The Effect of Temperature on the Population Health of *Tetrahymena thermophila*

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Abstract

A wide range of studies on *Tetrahymena* species have been done involving the cell's cilia and basal body movement, nuclear divisions (both the macronuclear amitotic and micronuclear mitotic divisions), temperature-sensitive mutation and growth response (Pennock *et al.* 1988). In our study, we incubated *Tetrahymena thermophila* cultures at 25ºC, 30ºC and 35ºC to investigate the relationship between an increase in temperature and population health. Our group defined population health by analyzing three different factors including: population cell counts, dividing cell counts and cell motility at three different temperatures. We collected data at four different times: after 3, 21, 27 and 39 hours of incubation. Video footage was captured using a Dinoxcope camera and later used to analyze cell motility speeds and quality. Population densities were determined by counting cells through compound microscopes and multiplying counts by their respective dilution factors. After 27 hours of incubation, the mean cell population density incubated at 35°C was significantly larger than the mean cell population density incubated at 25°C. This significant result coincides with the literature and therefore provides evidence supporting the alternative hypothesis stating that increasing the temperature of the environment will increase the population size of *T. thermophila*. After 39 hours of incubation, a significant difference between the mean speed of cells incubated at 25ºC and those incubated at 35ºC was observed. The cells incubated at 25ºC moved significantly faster than those cells incubated at 35ºC. Considering our population growth and motility results, we conclude that there could be a tradeoff between population growth and motility. At the highest treatment temperature, we observed higher densities of *T. thermophila* in our replicates and that the cells moved slower than the cells incubated at 25C.

Introduction:

Viability of a unicellular organism, such as its rate of cell division, is greatly dependent on various environmental or physical factors, such as temperature. The ciliated protozoan *Tetrahymena thermophila,* as the organism's name suggests, is known to tolerate temperatures as high as 40ºC; it multiplies extensively at this temperature (Frankel and Nelsen, 2001). It is considered to be one of the fastest dividing eukaryotes with a doubling time of only two hours. It can survive at temperatures ranging from 12ºC to 40ºC (Orias, 1997). Frankel *et al.* (1980) found wild type *T. thermophila* exposed to an optimal temperature of 37.5ºC to 39ºC provided for rapid exponential growth. However, it was also observed that a sudden switch to this temperature potentially resulted in long delays of cell division.

T. thermophila is also an important model organism known to swim unassisted at a speed of approximately 0.5mm/second (Kumano *et al.* 2012). They propel themselves through the water using their cilia, basal bodies and ciliary rows. A cartwheel protein, TtPoc1, plays a critical role in the assembly

and stability of centrioles found in *T. thermophila* and takes part in its ciliary-based movement and cilia formation (Pearson *et al.* 2009).

A wide range of studies on the *Tetrahymena* genus have been done involving the cell's cilia and basal body movement, nuclear divisions (both the macronuclear amitotic and micronuclear mitotic divisions), temperature-sensitive mutation and growth response (Pennock *et al.* 1988). In our study, we present our analysis of the changes in population health (in relation to population cell count, number of dividing cells and motility) brought about by various temperatures on our study organism, *Tetrahymena thermophila* at 25ºC, 30ºC and 35ºC. We tested our alternate hypothesis of whether an increase in temperature will improve the population health of the wild type *T. thermophila.* Our null hypothesis is that an increase in temperature will diminish or will have no effect on the population health of *T. thermophila.* More specifically our hypotheses are:

Ha_1: Increasing the incubation temperature will increase the population size of *Tetrahymena thermophila.*

Ho_1: Increasing the incubation temperature will decrease or have no effect on the population size of *Tetrahymena thermophila.*

Ha_2: Increasing the incubation temperature will increase the number of dividing cells in *Tetrahymena thermophila.*

Ho_2: Increasing the incubation temperature will decrease or have no effect on the number of dividing cells in *Tetrahymena thermophila*

Ha_3: Increasing the incubation temperature will increase the motility (faster movement) of *Tetrahymena thermophila*

Ho_3: Increasing the incubation temperature will decrease or have no effect on the motility of *Tetrahymena thermophila*

Methods:

We made the initial cell culture by determining the concentration of the *Tetrahymena* in the stock solution and then calculating the volume required in order to prepare 6 mL of 1 x 10^4 cells/mL. We prepared nine test tubes, each containing 6 mL of starting cell culture. We prepared three replicates for each of the following incubation temperatures: 25°C, 30°C and 35°C (Figure 1). We placed each set of replicates in three separate beakers and covered them with aluminum foil to ensure the light conditions were consistent at all treatment levels. We collected data at four times: after 3 hours, after 21 hours, after 27 hours and after 39 hours.

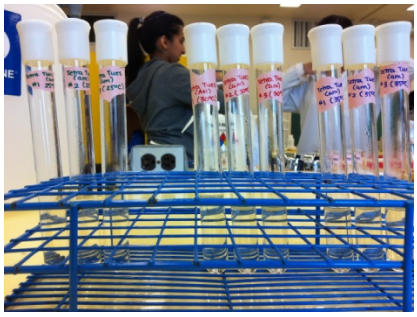

Figure 1: The three sets of replicates we incubated at 25°C, 30°C and 35°C.

To count cells, we fixed 150 µL of cell suspension with 5 µL of the fixative, Prefer. This was done for each of the nine cell cultures at each measurement time. For measurements taken after 21 hours, 27 hours and 39 hours of incubation, we diluted the fixed cell cultures with growth medium in order to easily count cell populations. At the second measurement time (after 21 hours), 5 µL of each of the mixtures incubated at 25°C and 30°C were diluted with 45µL of distilled water to make a 10x dilution. 5 µL of each of the mixtures incubated at 35°C were diluted with 495µL of growth medium to make a 100x dilution. At the third and fourth measurement times (after 27 and 39 hours), 10 µL of each of the eighteen prefer mixtures, which contained the prefer fixative and cell culture, were diluted with 90 µL of growth medium to make 10x dilutions.

Wet mount slides were prepared with 50 µL of fixed cell mixture from each of the centrifuge tubes. Compound microscopes set with the 10x objective lens (total magnification 100X) were used to systematically count the number of cells on each of the slides. We also recorded how many dividing cells

were on each of the slides. At the last measurement time, we took photos of dividing cells with a compound microscope (40x objective lens, total magnification 400X) using the DinoXcope (see Figure 3).

Figure 2: Stop watch and counter used to count cell densities and motility speeds.

Figure 3: DinoXcope; used to record video footage of cells.

At each of the measurement times, we recorded *T. thermophila* cell movement by making video files for each of our replicates. Wet mount slides were prepared by extracting 50 µL from each of the cell cultures (nine slides for nine cultures, three for each incubation temperature). The slides were placed on a dissecting microscope over a grid and we used the DinoXcope to capture 60-second video clips of each sample slide. If a slide dried out, 20 µL of growth medium was added to the slide in an attempt to rehydrate the samples. The video files were saved on a laptop and organized by date and measurement time.

To order to measure cell motility, we selected a cell from each replicate clip that moved one grid cell length (1500 µm or 1.5 mm). We used a timer to time how long it took the cells to travel across the grid square and recorded the data in a data table. We also made observations about the appearance of the cells and their mobility patterns at each temperature. After all data were collected, we converted all data to mm/s speeds and calculated means for each incubation temperature at each measurement time.

Results:

From the three different temperatures, we were able to observe and gather motility data both qualitatively and quantitatively as well as cell population counts. Figure 4 below shows the quantitative motility data recorded at 39 hours after incubation (which marks the end of our experiment). From this graph, the incubation at 25°C shows the highest motility speed amongst the other data points which is 0.78mm/second. The other two incubation periods showed similar motility speeds for *T. thermophila.*

From our qualitative observations, we found that at 25°C, the cells had an oblong and slender shape with movement similar to that of a fish swimming (moving its posterior half of body side-to-side to move forward). The movement was very fast compared to the 30°C treatment. The shape of these cells was also rounded and larger. In the 35°C treatment, the cells were moving much slower compared to the first two treatments and also had a rounded shape similar to those cells incubated at 30°C.

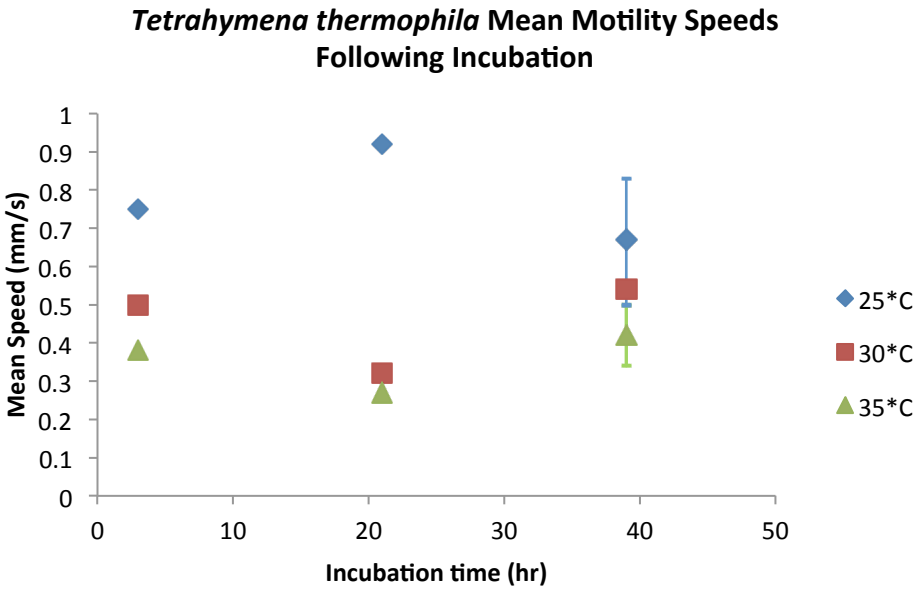

Figure 4: The motility speed (mm/second) at 25, 30 and 35°C. Bars represent 95% confidence intervals, n= 3.

The mean cell counts at all time intervals are presented in Table 1. We decided to focus on the mean population size after 27 hours of incubation at 25°C, 30°C, and at 35°C (see Figure 5). The mean cell counts are increasing, with the population size being the smallest at 25°C and highest at 35°C.

Figure 5 shows overlapping of the confidence intervals between 25°C and 30°C. The confidence interval at 35°C did not overlap with the other mean cell counts indicating a significantly higher mean population size at this temperature.

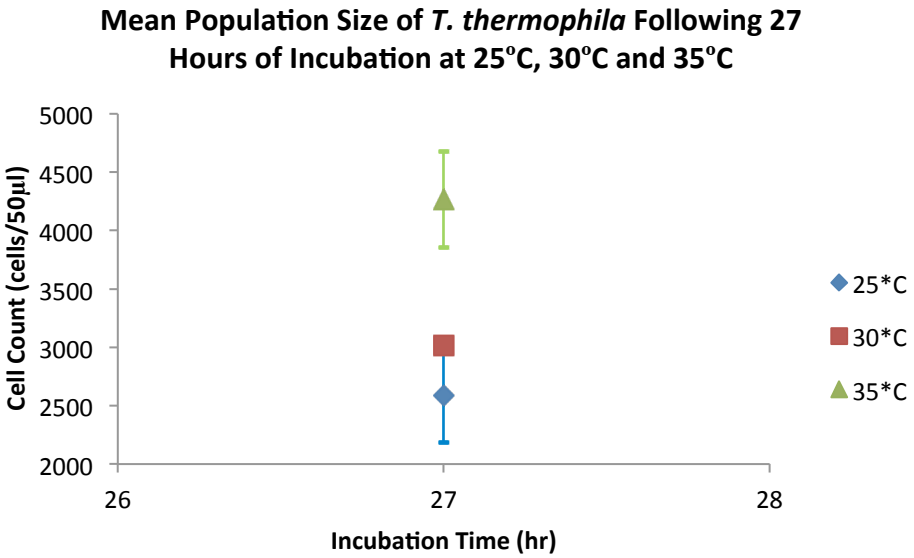

Figure 5: The cell counts (number of cells/50µl) at 27 hours at 25, 30 and 35°C. Bars represent 95% confidence intervals, n=3.

Table 1: Mean cell counts at 25, 30 and 35°C at the recorded times with 95% confidence intervals.

Temperature (ºC)	Time (hours)	Mean Cell Counts (cells/50µL)	95% Confidence Intervals
25	0	4514	
	3	353	(238, 467)
	21	3999	(3113, 4886)
	27	2590	(2190, 2991)
	39	4599	(-3048, 12245)
30	0	4514	
	3	137	(102, 173)
	21	12972	(-8182, 34125)
	27	3018	(2084, 3952)
	39	1241	(-999, 3479)
35	0	4514	
	3	256	(205, 308)
	21	3100	(973, 5228)
	27	4265	(3853, 4677)
	39	2370	(1301, 3440)

95% confidence interval sample calculation:

$$C.I. = \overline{X} \pm Z_{\frac{\alpha}{2}} \frac{\sigma}{\sqrt{n}}$$

where $\overline{X}$ = the sample mean
σ = the population standard deviation
$Z_{\frac{\alpha}{2}}$ = the Z value for the desired confidence level α (obtained from an Area Under the Normal Curve table)

Discussion:

During the incubation of *T. thermophila* for a period of 39 hours, the effect on motility, population size and the number of dividing cells present in each sample at each temperature was analyzed.

Motility

Based on our analysis, we failed to reject our null hypothesis stating, that increasing the temperature of the environment will decrease or have no effect on the motility of *T. thermophila*. Following 39 hours of incubation, the cells at 25ºC had a mean speed that was significantly faster than those cells incubated at 35ºC. Ito *et al.* 2002 found that the optimal growth temperature for *T. thermophila* is closer to 37ºC as compared to the optimal temperature we selected at 25ºC. As can be seen in Figure 5, we did not observe static *T. thermophila* populations; we found this to be consistent with Frankel *et al.* (2001). If we look further into this relationship, there is a possibility that at 39 hours of incubation at 35ºC, the cell population may have been dying. This may have caused a decrease in the cell motility observed. As the cell population was not observed at shorter increments of time, we cannot conclusively say that this occurred. One observation that could support this theory is that the observed motility of the *T. thermophila* cells that were incubated at 30 and 35ºC appeared to be affected by the cell's more rounded shape and increased body size. As a result the cells travelled in a more snake-like motion rather than a straight path which may have ultimately caused them to travel a further distance than measured. This could support the idea that as the cell cultures aged they experienced a decline in health that could possibly affect their motility. Furthermore, sources of error may have also led to the discrepancies in the findings. For example, in order to obtain the video footage required to measure the cells' swimming

speeds, we prepared wet mount slides beforehand. The time it took to prepare these slides and acquire the footage resulted in our slides drying out, which affected the swimming ability of the cells.

Population Size

With respect to the effect of temperature on population size, we reject the null hypothesis stating that, increasing the temperature of the environment will decrease or have no effect on the population size of *T. thermophila*. As previously mentioned, the optimal growing conditions for *T. thermophila* include a temperature close to 37ºC (Ito *et al.* 2002). Frankel *et al.* (1980) suggest that *T. thermophila* cell populations are at an optimal temperature around 37.5ºC to 39ºC, which provides a favorable condition for rapid exponential growth, and therefore results in larger population sizes. After 27 hours, we observed that the cell population incubated at 35ºC was significantly larger than the cell population incubated at 25ºC; all other data obtained were found to have no significant differences in their means. This significant result is consistent with the literature and therefore provides evidence supporting the alternative hypothesis stating that increasing the temperature of the environment will increase the population size of *T. thermophila*. Although we acquired a significant result, the lack of significant differences found at other sampling times and the trends observed in the changes in population provide valuable information regarding the biological occurrences. The experimental procedure required that the cells be incubated in large test tubes which contained liquid media within them and lids on them. We assumed that the cell populations had adequate space to grow and move, and that sufficient nutrients and oxygen were available. However, this may have not been the case. It is possible that with the extremely fast doubling rate of *T. thermophila,* that the population densities grew so large that the test tube could no longer provide them with optimal growing conditions. *T. thermophila* are said to have two distinct phases of exponential growth, a faster one at low densities and a slower one at higher densities and that population sizes are not stable (Frankel *et al.* 2001). This may provide a biological explanation for the observations we made. The drastic changes in population size observed at each temperature following the incubation periods support the idea that upon periods of fast exponential growth, cells within the populations may have experienced decreased access to nutrients, oxygen and space. This may have lead to the population dying and therefore reducing the population size drastically, in what is better known as a population crash. During the decrease in population size caused by cell death, a portion of surviving cells

would find themselves with more oxygen, nutrients and space to once again flourish leading to repopulation. Another possible reason for not encountering more significant differences could simply be a result of error in counting. All cell counts were done using a counter and microscope which leaves room for error in counting cells inaccurately, by counting some cells more than once or not at all.

Number of Dividing Cells Present Within a Population

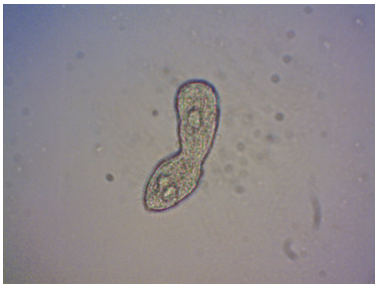

Figure 6: Dividing *T. thermophila*

The number of dividing cells present in each *T. thermophila* population was examined and no significant differences were observed in our data. Due to the lack of significant differences seen in the data, we fail to reject our null hypothesis stating that increasing the temperature of the environment will decrease or have no effect on the number of dividing cells within *T. thermophila* populations. The lack of significant differences in our data may have occurred as a result of experimental error. As mentioned previously, the method for counting the number of dividing cells required that a slide be made and that a microscope was used. Human error may have resulted in cells being counted more than once or not at all. Another source of human error may have resulted during the preparation of the slides themselves. When the cover slips were placed on the samples, some of the sample may have been pushed outwards and may have not been seen by the counter. Therefore, it is these sources of error that may have affected the accuracy of our results.

Conclusions:

Upon observation of the population health of our sample organism, *Tetrahymena thermophila*, we established that our experiment fails to support the hypothesis that an exposure to a higher temperature would cause the cell movement to increase in speed. In fact our data support a decrease in motility when we incubated the cells at a higher temperature. On the other hand, an exposure to a higher temperature

resulted in an increase in cell population, in agreement with current literature. However, the population health experienced a deterioration of cell density on our third measurement at a later time. Lastly, no significant conclusion can be made regarding the number of dividing cells present within our sample population.

Based on our data, we did not find any significant similarities or differences in the population health of *T. thermophila* cells that were exposed to different temperatures. Thus, we cannot state any conclusive relationship between increasing temperatures and the population health of *T. thermophila.* The currently accepted literature and our results suggest that several biological processes influence the overall population health of *T. thermophila*. Since no direct relationship can be observed between the increase in temperature and population health of *T. thermophila,* we hope that this study will stimulate further investigation to clarify whether an increase in environmental temperature does have a positive relationship on the cell dynamics and overall population health of *Tetrahymena thermophila*.

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