The Effect of Different Salinity Levels on Growth Rate of *Tetrahymena thermophila*

Christina Lee, Victoria Park, Ziyu Gan

ABSTRACT

Tetrahymena thermophila are single-celled organisms that are commonly used for research purposes due to their short doubling time. They are found in freshwater aquatic systems and are a food source for zooplankton, and juvenile salmons require zooplankton as their food source. The effect of different salt concentrations on the growth rate of the freshwater organism, *T. thermophila* were observed under different NaCl concentrations within the Biology department at the University of British Columbia. *T. thermophila* were exposed to four different salt concentration levels, 0%, 0.025%, 0.050%, and 0.100% over a 26-hour period. 100μL of samples were taken out every two hours during the first day to a total of eight hours, and the samples were taken out with a two-hour interval (24^{th} and 26^{th} hour of incubation) until the 26^{th} hour was reached on the second day of the experiment. Considering that *T. thermophila* live in freshwater environments that contain little to no salt concentration, they grew best when exposed to 0.05% NaCl. Based on the results, *T. thermophila* were not affected by NaCl concentrations during the 26 hours of incubation.

INTRODUCTION

Tetrahymena thermophila (T. thermophila) are a single-celled, ciliate model organism that live in freshwater systems such as streams, lakes and ponds (Collins and Gorovsky 317). The studies of *T. thermophila* have led to many explorations and insights about the conservation and recovery of aquatic ecosystems. Being mainly present within freshwater systems, *T.*

thermophila and other protists are a significant factor within the food web of bacteria and zooplankton (Wickham 419-420). Therefore, studying the reaction and behaviour of *T. thermophila* under different range of salt concentration will allow a better understanding of the ecology of salmon species.

Mainly inhabiting freshwater systems, the behaviour of *T. thermophila* is affected under different water salinity levels as a previous study showed that the maximum concentration that *T. thermophila* can tolerate is 0.12% NaCl or 1250ppm (Ayre et al. 1). This shows that high salinity levels are toxic to them and will eventually cause mortality in the organisms (Gilron et al. 1915). To relate this to the environment, it is observed that the global temperature is increasing and warming the waters, rising sea levels and increasing salt concentration within the water systems (Paolo et al. 7). To see whether water salinity levels affect the growth rate of *T. thermophila* and eventually the salmon populations, this study was done to see if there is a significant difference in growth rate when exposed to different NaCl concentrations. The hypotheses that were tested are:

Null hypothesis (H_0): There is no influence of water salinity level on the growth rate of *T. thermophila*

Alternative hypothesis (H_a): There is a significant influence of water salinity level on the growth rate of *T. thermophila*.

MATERIALS AND METHOD

In order to measure the effect of salinity level on the growth rate of *T. thermophila*, different concentrations of NaCl media were applied to the same concentration of *T.*

thermophila. The *T. thermophila* cultures, 0.2% NaCl media and standard media were provided by laboratory technicians from University of British Columbia.

Before all procedures, a sterile condition had been achieved through 70% ethanol and an open flame to prevent any contamination during the entire procedure. During the transfer of *T. thermophila* between different equipment, new tips of micropipettes were used, and the openings of test tubes and flasks were flamed.

The unknown concentration of the obtained stock of *T. thermophila* was calculated by applying an equipment called haemocytometer. Haemocytometer is a slide consisting two-etched grids that can trap a specific volume of a liquid. It is often used to count single-celled organisms ranging in size of 50μm to 100μm. The concentration of the organisms in the original solution can be calculated from the number of cells that are found on the grid of the haemocytometer. The recipe for standard media and the calculation of the original concentration of *T. thermophila* are both provided in Appendix on page 16.

Before pipetting 10μL directly from the Erlenmeyer flask which contained original stock of *T. thermophila*, the flask was mixed thoroughly by gently swirling the flask by holding onto the neck of the flask. Then, 10μL of the original stock of *T. thermophila* was extracted with a micropipette, then released below the coverslip through the top middle edge of the haemocytometer (**Figure 1**). Another 10μL portion of the sample was loaded onto the other grid of the haemocytometer using the same micropipette. Originally, 20μL are supposed to be released into each grid of the haemocytometer, but 10μL was enough to cover the entire grid. When the haemocytometer was completely loaded, it was placed on the stage of a compound microscope. Only the 10x objective lens was used when viewing the haemocytometer.

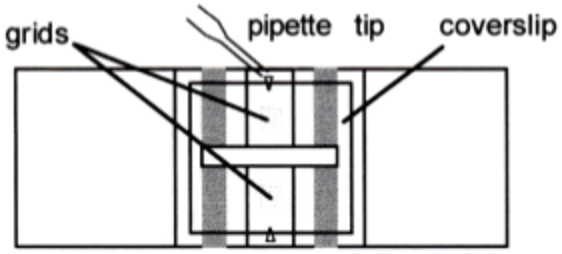

Figure 1. Components of the haemocytometer where the coverslip is placed on top and the sample is inserted underneath the coverslip with a pipette.

Once the haemocytometer was placed on the stage of the microscope, the haemocytometer was oriented so that one of four by four squares could be seen through the lens of the microscope (Figure 2). The number of cells within that specific four by four squares was counted and was recorded as 20 cells. Therefore, 1.0×10^5 cells/mL has been calculated to convert the number in terms of density and the calculation is provided in Appendix I. The original stock was diluted to achieve a final concentration of 4.0×10^4 cells/mL by adding 36mL of the standard media into a 150mL Erlenmeyer flask by using a 10mL pipette. Then, 24mL of the original stock of *T. thermophila* was added to the same Erlenmeyer flask to achieve a final volume of 60mL using a different 10mL pipette. The Erlenmeyer which contained the solution was thoroughly mixed by a gentle swirl.

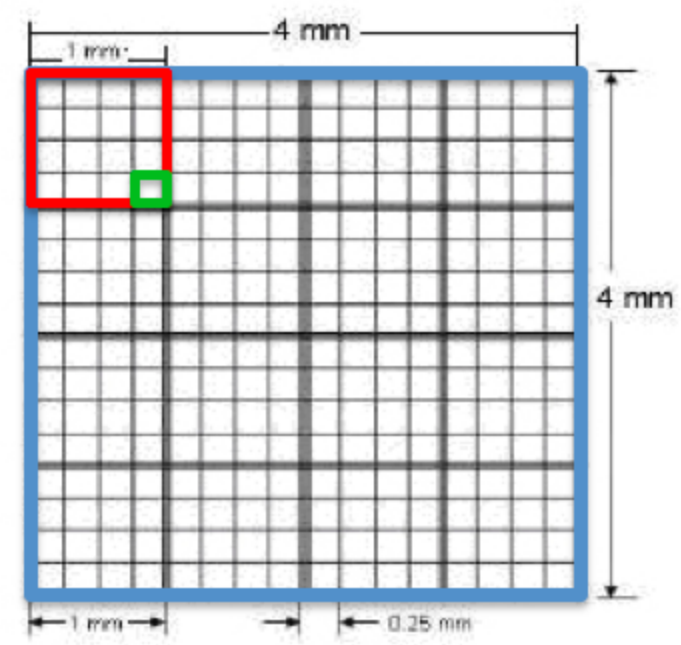

Figure 2. Grid Dimensions on haemocytometer provided from UBC lab.

To distribute the solution of the working stock of *T. thermophila* to 12 of 15mL test tubes, 4mL of the working stock of T. *thermophila* were pipetted into all of the 12 test tubes. The test tubes were labelled as the following:

T1- 0.025%, T1-0.050%, T1- 0.100%, T1-0% (control)

T2- 0.025%, T2-0.050%, T2- 0.100%, T2-0% (control)

T3- 0.025%, T3-0.050%, T3- 0.100%, T3-0% (control)

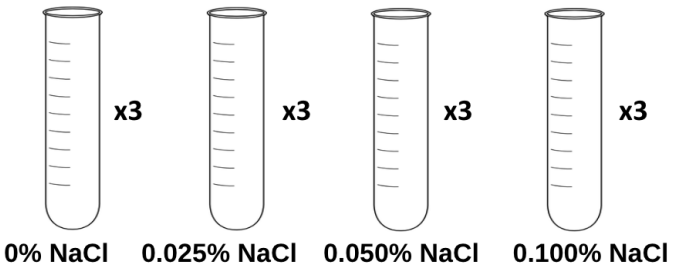

Figure 3. A brief layout designed by Group 3 of how the treatment groups were set up for the experiment.

In each test tube consisting 4ml of the working stock of T. *thermophila*, 4ml, 3ml, and 2ml of *T. thermophila* media were added into those test tubes which were labelled as “control”, 0.025%,

and 0.050% respectively. Then, 1ml, 2ml, and 4ml of the given 0.2% NaCl solution into test tubes labelled as 0.025%, and 0.050% and 0.1% respectively.

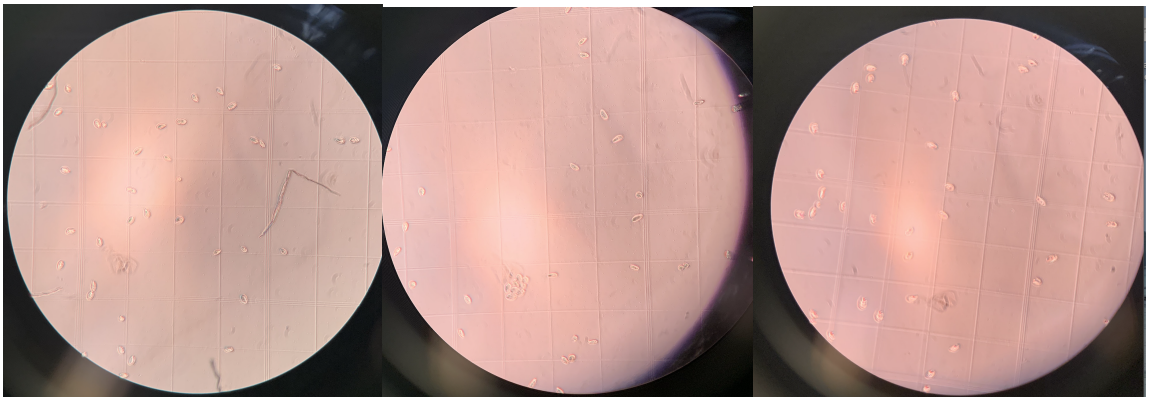

Figure 4. Fixed *T. thermophila* under 10X magnification using the haemocytometer grids. Placed under the ZEISS microscope provided from UBC Biology Laboratory. From left to right, (1) 0% NaCl at 6 hours of incubation, (2) 0.05% NaCl at 8 hours of incubation, and (3) 0.1% NaCl at 24th hour of incubation.

After all of the samples were prepared, 100µL from each test tube was extracted and released into a different Eppendorf tube. These Eppendorf tubes were also labelled corresponded to the labels of the test tube. The stack containing the test tubes was placed into an incubator which was set to 30 ˚C. 10µL of given fixative, 3% glutaraldehyde was added to each of 12 Eppendorf tubes containing the 100*u*L of each sample. Using a micropipette, 10µL of each sample was extracted from the Eppendorf tubes, then released onto each grid of the haemocytometer (**Figure 1**). The cell count was done for each sample in three separate grids and the same method for counting with the haemocytometer was carried out throughout the entire procedure for the 2nd hour, 4th hour, 6th hour, 8th hour, 24th hour, and 26th hour. The same fixative and the same 30 ˚C incubator were used throughout the entire experiment. As well as pipetting the samples up and down three times, a vortex was used to mix the samples prior to taking them out from the Eppendorf tubes.

After finished using coverslips for the haemocytometer, the used coverslips were discarded in the “used coverslip” container, which was placed on the bench. The

haemocytometer was rinsed with distilled water and dried gently with Kimwipe each time and stored back in a protective case after the experiment was complete.

The statistical method of analysis was applied as one-way ANOVA. This method was chosen because this statistical test can determine whether the responses of *T. thermophila* under three treatment levels are significantly different from one another. After getting p-values, the null hypothesis can be rejected if calculated $p \leq 0.05$, and alternative hypothesis can be supported, but if the calculated p is > 0.05, the null hypothesis is failed to be rejected.

RESULTS

The growth rates of *T. thermophila* under different NaCl concentrations were calculated to see if increasing water salinity influenced their growth rate. The results of the data obtained were shown in **Figure 5**, **6**, **7** and **8**. From **Figure 5, 6** and **7** it can be seen that the growth rate of *T. thermophila* in all of the three replicates followed a similar trend: from the 0^{th} to 24^{th} hour they showed an increase trend overall, after the 24^{th} hour, a decrease trend can be seen. The bar graph in **Figure 7** shows that the average growth rate of *T. thermophila* thrived under 0% NaCl at 0.065 cells per microliter per hour, while under 0.05% NaCl, they had the slowest growth rate at 0.0114 cells per microliter per hour. The p-value and F value obtained from one-way ANOVA analysis both came out to be 0.563 (see Appendix I on page 16).

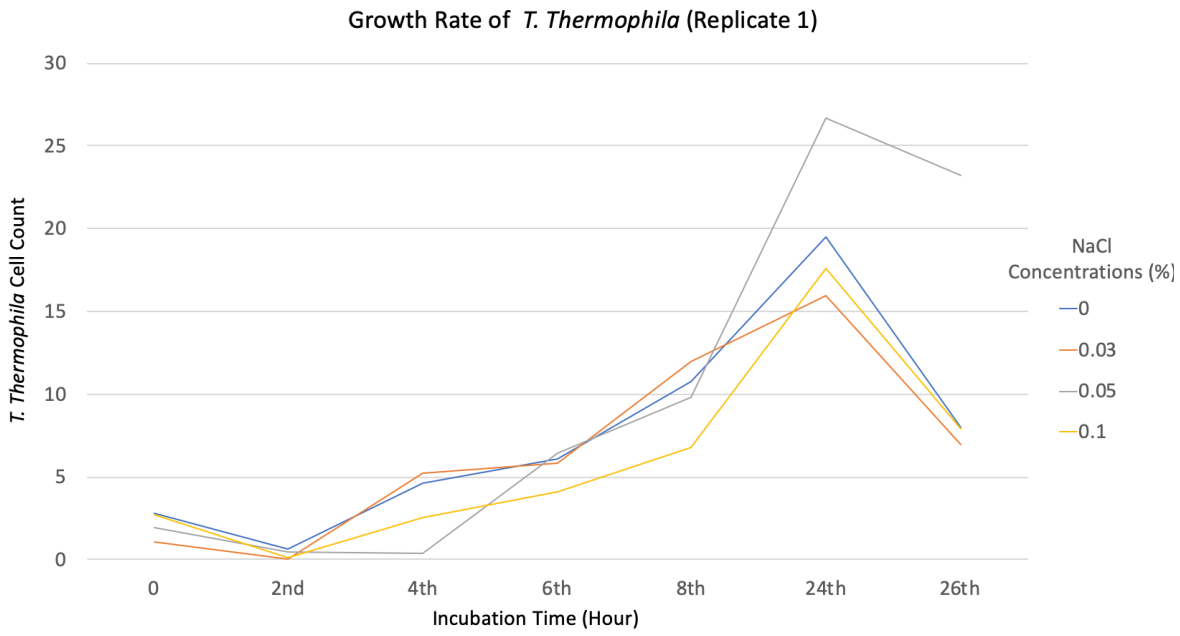

Figure 5. Line graph showing growth rate of *T. thermophila* in Replicate 1 under different NaCl concentrations at 0th hour, 2nd hour, 4th hour, 6th hour, 8th hour, 24th hour and 26th hour. The x-axis indicates different incubation time in hour and the y-axis indicates cell count in number of cells per microliter.

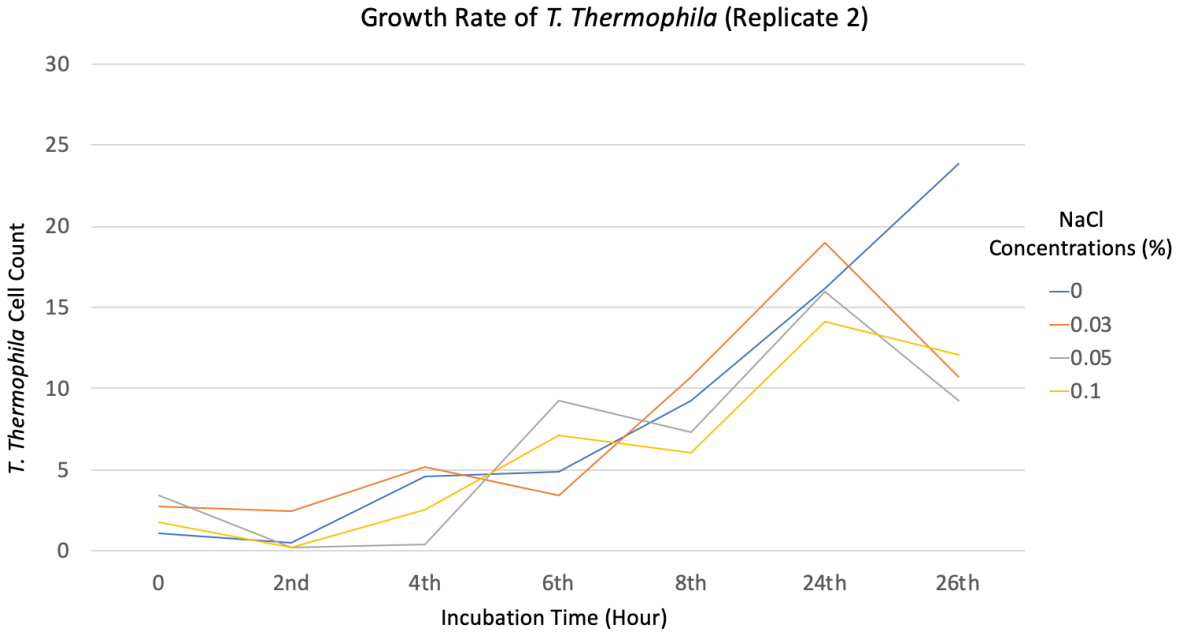

Figure 6. Line graph showing growth rate of *T. thermophila* in Replicate 2 under different NaCl concentrations at 0th hour, 2nd hour, 4th hour, 6th hour, 8th hour, 24th hour and 26th hour. The x-axis indicates different incubation time in hour and the y-axis indicates cell count in number of cells per microliter.

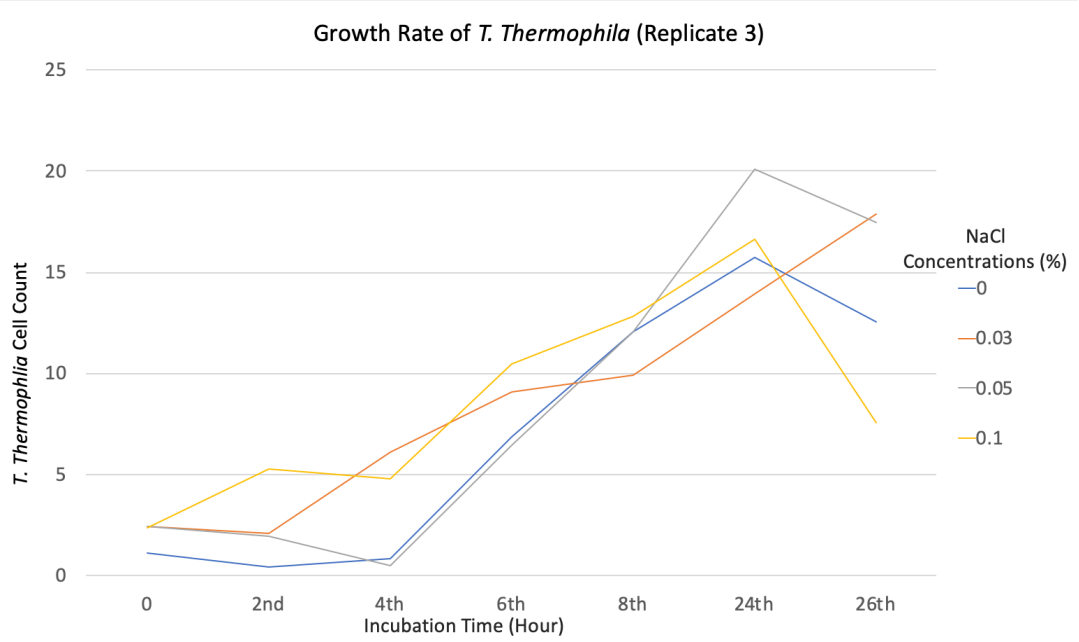

Figure 7. Line graph showing growth rate of *T. thermophila* in Replicate 3 under different NaCl concentrations at 0th hour, 2nd hour, 4th hour, 6th hour, 8th hour, 24th hour and 26th hour. The x-axis indicates different incubation time in hour and the y-axis indicates cell count in number of cells per microliter.

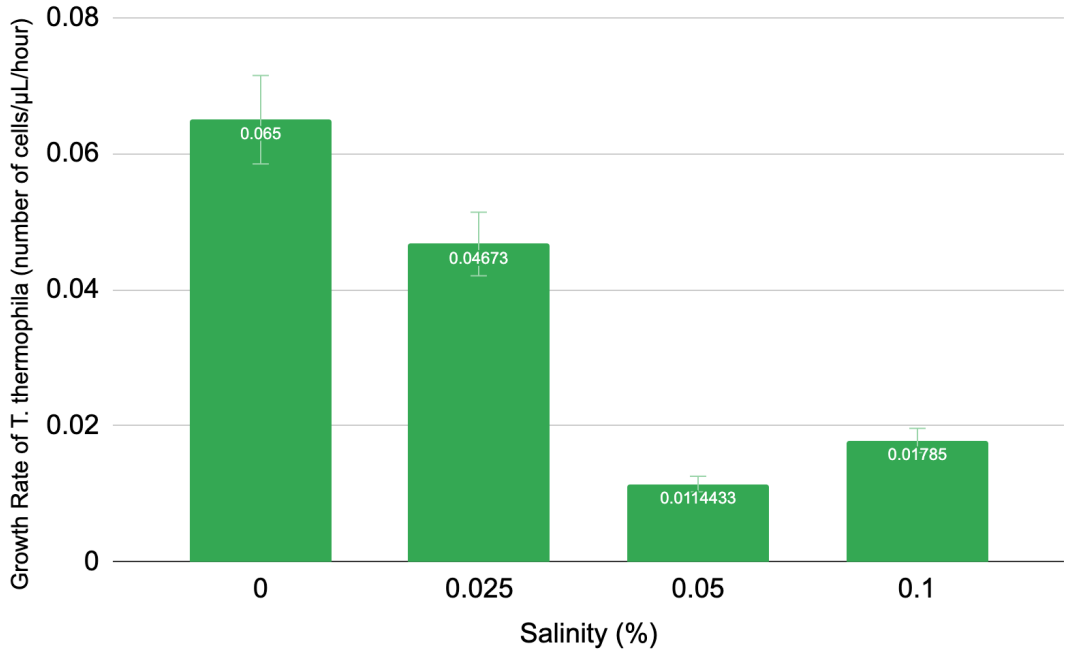

Figure 8. This bar graph shows the overall negative relationship between the salinity concentration and the growth rate of *T. thermophila*. The x-axis indicates the four different salinity concentrations in percentage and the y-axis indicates the growth rate of *T. thermophila* in number of cells per microliter per hour. The numerical values in white show the exact value of each growth rate.

DISCUSSION

The p-value was calculated to be 0.563, which is greater than 0.05 and the F-value obtained from one-way ANOVA analysis, also came out to be 0.563. This states that the null hypothesis is failed to be rejected. The overall results of the study do not show as predicted according to statistical test. **Figures 5**, **6**, and **7** showed greater increases in growth rate at NaCl concentrations of less than 0.1%, which is indicated as a yellow line in **Figure 5**, **6**, and **7**. It took about six hours of incubation for the cells to show a doubling effect in growth rate. In fact, when they were placed inside the incubator for about four hours, the number of cells started to vary and showed an increase in all of the treatment groups when compared to the initial group (zeroth hour inside the incubator). The incubation temperature was constant throughout the whole experiment of 26 hours and usually organisms in culture at an optimal temperature would increase in cell number; however, this was not the case in this experiment with the *T. thermophila*. There was a lag phase that was observed at around two to six hours of incubation **Figure 5, 6, 7** that did not show a significant increase in cell numbers. This could be explained by Cassidy-Hanley's paper of a doubling period *T. thermophila* undergo where they double in their cell number not at the moment, they are put into culture but at around two hours after (237).

T. thermophila are single-celled organisms that live in freshwater environments (Collins and Gorovsky 317) with low salt concentrations. From our results, it has shown that the growth rate of *T. thermophila* are affected by 0.05%, 0.025% and 0.1% NaCl concentrations. As studies have shown that the maximum concentration *T. thermophila* can survive in is 0.12% NaCl (Ayre et al. 1), this experiment shows that the concentration of 0.1% NaCl has a significant effect on

their growth rate. *T. thermophila* are vulnerable to the high salinity concentration due to their own programmed cell death after becoming stressed enough (Ayre et al. 1). There could have been various factors for this reason, but one main factor could have been due to the fact that they were incubated at optimal temperatures for 26 hours (Luan 5).

Overall, for this whole experiment, the null hypothesis is failed to be rejected. This shows that the overall results indicate that there is no effect of increasing water salinity on growth rate of *T. thermophila*. Although there are possible factors that have led us to fail to reject the null hypothesis, this area needs further investigation for other testable concentrations of NaCl that could alter their growth rate.

POTENTIAL ERRORS AND VARIATIONS

On the first day of this experiment, the number of *T. thermophila* cells in each replicate was measured by counting the number of organisms in three random grids (haemocytometer). This counting method might have led to sample error because the cells were not evenly distributed on the counting chamber. One grid might have contained the majority number of the cells in the sample while in another grid, no cell could have been found. Therefore, on the second day of lab, a new counting method was used. The new counting method was used: keep counting the cells until the number reached 100, then used this number to divide the number of red grids (haemocytometer) counted to estimate the cell growth. By counting in this way, sample error was reduced since there was more than 100 cells being counted for each of the sample and then the average was taken from the counted values. However, the cells were counted by an average of three different counts (for each group member), which could have introduced selection bias. One way used to reduce this selection bias was to make as many replicates as possible. As a result, to

reduce this bias, each of the samples had three replicates in each treatment group. During the first eight hours of incubation, the sample was not mixed well before being pipetting onto the haemocytometer because the first sample that was taken out had high cell count. Whereas, by the time the third sample was obtained, the number of cell count decreased tremendously. This might have caused sample error within our data because the cells could have accumulated at the bottom of the Eppendorf tubes due to gravity and most of the cells would have been taken out during the first sample count. The samples should have been well mixed before being pipetting onto the counting chamber. In order to reduce this error, the test tubes containing the samples were put on the vortex for three to five seconds and pipetted up and down a couple of times before the 24th and 26th hour incubated samples were taken out for counting.

CONCLUSION

The results of the experiment show that there is no effect of increasing water salinity on the growth rate of *T. thermophila* since the p-value suggests that the results are not significant. Therefore, the null hypothesis, which states that there is no influence of water salinity level on the growth rate of *T. thermophila* cannot be rejected. Therefore, our prediction that increasing NaCl concentrations will decrease the growth rate was not accepted.

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Mindy Chow and Chanelle Chow for providing our group with the materials and the preparation of the experiment. Finally, we would like to thank the University of British Columbia for allowing our group to take this course and conduct this experiment.

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APPENDIX

Ingredient of standard media:

2% Proteose Peptone, 0.1% Yeast Extract, 0.2% Glucose, and 33 μM $FeCl_3$ (chlamy stock)

Calculation of initial number of cells in the original stock:

number of cells x (5×10^3) *for one of 16 bigger squares (red square) - refer to **Figure 2**

= 20 cells x (5×10^3)

= 1.0×10^5 cells/mL

Calculation of initial concentration:

$C_1V_1 = C_2V_2$

C_1 (initial concentration of the original stock) = 1.0×10^5 cells/mL

C_2 (final concentration of working stock that we want to achieve) = 4.0×10^4 cells/mL

V_2 (final volume of working stock that we want to obtain) = 60mL

V_1 (initial volume of original stock used for dilution) = unknown

$C_1V_1 = C_2V_2$

$V_1 = (C_2V_2)/C_1$

= (4.0×10^4 cells/mL) x (60mL)/ (1.0×10^5 cells/mL)

V_1 =24mL

Summary of the Regression Output from Excel that was made for the data that was obtained experiment:

SUMMARY OUTPUT								
Regression Statistics								
Multiple R	0.63314688							
R Square	0.40087498							
Adjusted R S	-0.19825							
Standard Err	0.02058357							
Observations	3							
ANOVA								
	df	*SS*	*MS*	*F*	*Significance F*			
Regression	1	0.00028349	0.00028349	0.66910071	0.56352581			
Residual	1	0.00042368	0.00042368					
Total	2	0.00070717						
	Coefficients	*tandard Erro*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-0.043525	0.02520962	-1.7265233	0.33421517	-0.3638436	0.27679362	-0.3638436	0.27679362
0	0.3117619	0.38113366	0.81798576	0.56352581	-4.5310004	5.15452417	-4.5310004	5.15452417

Table 1: Results of a one-way ANOVA test done on Microsoft Excel.