

# **Effect of temperature and time on the ciliary function of *Tetrahymena thermophila* based on food vacuole formation**

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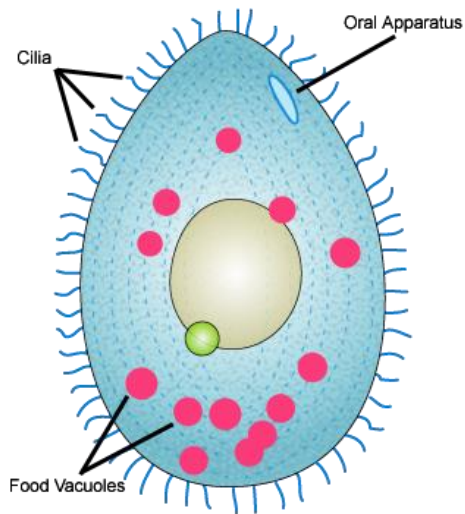
## **ABSTRACT**

The objective of this experiment was to determine the effect of time and temperature on the ciliary function of the single-celled eukaryote *Tetrahymena thermophila*. The movement of cilia is vital for food ingestion by phagocytosis in this organism, therefore, food vacuole formation was used as a measurement of ciliary action. Based on previous literature, we predicted that the number of food vacuoles formed would increase with temperature and time. We counted the number of food vacuoles formed in 10 cells at 15-minute intervals for one hour at 25°C, 30°C and 35°C. The number of food vacuoles formed increased over time at all treatment temperatures, and at 60 minutes 25°C had the highest number of food vacuoles while 30°C had the lowest. Cells in the 30°C and 35°C treatments displayed a sharp increase in number of vacuoles formed at 30 minutes and then smaller increases thereafter. Based on a two-way ANOVA we were able to show that temperature and time, when considered separately, had an effect on number of food vacuoles formed, and that there was no interaction between these two factors. Our observed trends were different than previous studies, likely due to uncertainty and variation in our experiment. Based on our results, we were unable to conclude that increasing temperature results in increased ciliary function.

## **INTRODUCTION**

### *General Information*

*Tetrahymena thermophila* is a unicellular eukaryote that is large in size among ciliates (Collins and Gorovsky 2005). On average, its dimensions are 30 µm wide by 50 µm long and it is found in temperate freshwater environments (Collins and Gorovsky 2005). It is a protozoan with a rapid growth rate and a doubling time of less than two hours (Collins 2012). *T. thermophila* feeds by phagocytosis using an oral apparatus, a funnel-like structure located in the anterior portion of the cell, as shown in Figure 1 (Gonda *et al.* 2000).



**Figure 1.** Anatomy of *T. thermophila*. Figure showing the cilia, food vacuoles and oral apparatus of our organism.

As depicted in Figure 1, *T. thermophila* is covered by about 1,000 cilia which are essential for the survival of *T. thermophila* because they aid with cell motility and phagocytosis (Rajagopalan *et al.* 2009). Food particles are swept into the cell by cilia that line the interior of the oral apparatus where food vacuoles are formed and then transported to the rest of the cell (Gonda *et al.* 2000). Food vacuoles range in size from 5  $\mu\text{m}$  to 15  $\mu\text{m}$  and decrease in size shortly after being formed (Nilsson 1977).

### *Our Model*

A study conducted by Lee (1942) on *Paramecium*, a unicellular eukaryote that has very similar characteristics to *T. thermophila*, noted that raising the temperature from 4°C to 35°C resulted in an increase in the number of food vacuoles formed. Cilia are required for food vacuole formation, therefore increased temperature implies an increase in ciliary movement (Lee 1942). As temperature increases from 22°C to 40°C, actin and myosin, the main components of cilia, can bind more effectively and this leads to increased ciliary movement (Okazaki *et al.* 2012; Jessen *et al.* 1979). Thus, we believe an increased number of food vacuoles would be observed at higher temperatures, as illustrated in Figure 2.



**Figure 2.** Flowchart of our research model. How temperature may affect the ciliary movement and number of food vacuoles formed in *T. thermophila* based on previous literature.

### *Our Experiment*

The main objective of our study was to investigate how temperature and time affect the ciliary function of *T. thermophila* based on food vacuole formation. The interest of the project lies in building on the work of Jessen *et al.* (1979) who found a correlation between temperature and food vacuole formation of *T. thermophila*. The action of cilia is necessary for ingestion by phagocytosis, one of the most important ways *T. thermophila* intakes nutrients (Mortimer *et al.* 2014). Jessen *et al.* (1979) found that the wild-type cells formed food vacuoles at all temperatures they studied, 22°C to 40°C. Since food vacuoles were forming at all these temperatures, we decided to use 25°C, 30°C and 35°C as our treatment temperatures. We chose to observe cells at 15-minute intervals over one hour based on a study done by Thurman *et al.* (2010).

Our experiment is important because the relationship between temperature and food vacuole formation allows us to infer the effect of temperature on the cilia of *T. thermophila* as shown by our model in Figure 2. Along with food vacuole formation, the cilia of eukaryotic cells are also crucial for motility, moving medium across its surface and connecting sensory cells with their receptors (Rajagopalan *et al.* 2009). Therefore, temperature's effect on food vacuole

formation of *T. thermophila* can be applied to all the functions of cilia which collectively are essential for its survival. In addition, observations in this study could have implications for human ciliated cells. Clary-Meinesz *et al.* (1992) found that as temperature increased, ciliary movement increased in the human nasal and tracheal cells until about 20°C, after which it leveled off until 45°C. Lynn (2008) also found that the cilia in some unicellular organism can be used to filter out particles just like human nasal and tracheal cells do.

### *Hypotheses and Predictions*

The hypotheses for our experiment are as follows:

**H<sub>0</sub>1:** Time has no effect on the number of food vacuoles formed in *T. thermophila*.

**H<sub>a</sub>1:** Time has an effect on the number of food vacuoles formed in *T. thermophila*.

**H<sub>0</sub>2:** Temperature has no effect on the number of food vacuoles formed in *T. thermophila*.

**H<sub>a</sub>2:** Temperature has an effect on the number of food vacuoles formed in *T. thermophila*.

**H<sub>0</sub>3:** There is no difference in the effect of time on the number of food vacuoles formed in *T. thermophila* in varying temperatures (25°C, 30°C and 35°C).

**H<sub>a</sub>3:** There is a difference in the effect of time on the number of food vacuoles formed in *T. thermophila* in varying temperatures (25°C, 30°C and 35°C).

With regards to our first set of hypotheses, we predicted that as time progresses the number of food vacuoles formed would increase. This was based on a study conducted by Lee (1942) who found this trend in *Paramecium* food vacuole formation. For our second set of hypotheses, we predicted that higher temperatures would result in an increased number of food vacuoles formed because higher temperatures cause ciliary molecules to work faster, as explained in Figure 2 (Okazaki *et al.* 2012). A study conducted by Lee (1942) found that number of food vacuoles formed by *Paramecium* showed the same trend over time, regardless of

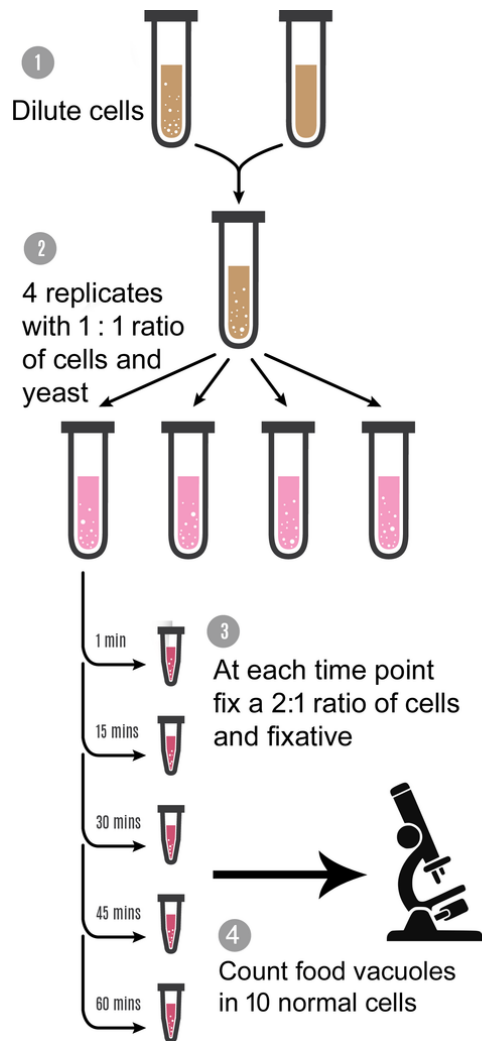
temperature. Based on this, we predicted that there would be no interaction between time and temperature with respect to the number of food vacuoles formed.

## **METHODS**

### *Preparations*

Originally, our procedure used India ink incubated at each temperature to stain the food vacuoles of *T. thermophila*. However, we noticed many cells were mutated in the treatments above 30°C and through further investigation (C. Pollock, BIOL 342 Course Coordinator, personal communication), we found that when India ink is heated to 30°C or above it is lethal to our organism. Thus, we changed our procedure to use Congo red-stained yeast with a concentration of  $9.5 \times 10^7$  cells/mL (C. Pollock, BIOL 342 Course Coordinator, personal communication).

For our experiment, we used 10mL of starting culture of wild-type *T. thermophila* that had been grown for one week in each of our treatment temperatures (25°C, 30°C (control) and 35°C) at a concentration of  $5 \times 10^4$  cells/mL for the 25°C and 35°C cultures and  $1 \times 10^5$  cells/mL for the control culture. Concentrations were counted using a haemocytometer with an Axiostar compound microscope at 100X magnification. We performed appropriate dilutions with cell medium to ensure equal starting concentrations as shown by the first step in Figure 3.



**Figure 3.** Flow chart illustrating procedure for one temperature treatment. Steps include: dilution to create stock solution, four replicates created from stock solution, time points to fix cells and finally, microscopy to count food vacuoles.

### *Food Vacuole Formation*

We began our experiment with the cells incubated at 25°C, and staggered the replicates by one minute using timers. First, we prepared 1.5 mL microcentrifuge tubes with glutaraldehyde fixative to prevent cells from forming additional food vacuoles and preserve existing stained ones so that counting could be conducted at a later time. Then, as shown in the second step of Figure 3, we added a 1:1 ratio of cells to stained yeast to a 6 mL test tube for replicate one. After the cells had been exposed to stained yeast for 1 minute, we transferred a 2:1 ratio of cells to fixative into a microcentrifuge tube. Immediately after this, we added the cells and stained yeast to the test tube for replicate two.

We repeated this procedure for replicates three and four. After replicate one had been exposed to yeast for 15 minutes, we took a sample, fixed the cells and

continued sampling at 15-minute intervals for a total of 60 minutes. This procedure of fixing, depicted in step three of Figure 3, was used for all replicates. We then repeated the entire procedure for cells at 30°C and 35°C.

To reduce potential variation, throughout our experiment, we finger vortexed test tubes for 10 seconds before micropipetting live cells so that they would be distributed evenly. Also,

when dealing with fixed cells, we mixed by pipetting to ensure the cells were evenly distributed. Finally, to reduce variability in temperature, we minimized the amount of time the cells were out of the incubators.

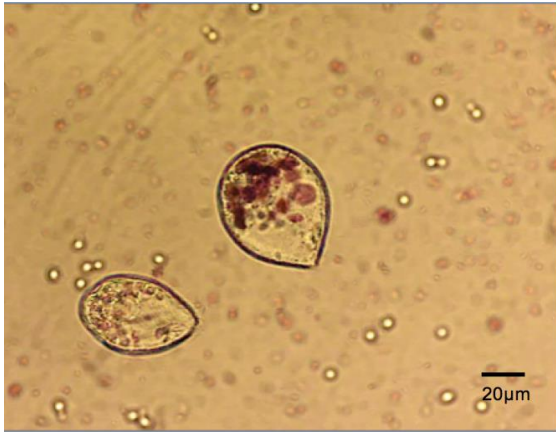
### *Microscopy*

The last part of experimentation, as depicted in step four of Figure 3, consisted of counting food vacuoles using an Axiostar compound light microscope. We made slides using cells fixed at each time and counted the number of food vacuoles in 10 cells on each slide. When counting, replicating cells were excluded because replicating *T. thermophila* do not form food vacuoles (Nilsson 1977). We also excluded circular cells because the oral apparatus flattens resulting in a circular shape during replication (Lynn 2008). In addition, ruptured cells were not counted. Cells that had no food vacuoles were also excluded because healthy *T. thermophila* cells create food vacuoles within the first minute (Nilsson 1977); if none were present at greater times, the cell had most likely died prior to beginning the experiment.

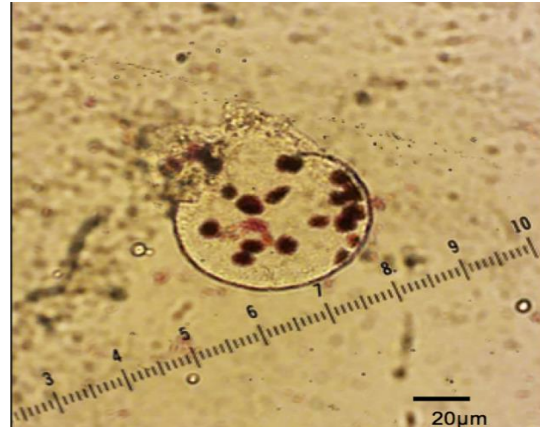
### *Analysis*

We averaged the food vacuoles from the 10 cells counted for each replicate. Then, we used these averages to complete a two-way ANOVA test with time and temperature as our two factors. The test provided us with three *p*-values, allowing us to say with 95% confidence if the means of our data were significantly different from each other. When conducting our statistical analysis, we excluded the number of food vacuoles formed at 1 minute. This amount of time that the cells spent in the yeast was very small compared to the length of our experiment; the amount of error introduced, especially in timing, was comparatively large.

## RESULTS



**Figure 4.** *T. thermophila* cells observed using an Axiostar compound microscope. Cells were grown at 30°C and were fixed 30 minutes after stained yeast were added to medium.



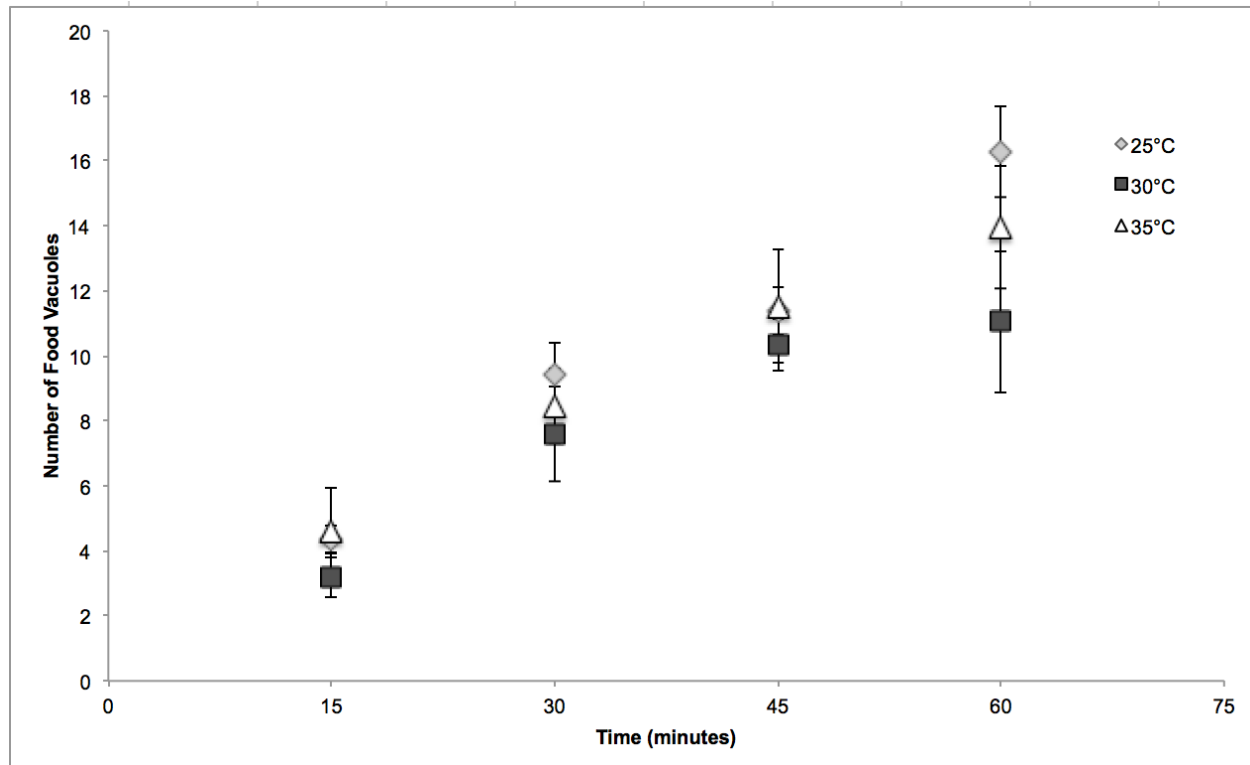
**Figure 5.** A *T. thermophila* cell with a ruptured membrane from a 35°C sample. Fixation was 15 minutes after exposure to yeast, staining food vacuoles dark pink.

Figure 4 shows cells observed at 30°C that were fixed 30 minutes after the addition of stained yeast. The stained food vacuoles can be seen quite clearly in the cell on the right, while the left cell has little or very light staining. We found that generally, the longer the cells remained in medium with the stained yeast, the darker the staining of their food vacuoles; cells fixed at 30, 45 and 60 minutes had darker, more distinct vacuoles than those taken out at 1 and 15 minutes. Figure 4 also shows the typical teardrop shape of the cells that we observed at 25°C and 30°C treatments. At 30°C however, *T. thermophila* cells were rounder in shape, and many were in the last stages of division. At 35°C, many cell membranes were ruptured, as depicted in Figure 5. Also, when counting the stained food vacuoles of fixed cells, we found that at 25°C there were more cells present on each slide compared to 30°C and 35°C treatments.

Using a two-way ANOVA test, we obtained a  $p$ -value of  $2.98 \times 10^{-18}$  for our first hypothesis, which was whether time had an effect on food vacuole formation rate. For our second hypothesis, whether temperature had an effect on food vacuole formation rate, a  $p$ -value



of  $9.82 \times 10^{-5}$  was obtained. Finally, for our third hypothesis of whether there was an interaction between time and temperature on the rate of food vacuole formation, we obtained a  $p$ -value of 0.064.



**Figure 6.** Time series of food vacuole formation in *T. thermophila* cells at 25°C, 30°C and 35°C. Four replicates of food vacuole counts were averaged at 15-minute intervals for each temperature. Error bars represent 95% confidence intervals. Using a two-way ANOVA,  $p$ -values obtained were  $2.98 \times 10^{-18}$  for effect of time,  $9.82 \times 10^{-5}$  for effect of temperature and 0.064 for interaction effect.

Figure 6 illustrates the average number of food vacuoles present in *T. thermophila* cells at all three temperatures (25°C, 30°C and 35°C) over 60 minutes. Cells at all temperatures show an increase in average number of food vacuoles over the one hour period. At 25°C, cells have the highest number of food vacuoles compared to the other treatments at 30 minutes (9.4 vacuoles) and 60 minutes (16.3 vacuoles). The cells at 35°C have the highest number of food vacuoles at 15 minutes (4.6 vacuoles) and 45 minutes (11.5 vacuoles). Of all three treatments, cells at 30°C have the lowest number of vacuoles at each interval over the hour.

For the 25°C treatment, there is a sharp increase in total number of food vacuoles from 15 to 30 minutes (5 vacuoles), a smaller increase from 30 to 45 minutes (2 vacuoles) and then another sharp increase from 45 to 60 minutes (4.9 vacuoles). At 30°C, there is also a sharp increase from 15 to 30 minutes (4.4 vacuoles), followed by food vacuole increases of 2.8 at 45 minutes and 0.7 at 60 minutes. Again, at 35°C there is a sharp increase from 15 to 30 minutes of 3.9 food vacuoles, and then smaller increases thereafter; 3 food vacuoles from 30 to 45 minutes and then 2.5 food vacuoles from 45 to 60 minutes. As evident in Figure 6, particularly at 30°C, these smaller increases in total number of food vacuoles after 30 minutes result in a plateau of the data.

## **DISCUSSION**

For hypothesis one, we are able to reject the null hypothesis ( $H_{01}$ ) and provide support for our alternative hypothesis ( $H_{a1}$ ), which is that time has an effect on the number of food vacuoles formed in *T. thermophila*, because the  $p$ -value is smaller than 0.05. Our results support our prediction that with increasing time there would be an increased number of food vacuoles formed. According to Figure 6, we see the number of food vacuoles increasing throughout the duration of the experiment (60 minutes). However, it is important to note that the number of food vacuoles formed starts to increase in smaller increments after 30 minutes. This can be explained by the fact that at 30 minutes, *T. thermophila* begin to defecate approximately 25% of their food vacuoles (Nilsson 1997). Thus, not only are *T. thermophila* making new vacuoles with the yeast incorporated, they are also expelling previously formed vacuoles causing the increase in the number of food vacuoles to slow down.

For hypothesis two, we are able to reject the null hypothesis ( $H_{02}$ ) and provide support for the alternative hypothesis ( $H_{a2}$ ), which was temperature has an effect on the number of food

vacuoles formed in *T. thermophila*, because we obtained a  $p$ -value less than 0.05. However, our prediction was not supported as we observed the highest number of food vacuoles at 25°C and 35°C. The counts were much larger at 30 and 60 minutes in the 25°C treatment which could explain the significant results we observed. These very large numbers may be because of sources of variation since all other counts at each time interval were very similar.

Due to a much higher concentration at 30°C, we had to perform a dilution to obtain an initial concentration that was similar to that at 25°C and 35°C. In doing so, a source of variation may have resulted from inaccurate pipetting. Since we did not measure the final concentrations before we began our experiment, we cannot be sure that they were the same at all temperatures. A higher concentration in any treatment would mean that there were fewer yeast available per cell, hence a lower number of stained food vacuoles observed.

To explain why 25°C had the highest counts at 30 minutes and 60 minutes and why 30°C had the lowest we need to consider that the range of temperatures we used may not be broad enough to observe a true change in the numbers of food vacuoles formed. We chose treatments of 25°C, 30°C and 35°C because Jessen *et al.* (1979) found food vacuoles were formed between 22°C and 40°C. In an experiment on *Paramecium* Lee (1942) found an increasing number of food vacuoles from 4°C to 35°C. However, the numbers of food vacuoles at 25°C, 30°C and 35°C were very close to one another (Lee 1942). Therefore, it is likely that small counting errors in our experiment could result in food vacuole numbers that do not follow the trend found in Lee's (1942) study (Figure 6). Counting errors are a very plausible explanation, especially at the 60-minute interval as seen in Figure 6, seeing as the 95% confidence intervals are so large, implying lots of variation. Also, since 25°C was our first treatment, more counting errors may have occurred because we may not have been comfortable with our procedure, thus resulting in

higher counts for this treatment at 30 and 60 minutes. Although we can say that temperature has an effect on food vacuole formation, future studies may wish to test a wider range of temperatures because we did not observe increasing food vacuoles with rising temperature.

Another explanation as to why the food vacuole numbers are very similar at all temperatures could be due to gene expression changes because of our long acclimation time. With shorter acclimation times, such as eight hours, gene expression changes are less likely to play a key role for an organism with a doubling time of two hours (Lee 1942). However, with an acclimation time of an entire week, as in our experiment, it is much more likely that gene expression changes occurred to accommodate the temperature change. If gene expression of actin and myosin changes, the functions they carry out may be altered. The binding affinity of actin and myosin increases with increasing temperature up to a threshold (Okazaki *et al.* 2012). However, if the proteins themselves have changed to accommodate differing temperatures, this trend may not be observed. Thus, ciliary action, which is controlled by actin and myosin, would not appear to increase with increasing temperature (Okazaki *et al.* 2012). As explained in our biological model (Figure 2), if ciliary action does not increase with rising temperature, neither will the numbers of food vacuoles formed (Gonda *et al.* 2000).

Another reason why ciliary action may not be increasing with increasing temperature in our experiment could be attributed to using high temperatures. After a threshold temperature of about 16°C to 20°C, ciliary action stabilizes and shows small variations as temperature increases further in *Peranema*, an organism similar to *T. thermophila* (Clary-Meinesz *et al.* 1992). We chose to use 25°C, 30°C and 35°C treatments because food vacuoles are always forming within this range and Lee (1942) found increasing numbers when using these temperatures; however, they are above the presumed threshold temperature of *T. thermophila* (Jessen *et al.* 1979; Clary-

Meinesz *et al.* 1992). This means that ciliary action could have stabilized and, because cilia are needed to form vacuoles, produce similar numbers of food vacuoles at all temperatures we tested (Gonda *et al.* 2000; Clary-Meinesz *et al.* 1992).

For hypothesis three, we obtained a  $p$ -value of 0.5, which is greater than 0.05 so we fail to reject the null hypothesis ( $H_0$ ), that there is no difference in the effect of time on the number of food vacuoles formed in *T. thermophila* in varying temperatures (25°C, 30°C and 35°C). This is in agreement with our prediction. Our results show the same trend of increasing number of food vacuoles regardless of temperature treatment. A qualitative observation that supports our prediction is that while we were conducting our experiment, we saw that the food vacuoles became darker as time passed, indicating increased ingestion of yeast, at all temperatures (Figure 4). Therefore, based on our observations and previous literature we are able to say that time and temperature do not interact when we studied the number of food vacuoles formed (Nilsson 1977).

## **CONCLUSION**

In conclusion, we found our two factors of time and temperature each had a statistically significant effect on number of food vacuoles formed by *T. thermophila*. Also, as we predicted, these two factors are not interacting, the trend of vacuoles formed over time is the same regardless of temperature. With our results, we were also able to support our prediction that as time progresses the number of food vacuoles formed increases. However, we did not find what we predicted for temperature; instead we see the highest numbers of food vacuoles formed at 25°C, then 35°C, and finally the lowest at 30°C.

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