Effect of Varying Concentrations of SSP Medium on the Chemotactic Response of *Tetrahymena thermophila*

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Abstract

This study investigated the chemotactic response observed in hunger-induced *Tetrahymena thermophila* (*T. thermophila*) when exposed to varying concentrations of a chemoattractant. The chemoattractant used was SSP medium, which was then diluted 10-fold and 100-fold. It was predicted that at high concentrations of the chemoattractant, there would be large chemotactic movement, which would then decline as the concentrations of SSP medium become more dilute. To do this, the preparation of *T. thermophila* focused on placing them in the starvation medium (10 mM Tris-HCl (pH=7.5) to deprive them of nutrients. A 2-chamber assay apparatus was then used to observe the movement of *T. thermophila* when varying concentrations of SSP medium were introduced. The highest average count of *T. thermophila* was found allocated in the maximum concentration of SSP medium (166533.3333 +/- 96904.35147 cells/mL), then followed by at a 10-fold concentration (51157.14286 +/- 32565.79838 cells/mL) and finally at a 100-fold dilution (23958.33333 +/- 23047.61828 cells/mL). There was a linear decrease of chemotactic movement as the concentration of SSP medium decreased. After conducting a one-way ANOVA test and a Tukey's HSD test, the p-value was found to be 0.0882. These tests showed our results to be insignificant, with no relationships between any groups. We concluded that there is no significant relationship between the chemotactic response of *T. thermophila* and varying concentrations of SSP medium.

Résumé

Cette étude a enquêté sur la réponse chimiotactique observée chez les *Tetrahymena thermophila* (*T. thermophila*) induits par la faim lorsqu'ils sont exposés à des concentrations variables d'un chimioattractant. Le chimioattractant utilisé était le milieu SSP, qui a ensuite été dilué 10 fois et 100 fois. On prévoit qu'à des concentrations élevées du chimioattractant, il y aurait un important mouvement chimiotactique, qui diminuerait ensuite au fur et à mesure que les concentrations du milieu SSP deviennent plus diluées. Pour ce faire, la préparation des *T. thermophila* a consisté à les placer dans le milieu de famine (10 mM Tris-HCl (pH=7.5)) pour les priver de nutriments. Un appareil d'essai à deux chambres a ensuite été utilisé pour observer le mouvement des *T. thermophila* lorsque des concentrations variables de milieu SSP ont été introduites. Le nombre moyen le plus élevé de *T. thermophila* a été trouvé alloué à la concentration maximale du milieu SSP (166533.3333 +/- 96904.35147 cellules/mL), puis suivi par une concentration 10 fois supérieure (51157.14286 +/- 32565.79838 cellules/mL) et enfin à une dilution 100 fois supérieure (23958.33333 +/- 23047.61828 cellules/mL). Une diminution linéaire du mouvement chimiotactique a été observée lorsque la concentration du milieu SSP diminuait. Après avoir effectué un test ANOVA à sens unique et un test HSD de Tukey, la valeur p s'est avérée être de 0,08828. Par conséquent, nous n'avons pas réussi à rejeter notre hypothèse nulle à un niveau de signification de 5 % et nos résultats ont été jugés non significatifs. Nous avons conclu qu'il n'y a pas de relation significative entre la réponse chimiotactique de *T. thermophila* et les différentes concentrations du milieu SSP.

I. Introduction

Tetrahymena thermophila is one of the most well-studied protozoa, which make up the majority of the diversity in the eukaryotic kingdom (Coyne, Stover, & Miao, 2012). They are large unicellular ciliates (30 μm × 50 μm) (Collins & Brunk, 2005) that are commonly found in freshwater lakes, streams, and ponds in connection with emergent or abundant flora (Doerder, 2012). The survival of the Pacific salmon, a keystone species in British Columbia, is dependent on freshwater protozoa like *T. thermophila*. As a result, *T. thermophila* is critical to aquatic ecosystem health. By using macropinocytosis, they can remove bacteriophages and viruses from fluid and deactivate them (Pinheiro et al., 2007). *Tetrahymena thermophila* can also recycle mineral nutrients and digest decaying organic materials and their associated bacterial flora in their ecosystem (Pratt & Cairns, 1985). In freshwater, they can also detoxify heavy metals like cadmium and zinc (Dunlop & Chapman, 1981). Because *T. thermophila* are so vital to the health of aquatic ecology, understanding their behavioural physiology under different nutrient levels is crucial.

Tetrahymena thermophila tend to swim around their environment when hungry, changing their shape and expanding along the posterior cilium to help with coordinated propulsion (Collins, 2005). Chemotaxis is defined as single-cell directional movement in the presence of chemical concentration gradients (Larsen et al., 1990). Chemotaxis allows *T. thermophila* to migrate for feeding (Szemes et al., 2015), temporal and geographical sensing (Tan & Chiam, 2018), and avoidance of harmful toxins (Szemes et al., 2015), making it essential for their survival. Analyzing how *T. thermophila* behaves in different environments can reveal behavioural patterns that aid in the maintenance of a healthy aquatic ecosystem.

Larsen et al. (1990), showed that swimming speeds of *T. thermophila* remain unaffected by varying concentrations of attractant. However, the amount of cells that exhibit chemotaxis varies depending on the attractant concentration. In the presence of a chemoattractant, T*. thermophila* is an ideal organism to monitor relative movement. A robust and rapid reaction to a chemoattractant can be induced by starving the cells in a Tris-aminomethane hydrochloride solution (Tris-HCl) first (Chen & Leick, 2004). As a result, starving *T. thermophila* before adding an attractant will create optimal conditions for

observing chemotaxis. The chemotactic response of *T. thermophila* has been investigated when in the presence of various nutrients such as proteose peptone, amino acids, glucose, and proteins (Larsen et al., 1990; Szemes et al., 2015; Hellung-Larsen et al., 1986). So, utilizing an SSP medium that contains proteose peptone (2%), glucose (0.2%), yeast extract (0.1%) and $FeCl_3$ (0.003%) would be ideal to observe chemotaxis in *T. thermophila* (Gorovsky et al., 1975). A study by Hellung-Larsen et al. (1986) found that peptides in proteose peptone initiate chemotaxis at 10^{-6} M. Also, the optimal glucose concentration for chemotaxis was found to be 10^{-7} M (Szemes et al., 2015). Taking this into account, the concentration of the SSP medium utilized might be diluted in relation to *T. thermophila* mobility. This formed the model of this study, as we note how as the concentration of SSP medium decreases, there should be a linear decrease of chemotactic movement. Because the SSP medium contains both proteose peptone and glucose, diluting the entire medium rather than individual nutrients should produce distinct results. Both experimental protocols used a 2-chamber assay device to examine the following alterations, which served as a foundation for our approaches.

The purpose of this study was to investigate whether *T. thermophila* exhibits chemotaxis when in the presence of varying concentrations of a chemoattractant, this being the SSP medium. The rate of chemotaxis was measured by the number of *T. thermophila* cells that migrate towards each concentration of the medium. Based on previous studies, we hypothesized that if *T. thermophila* is subjected to high concentrations of SSP medium, then it will exhibit more chemotaxis. This is because there is an increased concentration of chemoattractants. As the medium becomes more diluted, there will be reduced chemotaxis observed, since there is a decrease in the concentration of chemoattractant.

II. Methods

Starvation of *Tetrahymena thermophila*

Tetrahymena thermophila were obtained and initially starved in order to have a sample from which appropriate observations of chemotaxis could be made. In order to maintain sterility, all materials were flame sterilized using a Bunsen burner. To be able to observe significant chemotaxis, the sample was equally separated by volume into two 50 mL centrifuge tubes. Both tubes were centrifuged for 15 minutes at 13000 rotations per minute. Through this, a supernatant layer, containing the buffer solution in

which they were initially obtained, rose to the top in each tube, which was quickly extracted using a 10 mL glass pipette. This step was done with care to avoid touching the pellet that had collected at the bottom of the tubes. The remaining pellet that was left untouched was then combined into a single centrifuge tube to avoid having unequal amounts of cells of *T. thermophila*. In this centrifuge tube, 25 mL of 10 mM Tris-HCl (pH=7.5) were added using a 10 mL glass pipette. This solution served as a starvation medium for *T. thermophila*. Within this centrifuge tube, the same 10 mL pipette was used to resuspend the medium and culture to mix them. Again using the same 10 mL pipette, 2 mL of this mixed solution were extracted and added into an empty 6 mL test tube. This process was repeated so that there were twelve 6 mL test tubes, each with 2 mL of *T. thermophila* cells suspended within the starvation medium, which were then placed into a test tube rack and incubated at 25°C for 27.5 hours.

Preparation of Treatments

The experiment involved exposing samples of *T. thermophila* to four different treatments. The relative concentration of chemoattractants in each treatment can be seen in Table 1. The first of these was our control, for which we used a starvation medium (10 mM Tris-HCl (pH=7.5)). The second treatment was the standard 1X concentration of the SSP medium. From this standard concentration of SSP medium, two serial dilutions were made. The first of which was a 10-fold dilution, which acted as the third treatment, and the second of which was a 100-fold dilution, which acted as the fourth treatment. 4 mL of each of these serial dilutions, which were diluted using sterile dH_2O, were created through the formula $C_1V_1 = C_2V_2$.

Table 1. Concentrations, in percentages, of proteose peptone, glucose, yeast extract, and $FeCl_3$ in 10 mM Tris-HCl, in a standard concentration of SSP medium, in a 10-fold dilution of SSP medium, and in a 100-fold dilution of SSP medium.

Treatment	Proteose Peptone (%)	Glucose (%)	Yeast Extract (%)	$FeCl_3$ (%)
10 mM Tris-HCl (pH=7.5)	0	0	0	0
Standard concentration of SSP medium	2	0.2	0.1	0.003
10-fold dilution of SSP medium	0.2	0.02	0.01	0.0003
100-fold dilution of SSP medium	0.02	0.002	0.001	0.00003

Setup of 2-Chamber Assay Apparatus

After 27.5 hours had passed, the test tubes were taken out of the incubator and labelled. Three of them were labelled “control”, another three were labelled as “normal concentration, another trio were labelled as “10-fold”, and the last three were labelled as “100-fold”. In addition to this, each test tube in each trio of test tubes was labelled with a number from 1 to 3 to represent the number of replicates. Using a 10 mL glass pipette, 1 mL of the starvation medium was suspended and subsequently placed into a test tube labelled “control”, being careful not to eject the contents of the pipette into the test tube and to place the pipette at an angle relative to the test tube to allow *T. thermophila* to swim up, as can be seen in Figure 1. Following this, a timer was set for 18 minutes to allow *T. thermophila* to swim into the suspended pipette.

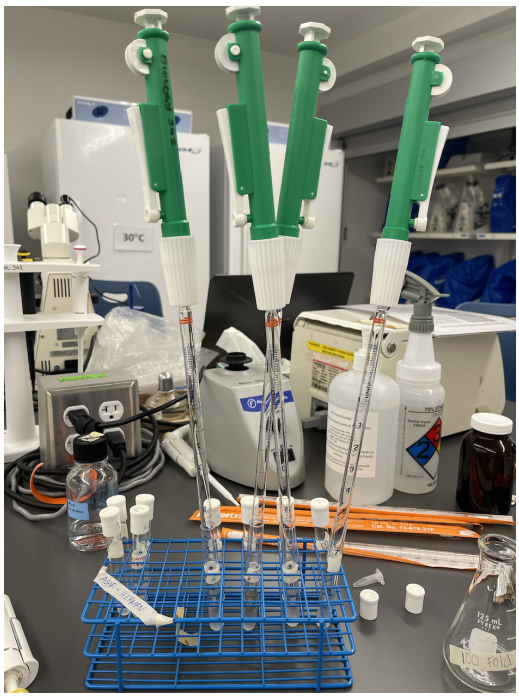

Figure 1. Two-chamber assay apparatus. 10 mL glass pipettes, containing treatments, are suspended within test tubes, containing *Tetrahymena thermophila* suspended in a starvation medium (10 mM Tris-HCl (pH=7.5)). Pipettes are placed at an angle relative to the test tube to allow *T. thermophila* to swim up.

After 18 minutes, the pipette was removed from the test tube and its contents were then dispensed into a 2 mL Eppendorf tube. 200 µL of Iodine-Potassium Iodide (IKI), which acted as a fixative, were then micropipetted and inserted into this Eppendorf tube. This process was then repeated for the

three other treatments. Three replicates of this process were done, resulting in twelve labelled Eppendorf tubes. After fixing the cells using IKI, a haemocytometer was used to count the number of cells in each Eppendorf tube. The haemocytometer slides were prepared by micropipetting 20 µL of the fixed sample from an Eppendorf tube under a coverslip on top of the slide. A compound microscope was set up at 100X magnification (10X objective lens) to count the cells. Particles that appeared burst were not counted as cells, as only whole cells were considered, which can be clearly seen in Figure 2, to avoid accounting for additional noise. This process was repeated for the other eleven Eppendorf tubes.

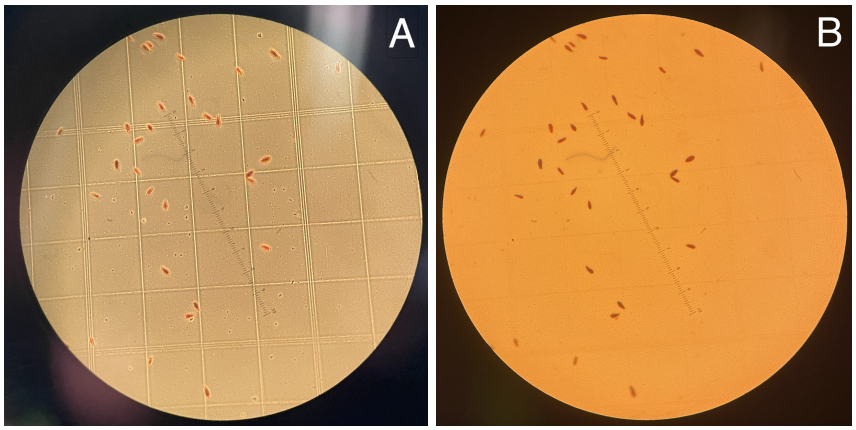

Figure 2. Fixed *Tetrahymena thermophila* in a haemocytometer slide at 100X magnification. Dark, oval shapes are individual *T. thermophila*, shown in both phase 1 (A) and phase 2 (B). Single-lined borders outline the 0.25 mm x 0.25 mm squares which were used for calculations and triple-lined borders outline the larger 1 mm x 1 mm squares.

In order to obtain a concentration of *T. thermophila*, in cells/mL, the number of cells counted was divided by the number of squares and then multiplied by the correction for the fixative, which in this case was IKI, and also multiplied by the dilution factor of the square:

$$\frac{cells}{mL} = \frac{\#\ cells}{\#\ squares} \times (correction\ for\ fixative) \times (dilution\ factor\ of\ the\ square)$$

Following this, a one-way ANOVA test and Tukey's HSD test were performed using the socscistatistics program to investigate the significance of the results.

III. Results

The values acquired from collecting data using the haemocytometer were modified so the concentration of *T. thermophila*, in cells/mL, could be obtained through a simple calculation for each measurement. A sample calculation for the first replicate of the control treatment is:

$$\frac{cells}{mL} = \frac{128}{5} \times (1.2) \times (8\,x\,10^{4}) = 2457600\ cells/mL$$

This calculation was repeated for all other samples. These calculated values were then used to create Figure 3 and Table 2. The treatment that used the standard concentration of SSP medium, had the highest mean (166533.3333 cells/mL) and median (180000 cells/mL). The Tris-HCl treatment (control), followed behind with the second highest mean (84150 cells/mL) and median (60600 cells/mL). With the second-lowest mean (51157.14286 cells/mL) and median (44400 cells/mL), the treatment with the 10-fold dilution of the standard concentration of SSP medium, followed right after it. Finally, the treatment using the 100-fold dilution of the standard concentration of SSP medium, had the lowest mean (23958.33333 cells/mL) and median (12375 cells/mL).

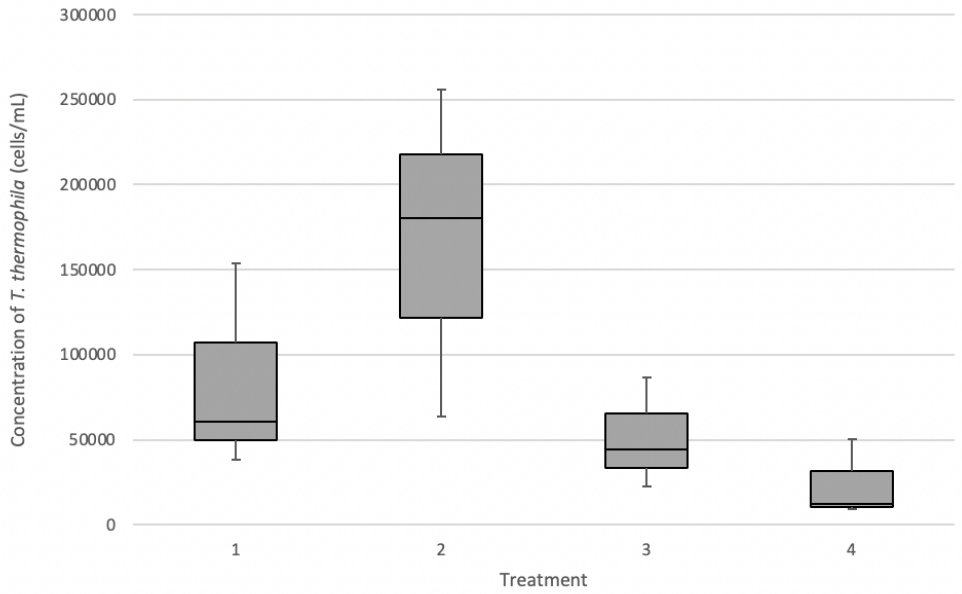

Figure 3. Median concentration of *Tetrahymena thermophila*, in cells/mL, in a starvation medium 5 mM Tris-HCl (pH=7.5) (treatment 1, IQR=57675 cells/mL), standard concentration of SSP medium (treatment 2, IQR=96200 cells/mL), in SSP medium diluted 10-fold (treatment 3, IQR=32035.7143 cells/mL), and in SSP medium diluted 100-fold (treatment 4, IQR=20750 cells/mL) (*N*=3). Hinges of boxplots represent interquartile ranges (IQR) and the bold line within each boxplot represents the median concentration of *T. thermophila* (treatment 1=60600, treatment 2=180000, treatment 3=44400, treatment 4=12375). Whiskers represent minimum and maximum values. (*F*=3.11573, *p*=0.08828)

Table 2. Mean concentration of *Tetrahymena thermophila*, in cells/mL, in a starvation medium Tris-HCl (treatment 1), in standard concentration of SSP medium (treatment 2), in SSP medium diluted 10-fold (treatment 3), and in SSP medium diluted 100-fold (treatment 4) with standard deviation (SD) (*N*=3).

Treatment	Mean concentration of *T. thermophila* (cells/mL)	Standard deviation of the mean concentration of *T. thermophila* (cells/mL)
1	84150	61174.81099
2	166533.3333	96904.35147
3	51157.14286	32565.79838
4	23958.33333	23047.61828

Interestingly, the amount of variation observed followed a similar pattern. In Figure 3, the interquartile range decreases as it goes from treatment 2 (96200 cells/mL), to treatment 1 (57675 cells/mL), then treatment 3 (32035.7143 cells/mL) , and finally it is the smallest for treatment 4 (20750 cells/mL). Standard deviation, as can be seen in Table 2, follows the same pattern as it is the smallest in treatment 4 (96904.35147 cells/mL), comparatively slightly larger in treatment 3 (32565.79838 cells/mL), then is the second largest in treatment 1 (61174.81099 cells/mL), and is the largest in treatment 2 (23047.61828 cells/mL).

Through performing a one-way ANOVA test, a p-value of 0.08828 was obtained. Additionally, a Tukey's HSD test was also performed to investigate whether there were any statistically significant results within two sets of data. This test revealed all p-values to be above the alpha value of 0.05.

IV. Discussion

Through the use of a one-way ANOVA test, the F-value was found to be 3.11573 and the p-value was found to be 0.08828. Since this p-value is above the alpha value of 0.05, as set by a 5% significance level, our results were deemed to be insignificant. As a result of this, we failed to reject our null hypothesis, which stated that *T. thermophila* would display the same level of chemotaxis regardless of varying concentrations of chemoattractant. Our results fail to support our alternate hypothesis, which stated that when *T. thermophila* is exposed to high concentrations of chemoattractant, it will display higher levels of chemotaxis.

As can be seen in Figure 3, our results do display a pattern of the concentration of *T. thermophila* decreasing as the concentration of the SSP medium decreases. However, statistical analysis revealed

that this was not statistically correlated as the results were insignificant. In addition to this, a Tukey's HSD test was performed, and all of the p-values calculated were greater than 0.05, displaying that there are no relationships between any of the groups that are significant. From this, we can say that we did not meet our predictions.

Based on the initial literature review, we believed that when in the presence of a strong concentration of a chemoattractant, the largest number of *T. thermophila* would be observed displaying chemotaxis towards it. So using the model of a standard concentration of SSP and subsequent 10-fold and 100-fold dilutions, the trend should have been to see a decline in *T. thermophila* numbers, as fewer cells display chemotaxis. Although observations appeared to support this prediction, the results were found to be insignificant. The variation, which can be seen through calculated values of standard deviation (Table 2) and observable interquartile ranges (Figure 3), is likely due to possible sources of error that occurred during the experiment.

One issue that may have caused insignificant findings is the number of replicates. Since this was a short-term experiment, there were a limited number of replicates that were possible given the time frame. Doing three replicates may have caused the sample size to be too small, thus any deviation from the mean would be large. Instead of doing three replicates, there could have been five to ten replicates. This could have reduced the variation found in the results. Secondly, the duration for migration of *T. thermophila* may have been too short. In each treatment, 18 minutes were allocated for the movement of *T. thermophila* under differing concentrations of SSP medium. This may have been too short of a duration to observe the maximum amount of chemotactic movement, and thus some *T. thermophila* were unable to enter the 10 mL pipette, causing variation in the subsequent cell count. To account for this, migration times should be increased to 25 minutes. This can ensure that more *T. thermophila* have the opportunity to migrate up the pipette. Furthermore, since the 10 mL pipettes were quite large, it was difficult to place them at an angle within the test tube, so they remained slightly upright for the duration of the migration. This may have made the angle too steep for *T. thermophila* to swim up towards the SSP medium. In the future, it would be beneficial to utilize smaller-sized pipettes, to ensure they can be placed at an angle relative to the test tube. Also, it is notable that for the control, a starvation medium (10 mM Tris-HCl

(pH=7.5)) was utilized to maintain consistency with the medium that the *T. thermophila* was already in. However, the 10-fold and 100-fold dilutions utilized sterile dH_2O instead of more starvation medium to dilute the SSP medium. The difference in using sterile dH_2O compared to the starvation medium for dilution may have altered the chemical composition of the SSP medium altering the movement of *T. thermophila* away or towards the pipette at unequal rates.

There were various changes in the experimental setup of this study when compared to other literature, which could have produced further variation in cell counts of *T. thermophila*. In our 2- chamber assay apparatus, we had 10 mL pipettes positioned at an angle relative to a test tube. In an experiment by Leick & Helle (1983), they arranged their 2-chamber assay apparatus using an alternative method. Instead of utilizing 10 mL pipettes, capillary tubes were used to poke holes in the two chambers. *Tetrahymena thermophila* was able to swim horizontally rather than at a steep upward angle as a result of this. Furthermore, our study left the samples to starve for 27.5 hours. However, in a similar study by Atienza, Huynh, & Lee (2022), *T. thermophila* were starved for 24 hours. They also had an overall higher count of individuals. By allowing *T. thermophila* to starve for longer than 24 hours, it may have led to increased variation as individuals that had eaten more recently were more likely to survive than those that had not eaten as recently prior to the starvation period.

There could also be some biological variation that could have impacted the results of this study. Although the utilization of replicates partly compensates for any biological variation, there could still be other contributing factors related to the chemotaxis of *T. thermophila*. A study by Cole (2000) found that younger *T. thermophila* are able to swim faster than older *T. thermophila*. Without any way to confirm the maturity of the cells in our study, some of them may not have been able to swim fast enough toward the attractant, which could have introduced variation to the measurements. However, our study did agree with the experimental observations of Cole (2000) that *T. thermophila* move when hunger-induced. When starved, *T. thermophila* swim faster and at larger lengths due to how they are deprived of nutrients. This is caused by physiological changes that induce increased length and number of cilia (Cole, 2000). This was observed when counting cells, as the most starved individuals were found in the highest concentration of SSP medium.

V. Conclusion

The goal of this study was to observe the chemotaxis of *T. thermophila* in the presence of the highest concentration of the chemoattractant (SSP medium), as well as subsequent 10-fold and 100-fold dilutions of the same chemoattractant. When hunger was induced, it was hypothesized that *T. thermophila* would exhibit the most chemotaxis in the presence of high chemoattractant concentrations. As the chemoattractant concentration declined, there should have been less chemotaxis due to decreased nutrient availability. The study's p-value was found to be 0.08828. As a result of the p-value failing to meet the 5% significance level, the study's findings were deemed to be insignificant. This study's experimental approach could serve as a good platform for future research on *T. thermophila* and chemotaxis. Due to the critical role of *T. thermophila* in the aquatic ecosystem, further research will allow us to better understand and protect aquatic life.

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