Tetrahymena thermophila population growth under salt stress

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Abstract

Substances such as salt can have adverse effects on a range of organisms. We were interested in demonstrating possible toxic effects by observing population differences in the fresh water species, *Tetrahymena thermophila* We counted cells, which were in varying salt concentrations, every two hours (doubling time of *T. thermophila*) Our results suggest that increasing concentrations of salt in the environment decreases the population of *T. thermophila*. At a higher concentration (1.20%) salt, populations decrease at a faster rate, but soon follow the trend of the lesser concentrated samples. We determined that salt does in fact have a detrimental effect on *T. thermophila* populations; more so at higher concentrations until a toxic concentration is reached.

Introduction

Population growth has been studied extensively to determine the effects of varying environmental settings on organisms, the degree to which environmental conditions can be of influence on a short and long-term basis, and if there is a measure of sensitivity and specificity. *Tetrahymena thermophila*, a unicellular, ciliated protozoan with an optimal temperature ranging from 19^{o}C to 31^{o}C, is our study organism citation. This eukaryote inhabits fresh water;, therefore, we set out to determine a maximum toxicity level threshold of salt for *T. thermophila.* Specifically, the purpose of this experiment is to determine the population growth pattern of *T. thermophila* in response to increasing salinity in their environment. Because their natural environment is fresh water,, we expect them to exhibit osmoregulation under hypertonic conditions. However, this ability to regulate should be relatively low. The eukaryote *Paramecium multimicronucleatum* contains contractile vacuoles that allow osmoregulation until the threshold concentration of ions is reached (Stock *et al.* 2002). Due to similarities between *Paramecium* and *Tetrahymena*, we believe *T. thermophila* will also use contractile vacuoles to osmoregulate.

Considering the ideal habitat of *T. thermophila*, we predicted that with increasing salinity, the population of *T. thermophila* will decrease. Thus our hypotheses were:

H_a: With increasing salinity, population growth of *T. thermophila* will decrease.

H_0: With increasing salinity, population growth of *T. thermophila* will increase or remain the same.

Gilron *et al.* (2009) conducted a toxicity test on *T. thermophila* to observe the possible adverse effects on the ciliated protozoan's behavioural responses and population yield. They found that a concentration of 1250 ppm NaCl (0.12% of NaCl) was toxic enough to cause abnormalities in the organism. This supports the idea that there exists a threshold below which *T. thermophila* can effectively osmoregulate. We believe that the population yield of *T. thermophila* will decrease with increasing salinity due to their established living lifestyles in fresh water.

The doubling time range of *T. thermophila* is two to four-hours, which that makes observing population growth over a short time span ideal. Incubating the cells at 30^{o}C will increase reproduction rates and will favour the two-hour doubling time citation. This advantage allows for a large accumulation of data during a short period of time which can be extrapolated for long-term trends.. Short term analysis will be conducted to observe the rate at which population sizes decrease at the onset of introduction of salt, while longer- term analysis will provide information on survivability conditioning.

Materials and Methods

Cells and culture medium

Investigation was carried out with *T. thermophila*, prepared by a laboratory technician from the University of British Columbia. We labeled three sets of four 10 mL test tubes and added 2 mL *T. thermophila* to each. Test tube 1 was labeled as the positive control and 8 mL standard medium was added. Test tubes 2 to 4 also had 1 mL 200% growth medium (i.e., twice as concentrated) and 6 mL 100% growth medium was added. Stock solution of NaCl was provided at 12.0% salinity. We diluted the stock solution into 1.20% and 0.12% for the culture medium. In test tube 2, 1 mL of 0.12% NaCl solution was added; in test tube 3, 1 mL of 1.20% NaCl solution was added; in test tube 4, 1 mL of 12.0% NaCl solution was added. Cells were kept at 29°C to maximize growth, except when removed for observation and counting.

Observation and Data Collection

At each observation time, 1 mL of each sample from the 10 mL test tubes was transfered into a fresh sterile test tube. Then 25µL of fixative were added and the sample mixed well. Immediately, 50µL of was transfered from the test tube and to a glass slide. A cover slide was added and the slide was observed under the microscope (Fig. 1). Most cells remained in their original shape, although some lysed due to hypertonic environment. Any lysed cells that were incomplete in their shapes were discarded and not considered.

We used a click counter an a magnification of 10 or 100 times to count the total number of cells in the 50µL samples. We collected data every two hours for a six-hour period.

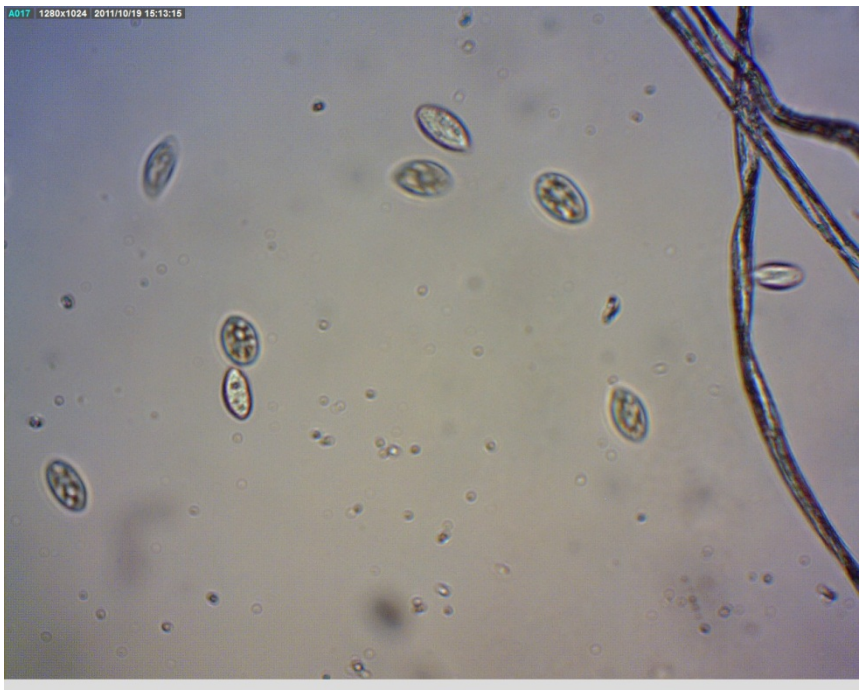

Figure 1. *T. thermophila* under 100X magnification with cotton to slow down movement for observations.

Statistical Analysis

We averaged the data collected from each set of the three replicates. and calculated the standard deviations and 95% confidence intervals.

Results

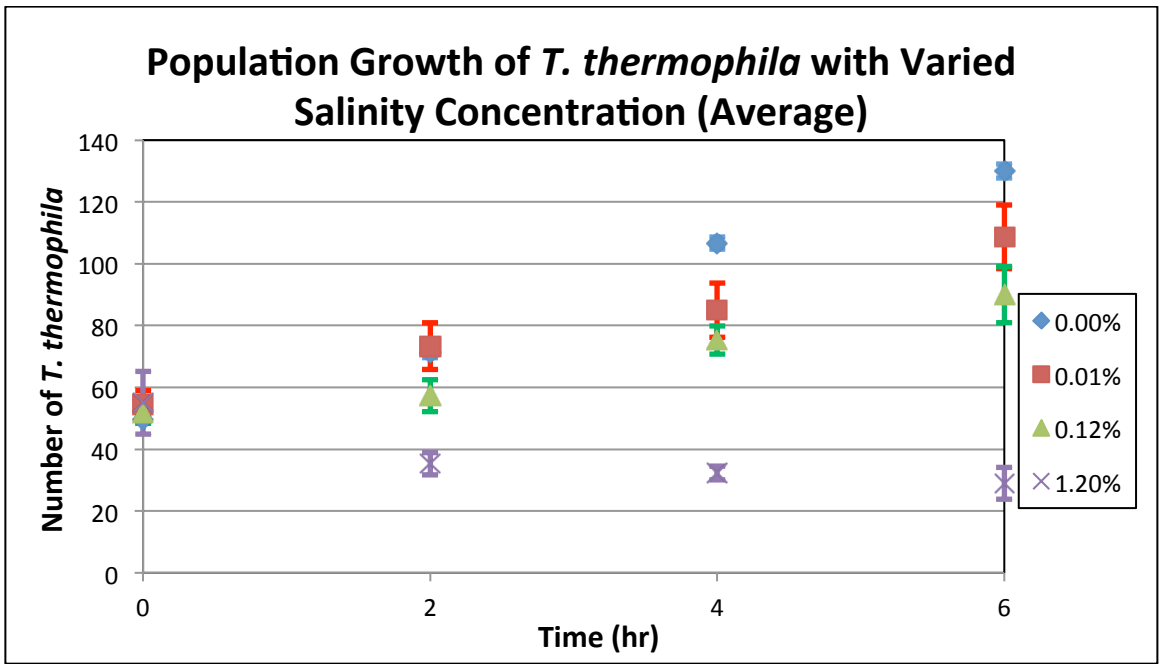

Figure 2. Population growth of *T. thermophila* in varying salinity (0.00%, 0.01%, 0.12% and 1.20%) (average of three replicates over six hours at 29°C.

At 0.00% salinity, the population of *T. thermophila* showed exponential growth, from 50 cells to 130 cells per 50µL. However, at 0.01% and 0.12% salinity, the population growth rate was reduced to a steady linear increase, from 54 to 109 cells and 52 to 90 cells in 50µL respectively. At 0.01% salinity, the number of cells increased by 55 cells, as compared to 38 cells in 0.12% salinity. *T. thermophila* in 1.20% salinity experienced a decrease in population size during the period of observation. The number of cells decreased from 55 cells to 29 cells per 50µL by the end of six hours. By the end of the observation session, the ranking of cell count corresponded to the salinity levels.

Table 1. Number of *T. thermophila* in three replicates after 18 hours of initial recording.

Replicate	**Salinity (%)**	
	0.00 (Growth medium)	1.20
1	>1000	426
2	>1000	440
3	>1000	432
Average	>1000	433

Eighteen hours after the initial recording of cells, an additional count was completed to observe the number of cells in the lowest and highest salinities. At 1.20% salinity, 433 cells were observed per 50μL, whereas at 0.00% salinity, the cell count was over 1000 (see Table 1).

Sample Calculation

Mean:

$$\frac{49+49+51}{3}=50$$

Standard Deviation:

$$\sqrt{\frac{(49-50)^2+(49-50)^2+(51-50)^2}{3-1}}$$
$$=\sqrt{\frac{3}{2}}$$
$$=1.154701$$

95% Confidence Interval:

$$=50\pm1.96*\frac{1.154701}{\sqrt{3}}$$
$$=50\pm1$$

Discussion

Based on the observations and data, H_o can be rejected since the data indicated a significant decrease in population growth as salinities were increased. In addition, this significant decrease in population growth supports H_a, since *T. thermophila* population growth was expected to decrease as NaCl concentrations increased. This has been directly or indirectly observed by

experiments done on protozoa by other scientists. The significantly lower population increase of 0.12% NaCl samples is in agreement with Gilron *et al.* (2009) on *T. thermophila* although the specific cell mechanisms were not discussed in that paper. We suggest that as the concentrations of salt increased, the *T. thermophila* started to die off due to the hypertonic environment inducing crenation – "shrivelling" – of the cells. This decreased cytosol volume would then inhibit cellular processes such as cellular respiration. This is supported by Allen and Naitoh (2003) who observed that the contractile vacuoles of various protozoa disappeared and cellular mechanisms were inhibited at high levels of salt. With these in mind, it can be safely stated that as freshwater protozoa, *T. thermophila* function optimally in isotonic conditions and cannot live in high salinity without resistance.

The above statement, however, can be contradictory since according to the data, many cells are still living at 1.2% concentration population growth occurs after 18 hours (nine generations). This can be explained by biological variation and natural selection principles. It is entirely possible that either the *T. thermophila* samples used for this experiment already had some cells that had a genetic mutation resulting in resistance to salinity, or, that the mutation occurred during the 18 hours of experimentation and natural selection favoured these cells This would mean that the mutation would have to target certain proteins of the *T. thermophila* that regulate salt intake – or osmoregulation. Perhaps the mutation had altered protein channels for sodium and chloride ions. This mutation would cause the cell to expel salts continuously or at a greater capacity in order to maintain cell integrity in high salt concentrations. While this is not favourable in normal conditions, this is one of the few options that would enable the cells to survive at the given conditions. The concept of cells expelling salts to osmoregulate is not unheard of Serrano (1996) where osmoregulation of similar nature was observed in

Saccharomyces cerevisiae. As time progressed in our experiment, natural selection favoured the *T. thermophila* with the mutation in the 1.20% NaCl samples since these strains would be able to survive in high salt environments and therefore produce offspring and pass on the mutation. The population growth of the 1.20% samples at the 18th hour may also have been caused by the *T. thermophila's* programmed cell death as observed in the experiment done by Beyer *et al.* (2001). As the cells become stressed in the high salt conditions, the quorum sensing mechanism Beyer *et al.* (2001), occurs where cells release a signal for cell death after replication to leave enough nutrients for the next generation. In the 1.20% samples, the continuous cell death following each generation would accelerate natural selection favouring the mutant strains by eliminating the cells without the resistance quickly and increasing the replication between those with the resistance. The 1.20% data points in Figure 2 supports this since each data point is not significantly different from each other – where the points for Hour 2, 4 and 6 have overlapping 95% confidence intervals but are significantly different than Hour 0. This can be interpreted that at the first four hours, the cells without the resistance for salinity are already killed off and the sample after this time only contain those resistant to the salt.

These results, however, are subject to errors and variation. The counting method, for example, was not standardized as in there were no set procedures on how to count each organism. Since there were three different individuals counting the organisms on a slide, each method may differ from person to person which would create a wider spread of data (a higher variance value). Moreover, the slides used in the experiment were not marked with any grid or guidelines and therefore when we scanned the slide under a microscope, the thoroughness of the scan for each replicate might not be consistent. These factors would affect the cell count data for

each treatment and replicate. This would affect the entire data in Figure 2, and therefore affect the supporting evidence for the H_a and the H_o.

Conclusion

As hypothesized, the results show that with an increased salinity, the population of *Tetrahymena thermophila* decreases. Due to natural selection phenomena, *T. thermophila* in the 1.20% salt environment experienced a decrease in reproduction, however soon began to reproduce but at a reduced rate within 18 hours. Therefore all samples of *T. thermophila* experienced a decrease in reproduction hence lower population counts. Furthermore, samples with higher concentrations demonstrated a period of inhibited growth before showing signs of resuming reproduction.

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