We would like to acknowledge that our class, research, and the paper was conducted on the traditional, ancestral, and unceded lands of the xʷməθkʷəyəm (Musqueam), sḵwxwúmesh (Squamish), and selílwitulh (Tsleil-Waututh) peoples.
For these Indigenous groups, salmon are an important aspect of their culture, and identity, as well as a major food source. Though they were, and still are a marginalized group, the Coast Salish peoples continue to spearhead advocacy for sustainability. We invite the reader to be mindful of the land upon which they currently reside, and the history of oppression and marginalization faced by its peoples.

Effect of Varying Glucose Concentration Levels on Speed and Direction of *Tetrahymena thermophila*

Anthony Ho, Jennifer Jung, Viktorija Juciute

I. ABSTRACT

Freshwater protozoan, *Tetrahymena thermophila*, show ability to move towards their primary source of food, such as bacteria in water. Glucose present in the cytoplasm is thus considered to be a chemo attractant for *T. thermophila.* The purpose of our study was to expose starved *T. thermophila* to glucose concentration levels of $4.4 \cdot 10^{-3}$ M, $2.2 \cdot 10^{-3}$ M, $2.2 \cdot 10^{-5}$M, $2.2 \cdot 10^{-8}$M, $2.2 \cdot 10^{-11}$M, $2.2 \cdot 10^{-14}$ M, and measure their speed and direction. We expected to see the greatest speed towards glucose at $2.2 \cdot 10^{-8}$ M concentration level based on previous findings, and hypothesized that the speed and direction of *T. thermophila* would be dependent on the varying glucose concentration levels. However, using a one-way ANOVA test, we found our p-value to be 0.2483 and failed to reject our null hypothesis at 5% significance level. Therefore, we concluded that the mean speed and direction of *T. thermophila* are independent of the six varying glucose concentration levels.

II. INTRODUCTION

Protozoa are an integral part of aquatic food chains (Pratt, J.R., Cairns J. 415-423). Salmon, the keystone species in British Columbia, rely on various freshwater protozoa for survival. One example of such species is *Tetrahymena thermophila* (Zhang, S., Ling, Z.,et al.). Besides being a part of salmon food chain, *T. thermophila* are shown to reduce the number of bacteria and viruses in the streams (Pinheiro, M., Power, M. et al., pp. 643-649.), and detoxify potentially toxic heavy metals such as zinc and cadmium in freshwater (Dunlop, S., Chapman, G., pp. 264–274). Due to their importance in aquatic ecosystems, understanding the behaviour of *T. thermophila* under varying conditions will result in better understanding of environmental conditions beneficial to salmon ecology.

Tetrahymena tend to switch between phagocytosis and surface absorption (Cassidy-Hanley, D.M. pp. 237-276). Previous research shows that *T. thermophila* uses bacteria and and detritus as their food source (Pratt, J.R., pp. 415-423). The variability of *T. thermophila* food sources shows that they are able to consume food in various forms. Hence, they can absorb glucose present in other organisms, as well as glucose dissolved in water. In addition, *T. thermophila* has been shown to move towards their food source (Hellung-Larsen, P., pp. 229-233). Being a heterotroph, *T. thermophila* shows chemotactic preference towards glucose (Hellung-Larsen, P., pp. 229-233), and obtain it through surface absorption. They can withstand relatively high concentrations of nutrients due to their contractile vacuoles that function as osmoregulators (Allen, R.D., pp. 351-394). *T. thermophila* show chemotaxis with increasing levels of glucose until a threshold concentration is reached (Kohidai, L., et al. pp. 467-476). Due to the role of *T. thermophila* in salmon ecosystems, it is of interest to explore their behavior as the concentration of nutrients is changing.

Based on the findings above, the speed and direction of *T. thermophila* exposed to varying concentrations of glucose were tested. The concentration levels were chosen based on a previous research suggesting that optimal glucose concentration for chemotaxis in *T. thermophila* is $1 \cdot 10^{'}$ M (Szemes, A., et al. pp. 158-165). We propose our hypotheses as the following:

H_0: The speed and direction of *T. thermophila* towards glucose is independent of the six glucose conentrations: 4.4×10^{-3} M, 2.2×10^{-3} M, 2.2×10^{-5} M, 2.2×10^{-8} M, 2.2×10^{-11} M, 2.2×10^{-14}M

H: The speed and direction of *T. thermophila* towards glucose is dependent on the six glucose conentrations: 4.4×10^{-3} M, 2.2×10^{-3} M, 2.2×10^{-5} M, 2.2×10^{-8} M, 2.2×10^{-11} M, 2.2×10^{-14}M

III. METHODS AND MATERIALS

Varying Glucose Concentrations and Control

The starting glucose concentration was 0.02M, on which serial dilutions were performed to make 1000μL = 1 mL of $1 \cdot 10^{-2}$ M glucose solution. Serial dilutions were performed on this newly diluted $1 \cdot 10^{-2}$ M solution to make $1 \cdot 10^{-4}$ M glucose solution. This process was repeated until all five concentration levels of the glucose solution were prepared. To calculate volumes for dilutions, the following formula was used:

$$C_1 V_1 = C_2 V_2$$

Glucose-free nutrient medium was used as control to observe the response of *Tetrahymena* in the absence of glucose.

Preparing Tetrahymena

To ensure prompt responses from *T. thermophila* (strain B206) to to the changing concentration levels of glucose, they were starved prior to the experiment in a glucose-free nutrient medium since July 17th, 2017 until October 23rd, 2017.

Experimental Assay and Setup

The experimental assay comprised of a miniscule track connecting two chambers on either ends. The assay was placed on a glass slide (Figure 1). The assay-containing slide was placed over a micrometer (Figure 2). Increments of the micrometer (0.2 mm) were aligned with the track. The slide and the micrometer were then both placed under the dissecting microscope at a total of 24X magnification (15X ocular lens, 1.6X objective lens).

Prior to each treatment, the experimental assay was rinsed with glucose-free medium solution, and partially filled with 45μL of the same solution. Five μL of *Tetrahymena* solution was placed into one of the two chambers. Simultaneously, 10μL of glucose solution (varying concentrations) was placed into the other chamber. Movement of *T. thermophila* along the track was recorded for 3 minutes using DinoXcope.

For each treatment group, the same experimental assay and dissecting microscope were used. All trials were carried out on the same day to prevent contamination of solutions and ensure the most consistent results.

Data analysis

Recorded videos were analyzed with ImageJ. Displacement (μm) and time (s) were recorded for all moving *T. thermophila* observed during the 3-minute interval. The interval was chosen as an arbitrary time needed to normalize the results under assumption that equilibrium between the glucose-free medium and glucose-containing solution has not been established. In order to keep the concentration gradient and prevent equilibrium of glucose across the assay. Speed was calculated as displacement over time.

Direction was denoted by assigning the *T. thermophila* moving towards glucose a positive value. Negative value was assigned for *Tetrahymena* moving away from glucose. The vertical motion of *T. thermophila* was ignored since it did not indicate their chemotactic responses.

Figure 1. *A diagram representation of the experimental assay on a glass slide with two chambers, A and B. T. thermophila and glucose solutions are placed into chambers A and B, respectively. T.thermophila migrate from chamber A to chamber B via the connecting track.*

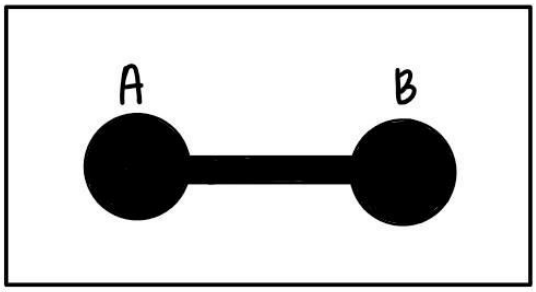
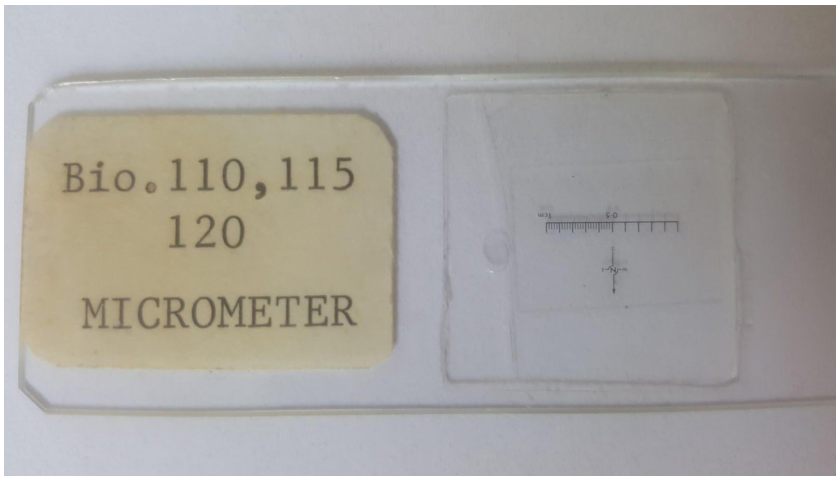


Figure 2. *Micrometer used to measure the distance travelled by each T. thermophila along the track in the experimental assay. One large increment is equivalent to 1 mm, and one small increment 0.2 mm.*



IV. RESULTS

The average speed for each concentration level and control group was calculated in order to evaluate whether there is a pattern in the response. The results are summarized in *Table 1*.

Table 1 *Mean speed and within-group standard deviation for each treatment group.*

	$4.4 \cdot 10^{-3}$ M	$2.2 \cdot 10^{-3}$ M	$2.2 \cdot 10^{-4}$ M	$2.2 \cdot 10^{-8}$ M	$2.2 \cdot 10^{-11}$ M	$2.2 \cdot 10^{-14}$ M	Control (0 M)
Mean speed	40.77	28.05	40.67	13.89	54.63	18.35	-2.427
Number of units	10	10	10	10	10	10	10
SD	53.36	22.40	76.89	17.91	90.98	13.34	37.30

The data is represented by side-by-side boxplots *(Figure 3)* to compare means and variations. Mean speed at $2.2 \cdot 10^{-14}$ M is the lowest, only slightly higher than the mean speed of the control, 0M. The highest mean speed was recorded at $2.2 \cdot 10^{-11}$ M. Even though the means differ across the groups (*Table 1)*, the variations between within-group Tetrahymena also are large. This can be seen from the vastly differing standard deviations (*Table 1*).The box plots of 4.4 x 10 -3 M and 2.2 x 10 -11 M groups appear to have large variations and are more heavily skewed to the right.

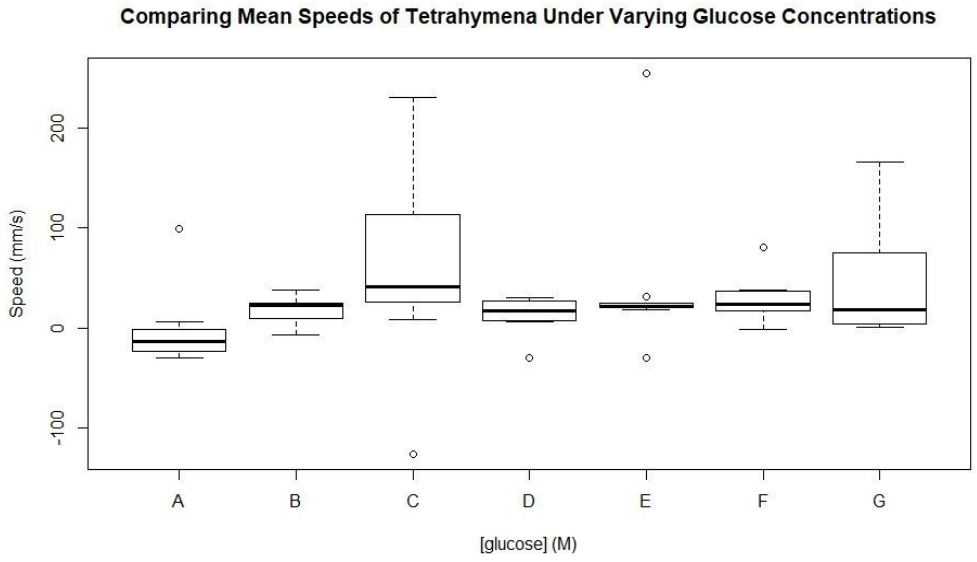
To investigate the significance in the differences of the means across the groups, one-way ANOVA was performed. Results of the test are summarized in table 2 below.

***Table 2**: ANOVA table for mean speeds of T. thermophila across groups of different glucose concentration levels.*

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-Ratio	P-value
Between	6	22504	3750.7	1.3512	0.2483
Within	63	174877	2775.8		
Total	69	197381			

P-value from the one-way ANOVA test is 0.2483 (*Table 2)*. It is much larger than our significance level of 0.05. Therefore, we fail to our reject the null hypothesis that the mean speed of *T. thermophila* is independent of the six glucose concentration levels, 4.4×10^{-3} M, 2.2×10^{-3} M, 2.2×10^{-5} M, 2.2×10^{-8} M, 2.2×10^{-11} M, 2.2×10^{-14}M

***Figure 3**: Side-by-side boxplots comparing mean speeds of T. thermophila at seven different concentration levels. Glucose concentration level is increasing from left to right. (A = OM (control), B = 2.2×10^{-14}M, C = 2.2×10^{-11}M, D = 2.2×10^{-8}M, E = 2.2×10^{-5}M, F = 2.2×10^{-3}M, G = 4.4×10^{-3}M)*



V. DISCUSSION

It was observed during the experiment that *T. thermophila* generally moved from well B (glucose-free medium) towards well A (the medium with varying concentrations of glucose), except for the control group, which was without glucose (*Table 1)*. Although some control-group *Tetrahymena* were moving towards well A, the movement was most likely random. Hence, lack of (differences in) speed in different glucose concentrations does not necessarily represent lack of chemotactic response, as discussed by Hellung-Larsen (1990). The chemotactic response is important for the role of *Tetrahymena* in freshwater ecosystems, such as being a part of a lower foodchain (Zhang, S., Ling, Z., et. al.), and reducing the number of salmon pathogens in water (Pinheiro, M., Power, M., et al., pp. 643-649). *Tetrahymena* response to different nutrient concentrations is directly related to their ability for surface absorption (Cassidy-Hanley, D.M., pp. 237-276). However, the speed of *Tetrahymena* is likely more affected by intracellular processes, such as phosphorylation of amino acids (Leick, V. et al., pp.249-260).

Sources of Error and Variation

Careful steps were taken to minimize variation within each group. At least 10 replicates were collected within each group to obtain well-represented average values. In cases where there were more than 10 replicates, 10 data points were selected at random to form a dataset. Variables such as lighting, the dissecting microscope, and experimental assay, were kept constant. For each trial, the experimental assay was rinsed twice with the control solution to ensure it was not contaminated with glucose concentration from the previous trial. All trials were performed within 2 hours in order to minimize variations in time of the day and contamination from prolongedly exposing solutions to the air.

However, despite these efforts, variation within some of the groups, such as in the $4.4 \cdot 10^{-3}$ M and $2.2 \cdot 10^{-11}$ M (*Table 1*), was particularly large. It is likely that there may have been variations caused by currents caused by the solutions added to the chambers. The currents might have pushed *T. thermophila* and caused them to move at the speed independent of their preferences. These currents might not have been visible, but might have had an impact on *Tetrahymena* since they are smaller and more sensitive to changes in its environment. To minimize the response variation, similarly-sized tetrahymena should be selected for the experiment, or a bigger sample size should be used. Differences in angles at which the solutions were being released from the micropipettes may have made the currents differ among different treatment groups, further increasing the variation in results.

The experimental setup was not aimed at using ImageJ as the observation software. Manual counting of tetrahymena is likely to have introduced selection bias: faster-moving and bigger *Tetrahymena* were preferred while taking measurements. ImageJ has an option to automatically track moving objects by distinguishing different-colored objects from the background and measuring change in position over time. The presence of a micrometer under the assay introduced extra noise which prevented automation of data collection. This resulted in the necessity to manually select data and hence likely introduced selection bias. Based on these observations, the experimental set-up might be improved by attaching a scale to the top of the experimental assay as opposed to using a micrometer underneath it. This would prevent the selection bias and will likely give more consistent results.

Some samples did not have enough *Tetrahymena* passing through even after the 3-minute trial time (this occurred with the $2.2 \cdot 10^{-11}$ M glucose solution group). In order to collect more data, another trial was performed with the same concentration of glucose and under the same conditions. During data analysis, Tetrahymena from both groups were used to calculate their average speed.

V. CONCLUSION

The results of the experiment suggest that the speed of *T. thermophila* is independent of the changing glucose concentration levels at

4.4×10^{-3} M, 2.2×10^{-3} M, 2.2×10^{-5} M, 2.2×10^{-8} M, 2.2×10^{-11} M, 2.2×10^{-14}M.

However, there were many sources of variation such as selection bias introduced by manual measurements. Thus, to evaluate *T. thermophila* chemotactic response it might be more accurate to examine the proportion of migrated *Tetrahymena* or their angle orientation (Hellung-Larsen, P. et al., pp. 229-233) towards glucose solution.

VI. ACKNOWLEDGEMENTS

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