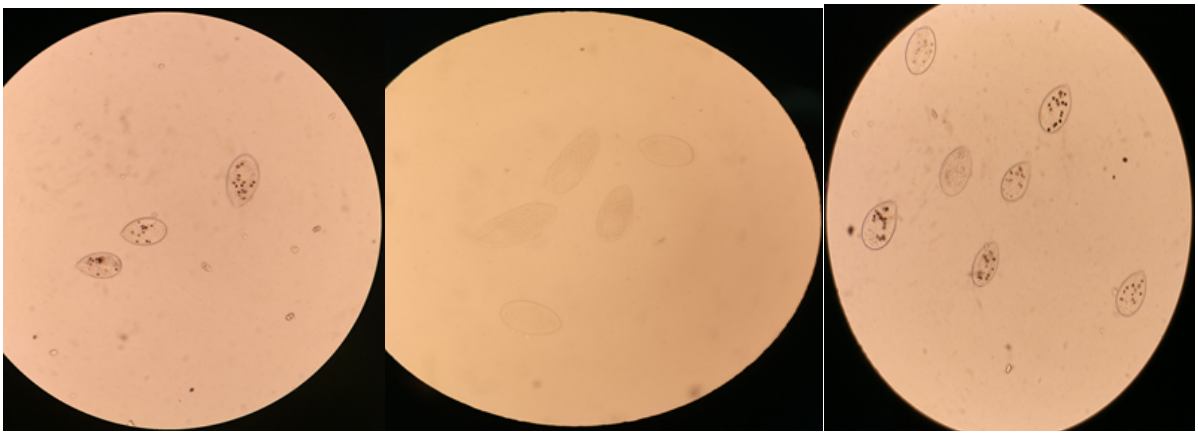

Figure 2. *T. thermophila* under 100X magnification within the second replicate. From left to right, (1) control at 35 min., (2) high salinity at 15 min., and (3) low salinity at 40 min. Imaged using the OnePlus 3 phone camera positioned over the objective lens.

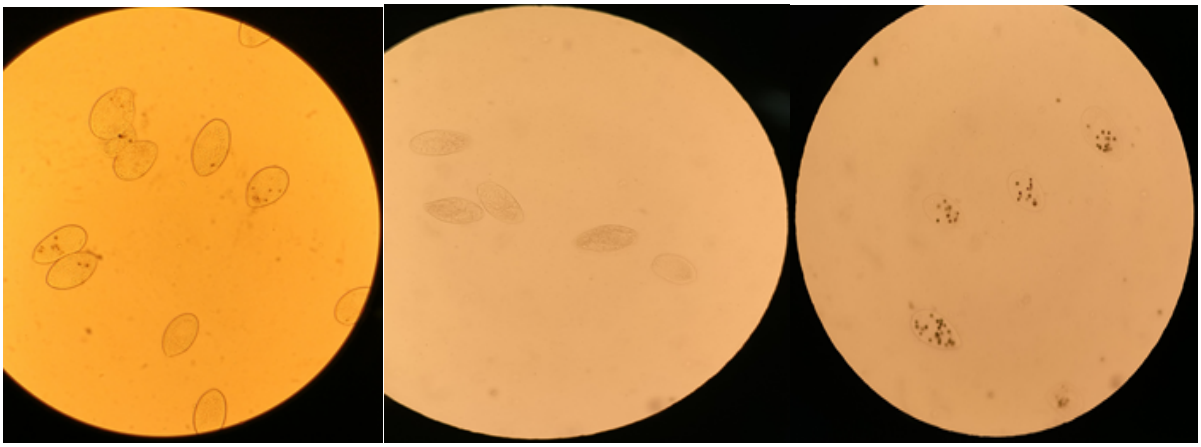

Figure 3. *T. thermophila* under 100X magnification within the second replicate. From left to right, (1) control at 20 min., (2) high salinity at 25 min., and (3) low salinity at 35 min. Imaged using the OnePlus 3 phone camera positioned over the objective lens.

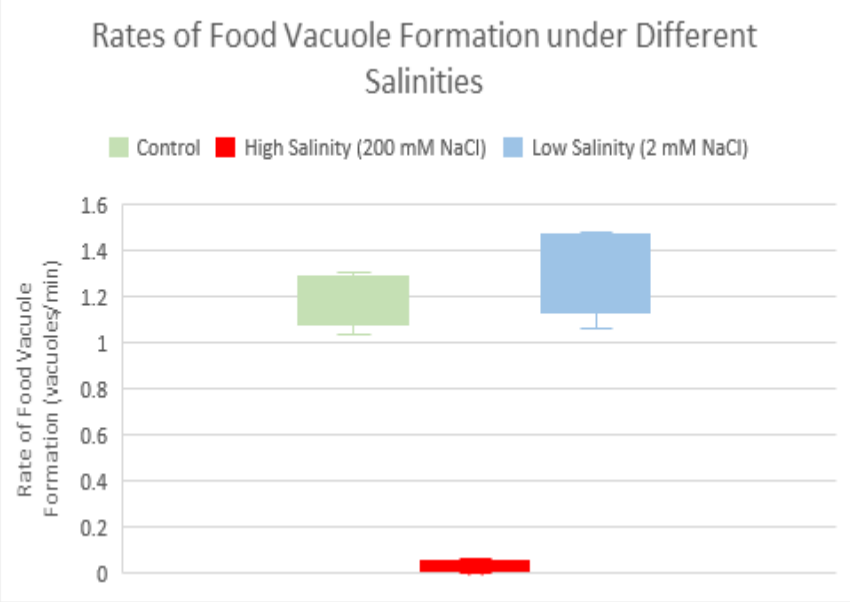

Fig.4 . Analysis of rates of food vacuole formation under different salinity conditions done by one-way ANOVA testing

Our data for each treatment level was averaged in Table 2 from Appendix. Afterwards, we performed a one-way ANOVA test using the Data Analysis function on Microsoft Excel. A one-way ANOVA test was chosen because our experiment has one independent variable (time) with more than two levels of treatments (Control, High Salinity, and Low Salinity). There was a statistically significant difference between groups as determined by one-way ANOVA (p = 9.89 x 10 ^-5). The p-value (9.89 x 10

^-5) is well below 0.05, and therefore there is a statistically significant difference between the average number of vacuoles in our treatments. Therefore, we reject H_0, and support H_a.

Discussion

Based on the data and observations, we can reject H_0, because the data indicated a significant decrease in number of vacuoles under high salinity conditions. In addition, this significant decrease in number of vacuoles supports H_a, as *T. thermophila* activity was expected to decrease under increased salinities. As observed in our data for our control and low salinity treatments, *T. thermophila* culture can better osmoregulate in environments with little to no salt as evidenced by the high numbers of dyed vacuoles found in these treatments compared to the high salinity treatment. In the high salinity treatment, *T. thermophila* was unable to adjust to the extreme environment and could not osmoregulate. This observation correlates with their known freshwater habitats in nature.

Our project faced many challenges in the structure and design of the experiment, some of which we were unable to anticipate until attempting it in lab. Therefore, with the guidance of instructors, we adapted a number of adjustments to our experiment. Initially our lab group was provided red dye to perform the experiment, but we were not able to observe vacuoles within *T. thermophila* due to its low opacity even after waiting for an hour. We switched to black dye which was significantly opaque and allowed us to count vacuoles. It should be noted that the black dye was effective enough to be observed in cells within 5 minutes, and we adjusted our sampling intervals to reflect it. We reduced the amount of fixative to a 1:10 fixative:sample ratio to prevent cell lysis. We used fresh culture and solutions every lab session to prevent contamination of our specimens. Lastly, we only created slides when we were counting that particular treatment to prevent the specimen from drying out.

We recognize that our experiment is limited in scope and propose the following improvements for further study. In our experiment we only tested two salinity treatments with our control following instructor's advice for the scope of Project 3. Testing more than two salinity treatments, especially one between 2 mM and 200 mM, would have given more detailed results about *T. thermophila* vacuole formation. The number of cells counted could also be increased (>9) and the number of sampling intervals could also be increased for more detailed results. The margin of human error could be reduced if there was only one group member in charge of counting vacuoles. However, in our experiment we

assigned two people to count as the black dye made the vacuoles extremely distinct and we had practiced counting beforehand to agree on a standard of measurement.

Conclusion

As hypothesized, our results show that the number of vacuoles produced by *Tetrahymena thermophila* decreases under an increased salinity. It is due to the organism's decreased reproduction in high salinity conditions, leading to lower cell activity and thus lower number of vacuoles found within the cell. Furthermore, *T. thermophila* under extreme salinity condition, for example 200 mM NaCl, showed an inhibited cell activity, since almost none of vacuoles was observed in all three replicates of samples, supporting our alternative hypothesis.

Acknowledgements

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References

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Appendix

	Replicate 1			Replicate 2			Replicate 3		
Time	Control	HS	LS	Control	HS	LS	Control	HS	LS
0	1.7	1.2	1.8	1.7	0	2.9	1.5	0	1.2
5	2.6	0.1	1.8	2.5	0	2.6	2.4	0	1.8
10	2.9	0.2	3.6	4.1	0.3	4.6	5.1	0	4.3
15	4.9	0	3.8	5.8	0	5	4	0	6.5
20	4.5	0.1	5.1	7.1	0.1	5.5	5.4	0	5.6
25	3.3	0	3.7	6.7	0	8.9	6.1	0	6.4
30	5.2	0	5.1	6.2	0	8.5	6.8	0.2	9.7
35	9.8	0	8.2	7.2	0	9.9	10	0.1	12.5
40	6.6	1.1	9.5	11	0	11.5	8.6	0	9.7

Table 1. Showing the average number of vacuoles counted in 10 T. thermophila cells in each time interval (in minutes) and in each treatment (Control, High Salinity (HS), Low Salinity (LS)).

Average # vacuoles across all 3 replicates			
Time	Control	HS	LS
0	1.6333333	0.4	1.966667
5	2.5	0.033333	2.066667
10	4.0333333	0.166667	4.166667
15	4.9	0	5.1
20	5.6666667	0.066667	5.4
25	5.3666667	0	6.333333
30	6.0666667	0.066667	7.766667
35	9	0.033333	10.2
40	8.7333333	0.366667	10.23333

Table 2. Average number of vacuoles per treatment level (Control, High Salinity (HS), Low Salinity (LS)) across all three replicates.

Anova: Single Factor

SUMMARY

Groups	Count	Sum	Average	Variance
Control	3	3.5925	1.1975	0.0201
High Salinity (200 mM NaCl)	3	0.085	0.028333333	0.001152083
Low Salinity (2 mM NaCl)	3	3.9925	1.330833333	0.053452083

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	3.081234722	2	1.540617361	61.86873222	9.89139E-05	5.14325285
Within Groups	0.149408333	6	0.024901389			
Total	3.230643056	8				

Table 3. Results of a one-way ANOVA test done using the Data Analysis function on Microsoft Excel.