The effects of glucose concentration on the glucose-induced chemotaxis of *Tetrahymena thermophila*

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Abstract

Tetrahymena thermophila is a free-living, unicellular eukaryote (Collins & Gorovsky, 2005) that lives in freshwater environments, using its cilia to move through water and sweep food into its oral grooves (Bozzone, 2000). Chemotaxis is the movement of an organism in response to a chemical gradient of a particular substance. The glucose-induced chemotaxis of *T. thermophila* was investigated to identify which concentration of glucose solution elicited the greatest chemotactic response. *T. thermophila* were starved and incubated at 25 °C for 24 hours. After the starvation phase, *T. thermophila* were cultured in $1x10^{-1}$ M and $1x10^{-6}$ M glucose solutions for 5 and 30 minutes. Following fixation of the cells using IKI, the cells in each replicate were counted using a haemocytometer. A one-way ANOVA test was conducted for both time trials with the glucose concentration as the factor, which obtained p-values that were statistically insignificant. Therefore, the concentration of glucose solution does not have a statistically significant effect on *T. thermophila's* chemotaxis. We fail to reject the null hypothesis that the concentration of glucose will not affect the chemotaxis of *T. thermophila*.

Introduction

Tetrahymena thermophila is a free-living, unicellular eukaryote belonging to the phylum Protozoa (Collins & Gorovsky, 2005). *T. thermophila* is a ciliate that swims in freshwater environments, using its cilia to move through water and sweep food into its oral grooves (Bozzone, 2000).

Chemotaxis is the movement of an organism in response to a chemical gradient of a particular substance. Organisms may migrate towards or away from chemical stimuli as a chemosensory locomotive response. *T. thermophila's* chemotactic response is essential as it affects the survival of *T. thermophila* in varying environmental conditions. *T. thermophila's* locomotion can be measured using an extracellular substance such as sugar (Szemes et al., 2015).

T. thermophila perform crucial functions in aquatic ecosystems, such as the processing of dead organic material and recycling of mineral nutrients (Pratt & Cairns, 1985). As well, *T. thermophila* have been found to activate the chum salmon reovirus. While non-lethal, chum salmon reovirus has a visible cytopathic effect consisting of cells fusing together to form a cell mass which may affect the salmon embryos' success (Pinheiro & Bols, 2018). *T. thermophila* affects the survival of other marine organisms (Stoecker & Pierson, 2019), which includes the keystone species Pacific Salmon in British Columbia (Bass et al., 2017). Investigating the glucose-induced chemotaxis of *T. thermophila* can provide insight into its behaviour in varying environmental conditions, which may be beneficial in understanding its effects on salmon and the aquatic ecosystem.

To determine the impact of glucose concentration on *T. thermophila's* chemotaxis, the number of *T. thermophila* that had migrated to and were present in differing concentrations of glucose solutions were counted. The null hypothesis states that the concentration of glucose does not affect the chemotaxis of *T. thermophila.* We alternatively hypothesized that in the highest concentration of glucose solution, the chemotactic response of *T. thermophila* will be the greatest, and therefore the number of *T. thermophila* present will be the highest.

Methods

Our experiment consisted of three phases: starvation, treatment, and counting. For the starvation phase, a culture of *T. thermophila* was prepared for us. The culture was spun down for 20 minutes at full speed in a centrifuge. We decanted the natant layer with pipettes and combined the pellets of *T. thermophila* in a Falcon tube, rinsing the centrifuge tubes with starvation media

and adding the rinse to the Falcon tube. 45 mL of starvation media was pipetted into the Falcon tube and mixed with the *T. thermophila.* The sample was then incubated at 25 °C for 24 hours.

To prepare the different treatments of glucose solutions for the treatment phase, we used a 0.1 M stock glucose solution for one treatment. We then diluted the stock solution to make 5 mL of $1x10^{-6}$ M glucose solution. The control treatment was starvation media.

After 24 hours of incubation, we removed the starved *T. thermophila* from the incubator. 3mL of the starved culture was pipetted into each of the eighteen 10 mL test tubes. We then prepared three micropipettes with 400 uL of starvation media and three micropipettes with 400 uL of 0.1 M glucose solution. The micropipettes were set up on racks and test tubes of starved *T. thermophila* were placed below them. We ensured all the micropipette tips were submerged in the starved cultures and left them there for 30 minutes. After 25 minutes, the contents of the micropipettes were dispensed into 500 uL plastic tubes. 40 uL of iodine potassium iodide (IKI) fixative was added to each plastic tube to fix any *T. thermophila*. We repeated this process again with three micropipettes of 400 uL $1x10^{-6}$ M glucose solution to complete our 30-minute trials. The contents of each micropipette were dispensed into 500 uL plastic tubes as before, and 40 uL of IKI fixative were added to each tube. The above process was repeated for the 5-minute trials.

Once all treatments occurred, we were left with eighteen plastic tubes of fixed *T. thermophila* from each micropipette. The *T. thermophila* samples were resuspended with a micropipette to thoroughly mix the culture to ensure the cells were evenly distributed. To determine how many *T. thermophila's* migrated into the micropipettes, 20 uL of each fixed sample was dispensed into a counting chamber. Using a compound microscope set to phase 1 and a 10X objective lens, the cells were counted using a haemocytometer slide (Appendix A). Between every sample, the haemocytometer was rinsed with deionized water and dried with a

Kim wipe. The data from each sample was recorded in a data table (Appendix B).

Results

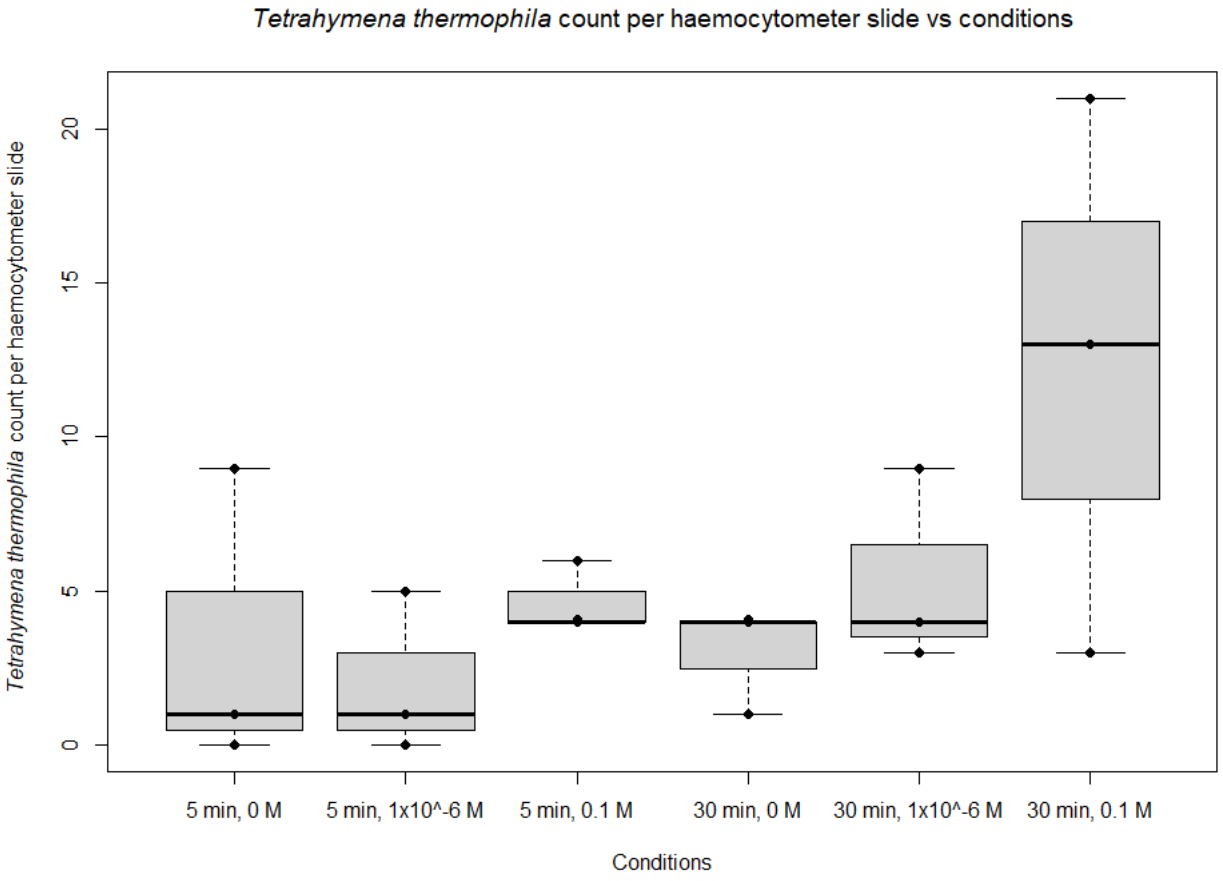

Figure 1. Tetrahymena counts at different conditions. The black line represents the median, the section above the line is the third quartile, and the section below the line is the first quartile. The whiskers represent the maximum and minimum values, any data points above the whiskers are considered outliers.

We conducted a one-way ANOVA using R at a 95% significance level (alpha of 0.05) for the data collected at 5 minutes and 30 minutes. Our null hypothesis states that the molar

concentration of glucose will not have a significant effect on the mean *T. thermophila* count and we can say that the difference in mean *T. thermophila* count across glucose concentrations is not statistically significant. It was alternatively hypothesized that the molar concentration of glucose will have a significant effect on the mean *T. thermophila* count, and the different mean *T. thermophila* counts across different molar concentrations of glucose is statistically significant. Upon conducting a one-way ANOVA for the counts at both 5 minutes and 30 minutes, with the molar concentration of glucose being the factor, we have obtained a p-value of 0.3494 and 0.0662, for 5 minutes and 30 minutes respectively. The median is 1 *T. thermophila* organism at 5 minutes, and 0 and $1x10^{-6}$ M, and 4 *T. thermophila's* at 5 minutes and 0.1 M. The median is 4 *T. thermophila's* at 30 minutes, and 0 and $1x10^{-6}$ M, and 13 *T. thermophila's* at 30 minutes and 0.1 M.

Discussion

From the one-way ANOVA statistical test with our experimental data, we found that our data is statistically insignificant with both p-values above 0.05. Therefore, different glucose concentrations ($1x10^{-1}$ M and $1x10^{-6}$ M) and different treatment times of 5 and 30 minutes did not result in a statistically significant difference in *T. thermophila's* chemotaxis. However, for the experiment completed at 30 minutes, the mean tetrahymena count at 0.1M was noticeably higher than the other concentrations within the same time trial, and this resulted in a p-value that was much closer to 0.05 than the p-value from experiment done at 5 minutes, being 0.0662 and 0.3494 respectively. A p-value that is close to 0.05 indicates the possibility of weak evidence against the null hypothesis, so we can conclude that the tests completed at 30 minutes hold more statistical significance than the tests completed at 5 minutes. In particular, the p-value for 30

minutes is lower than that of at 5 minutes, implying that the results for 30 minutes are more significant compared to that from 5 minutes. Both p-values are larger than the alpha of 0.05, so we fail to reject the null hypothesis which states that there is no significant difference in *T. thermophila* count across different molar concentrations of glucose and molar concentration does not have a significant effect on the *T. thermophila* chemotaxis.

Our results are similar to other experiments done in similar settings. In an experiment done with similar treatments, similar results were achieved where a statistically insignificant relationship between *T. thermophila* chemotaxis and glucose concentration levels were observed (Ho et al., 2018). The insignificant results can also be accredited to the various sources of error in their experiment, likewise to our experiment.

However, despite the statistically insignificant data, there is a noticeable trend in the graph. For the *T. thermophila* chemotaxis count with $1x10^{-1}$ M glucose solution at 30 minutes, we can see that most of the data count is significantly higher than the rest of the data. This shows that there may be a preference for higher concentrations of glucose for longer periods of time. This trend of data can be addressed and explored in future studies. As well, at 30 minutes with a glucose concentration of $1x10^{-1}$ M, the variance in our tetrahymena count is much higher than all the other conditions as indicated by the spread of points on our plot, and at 5 minutes with a molar concentration of $1x10^{-1}$ M, the variance is at its lowest.

There are multiple potential confounding variables that accompany our experimental design and performance. During our treatment phase, before the micropipette tip was inserted into the well with the glucose solution, that is after the glucose solution is in the micropipette, some of the micropipettes had air bubbles in the tip. This could have potentially caused trouble for the liquid to come in contact with the *T. thermophila* solution, restricting their movement into

the glucose solution. This could explain the overall large variability of our data, especially for the large variance at 30 minutes with $1x10^{-1}$ M glucose solution, as well as our insignificant data. Other potential sources of error include performance errors that may not be as impactful. One error was that we didn't resuspend the mixture thoroughly enough in the beginning of the experiment for the 5-minute trials. This could result in lower than expected numbers when we pipette from the middle of the vial. Other minor errors can include various human errors from pipetting and other actions.

Apart from experimental error, error could also be from preparation of the experiment. The condition of the *T. thermophila* starting stock solution we used in our experiment may have had a low concentration of organisms, which could explain the low numbers in our data. In a future study, significant results may be obtained if the starting stock solution of *T. thermophila* had a higher concentration of organisms. As well, we had to conduct our original planned length of spinning duration twice in the centrifuge and we still may not have collected maximum pellets which could explain our low data numbers.

Conclusion

In conclusion, although our experiment obtained statistically insignificant results, our data still display a trend that is worth expanding on. In the future, the experiment could be conducted at different time intervals and at higher concentrations in support of the data trend. Future experiments can also involve the development of a custom or better experimental site which minimizes human and experimental design errors that may impact the significance of the results.

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Appendix

Appendix A: Tetrahymena Counting Details

- Microscope C19
- cat. no. 3720
- counting chamber
- Hausser Scientific Partnership
- Horsham PA 19044

Appendix B: *T. thermophila* Counts

Number of *T. thermophila* with different time and concentration			
Concentration	Tube #	5 min	30 min
Control	Tube 1	0	4
	Tube 2	1	4
	Tube 3	9	1
0.1M	Tube 1	4	13
	Tube 2	4	21
	Tube 3	6	3
1 x 10-6M	Tube 1	0	3
	Tube 2	1	4
	Tube 3	5	9