The effect of increasing glucose concentrations on the swimming speed of *Tetrahymena thermophila*

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Abstract

Tetrahymena thermophila, a unicellular ciliate, is a good model organism for the study of cilia. This study investigates whether the concentration of glucose in the growth medium had an effect on the swimming speed of the organism. We treated *T. thermophila* with different glucose concentrations, 0.2%, 1%, 2% and 4%, and observed their behaviour and measured their swimming speed. We observed an increase in average speed in the treatments of 0.2% to 2% glucose concentration. We also found a decrease in speed when the concentration of glucose was 4%. One-way ANOVA gave a *p*-value of 1.12×10^{-3}. The increase in speed when glucose concentration increased from 0.2% to 2% may have been due to aerobic respiration, which upon generating ATP provides energy to power microtubules in the cilia to beat faster. The decrease in speed at 4% glucose concentration may have been due to oxygen depletion in the test tubes that resulted in *T. thermophila* producing less free energy.

Introduction

Tetrahymena thermophila are motile, single-celled eukaryotes that live in freshwater environments (Orias *et al*. 2011). These organisms have nuclear dimorphism which is defined as having both a micronucleus and a macronucleus. In addition, they are relatively big as their size varies between 30 to 50 μm, and they have hundreds of cilia that cover their bodies (Gavin 2009). The large number of cilia present on *T. thermophila* makes it a good model organism for investigation of this organelle. These structures are commonly known for propelling the cells around; however, they also play important roles in other basic cell functions such as conjugation (sexual reproduction), phagocytosis, and "rotokinesis," which is a mechanism that assists in cell division (Wloga and Frankel 2012).

Cilia are comprised of a doublet of microtubules that slide past each other to create a beating motion; this is the basis for the organism's movement (Lodish *et al*. 2000). Gibbons and Rowe (1965) identified a set of proteins called dyneins that power the sliding of the

microtubules. Dyneins use ATP hydrolysis to convert free energy into mechanical work (Gibbons and Rowe 1965). Since synthesis of ATP is crucial for cilia beating, it is important to understand the mechanisms used for its optimal production.

Catabolism, the breakdown of food to cellular energy, is a critical process for eukaryotic organisms since it leads to ATP production (Alberts *et al*. 2010). *T. thermophila* have a primitive metabolic system, which includes aerobic respiration (Blum 1970). This process starts with a glucose molecule in the glycolysis stage, which is broken down to form pyruvate and then oxidized in the Krebs cycle to form succinate (Alberts *et al*. 2010). Kobayashi (1965) found evidence that succinate is oxidized for the genus *Tetrahymena* and this pathway leads to the formation of ATP. Free ATP will then be used by the organisms to power their cilia and swim, as illustrated in Figure 1.

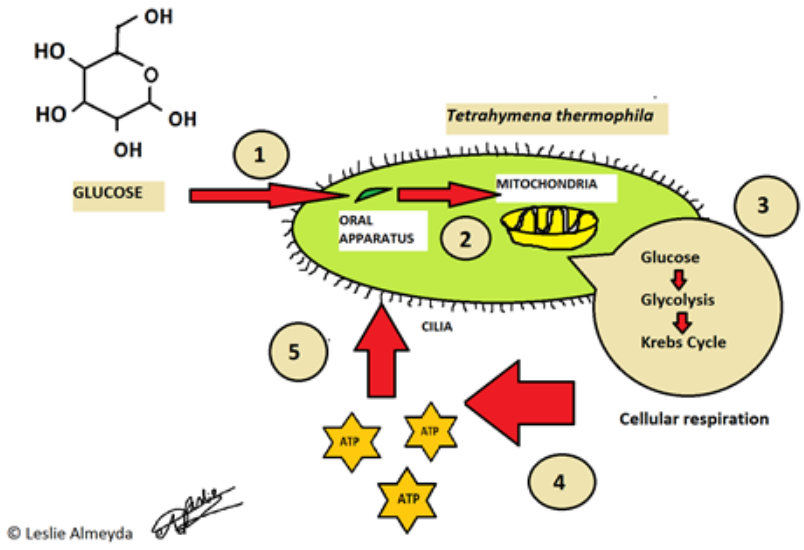

Figure 1. Model of how glucose affects the cilia movement of *T. thermophila*. (1) Glucose molecule is taken in by *T. thermophila* (either via oral cavity or surface uptake). (2) Glucose is ingested and is transported to the mitochondria for carbohydrate catabolism. (3) Cellular respiration uses the glucose molecule and (4) produces ATP as a final product. (5) ATP is used by the dyneins that are part of the structure of *T. thermophila* cilia.

Since *T. thermophila* live in an environment where swimming is required, we believe that finding a factor that causes faster cilia beating would be an important discovery. There are several studies that investigated the effect of glucose on the life cycle of *T. thermophila*; however, none focus on how glucose concentration affects their swimming speed. For our experiment, our null hypothesis is that glucose concentration in the growth medium has no effect on the swimming speed of *T. thermophila*. Consequently, our alternative hypothesis is that increasing glucose concentration has an effect on the swimming speed of *T. thermophila*. Based on the fact that the glucose consumed by the organism will be used for synthesis of ATP, which would then be used to power cilia, we predict that an increase in available glucose will lead to *T. thermophila* swimming at greater speeds.

Methods

Setup

The *T. thermophila* culture used was cultivated in standard SSP growth medium for 10 days before the experiment was conducted. SSP growth medium consists of 2% proteose peptone, 0.1% yeast extract, 0.2% glucose, and 0.003% sequestrene (Cassidy-Hanley 2012). A cell count was completed using an Axiostar compound microscope and a haemocytometer to find an initial count of 5.72×10^5 cells per mL.

To determine the effect glucose had on swimming speed, we introduced *T. thermophila* to four different glucose concentrations: 0.2%, 1%, 2%, and 4% in their growth media. The treatment with 0.2% glucose concentration is the control in the experiment, as this is the standard concentration of glucose in the growth medium that in which *T. thermophila* was cultivated. We made different concentrations of glucose for the treatments through serial dilutions. We diluted

concentrated glucose with SSP growth medium containing 0.2% glucose, to create intermediate concentrations needed for each treatment (Figure 2).

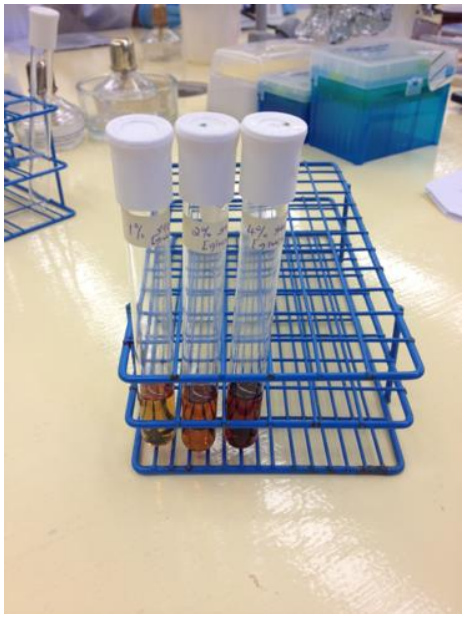

Figure 2. Intermediate glucose media for treatments with 1%, 2%, and 4% glucose concentration from left to right.

For each treatment, we used four replicates. Each replicate was made by adding 1 mL of the intermediate glucose concentration to 1 mL of the organisms from the stock culture, for a total of 2 mL in each test tube. By adding the organisms in standard SSP, the glucose concentration in the growth medium for each treatment was further diluted to create the desirable final concentrations for each treatment. For the 0.2% glucose concentration treatment, we added 1 mL of the culture to one mL of SSP growth medium containing no organisms to achieve the 0.2% final glucose concentration.

Once the organisms were added to the test tubes, the test tubes were immediately capped and placed in a 30^{o}C water bath for three hours so they would have enough time to ingest and utilize the glucose in the growth medium.

Data Collection

Replicates were removed from the water bath after three hours. The test tube was mixed by finger vortexing so that the organisms were spread out evenly throughout the growth medium. A 50 μL sample was taken from the top of the liquid and placed on a microscope slide and examined using an Axiostar compound microscope at 100x magnification. The shape, size, and swimming behaviours, including movement and pathways of the cells were observed and recorded. A 10-second video was taken of the sample using a DinoXcope. An additional two samples were taken from the same replicate, for a total of three samples to be used to determine swimming speed for each replicate. These samples were taken immediately after the replicate was removed from the water bath to minimize the effect that temperatures differences would have on swimming speed.

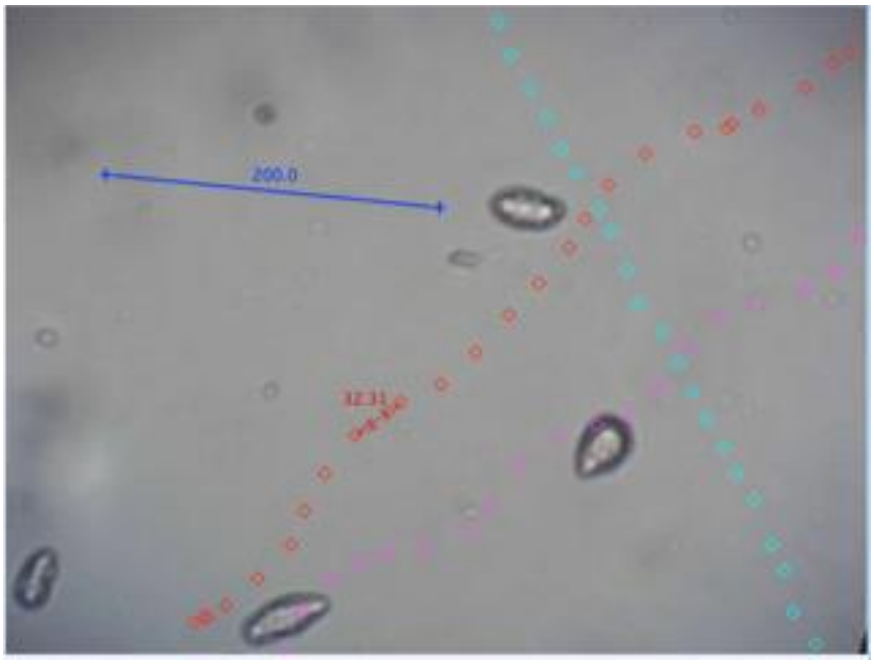

Figure 3. The tracks of three *T. thermophila* cells, at 100x magnification, used to find the average speed for a replicate in 1% glucose concentration using the computer program Tracker. The different colours indicate different cell paths.

From each video taken, we took the average speed of three random cells that swam on-screen and off-screen within the timeframe of the video. We used a video analysis and modeling computer program called Tracker. The size, shape, and swimming behaviour of each of the individual cells were also observed. The speed of each cell was taken by tracking the location of the same point on the organism in every frame that it was present in (Figure 3). The total distance that the cell swam was calculated by measuring the distance between all its location points. Once the total distance was found, it was divided by the total time the organism was present in the video to find the mean swimming speed. We averaged the speeds we took of the three cells for each video to find the mean swimming speed of the sample. Since we took three samples of each replicate, we averaged the mean speed found for each sample to find the mean swimming speed of the replicate (Figure 4).

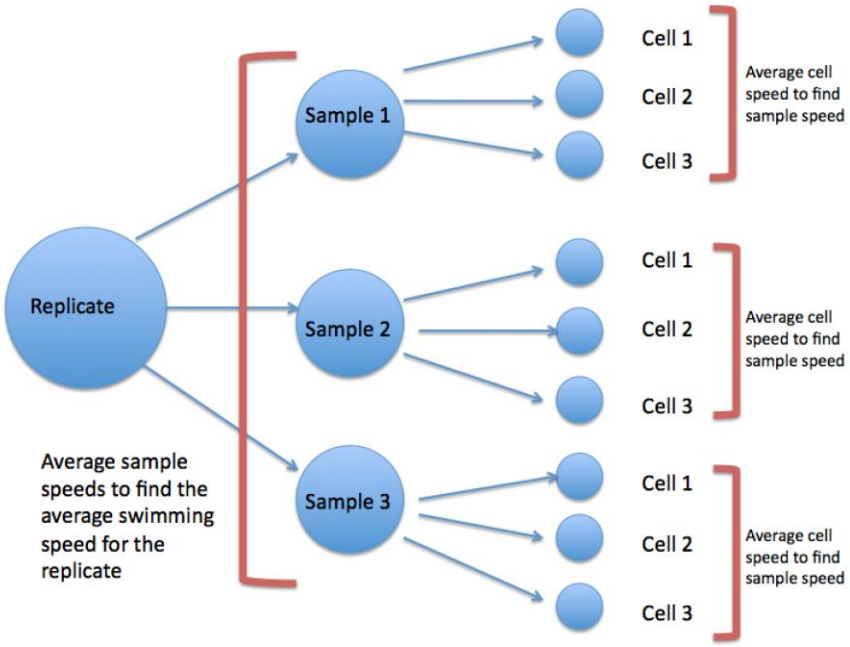

Figure 4. Process of determining the swimming speed of each replicate.

We used one-way analysis of variance (ANOVA) to determine if there was a significant difference in swimming speeds among the treatments. We then found the 95% confidence

interval for the mean of each of the treatments to determine whether the mean swimming speed found for each treatment was likely statistically significant from any others.

Results

In the different treatments, we observed cells of different sizes, shapes, and behaviour (Figure 5). The cell size of *T. thermophila* appeared to be bigger in the treatments with higher concentrations of glucose. The cells also became less oblong and more round as concentration increased. Cells in 4% glucose concentration were also observed to be more stationary as exemplified by the very close and overlapping location points in Figure 5d.

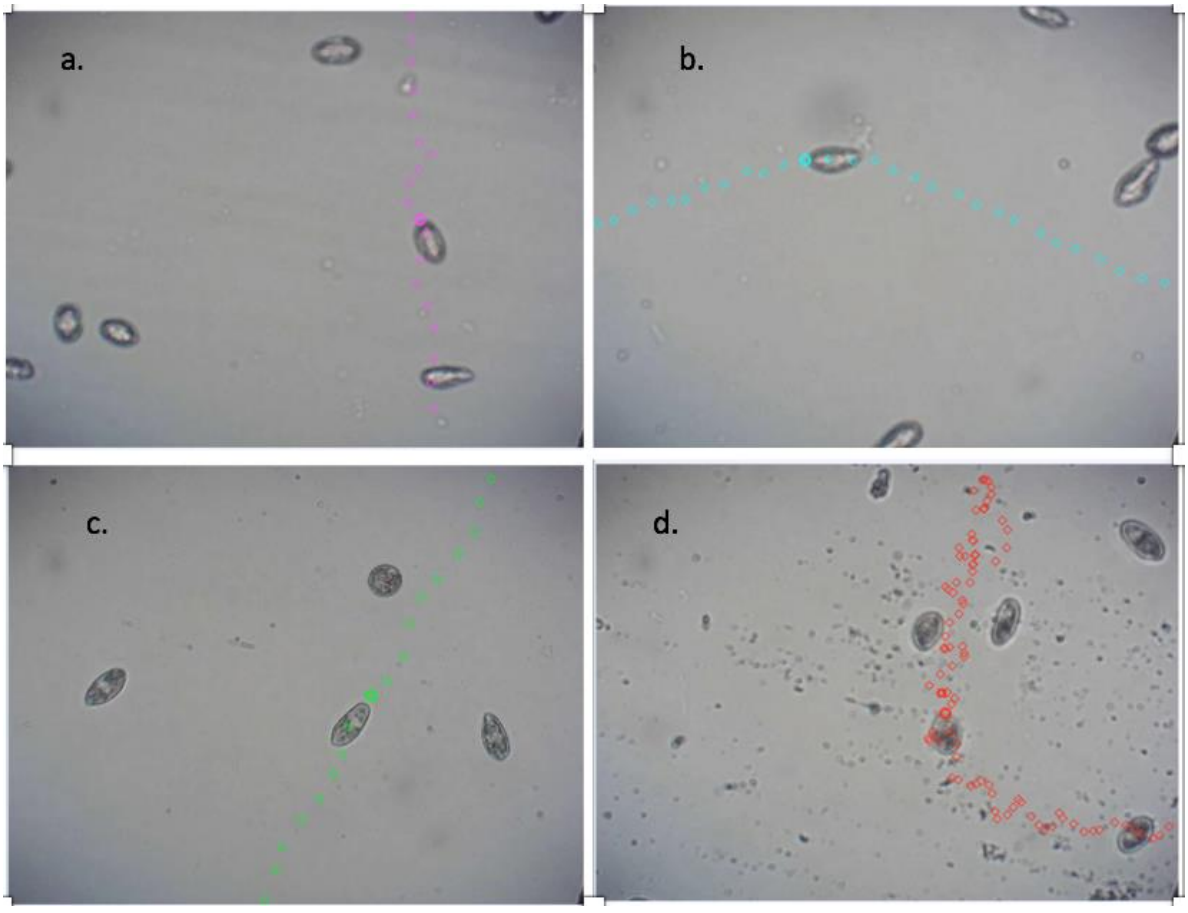

Figure 5. Pathways of *T. thermophila* at 100x magnification in a. 0.2% glucose concentration, b. 1% glucose concentration, c. 2% glucose concentration, and d. 4% glucose concentration.

The average swimming speeds and confidence intervals of *T. thermophila* for the 0.2%, 1%, 2% and 4% glucose concentration treatments respectively are: 62.13±7.11 μm/s, 71.37±5.10 μm/s, 80.79±3.84 μm/s, and 46.84±4.70 μm/s as shown in Figure 6. The swimming speed of *T. thermophila* increased from the control, 0.2% glucose concentration, to the fastest speed at 2% glucose concentration. The average speed increase from 0.2% to 1% was 9.24 μm/s, and the increase from 1% to 2% was 9.43 μm/s. However, the swimming speed decreased when the concentration of the glucose was 4% and a decrease of 33.95 μm/s was observed.

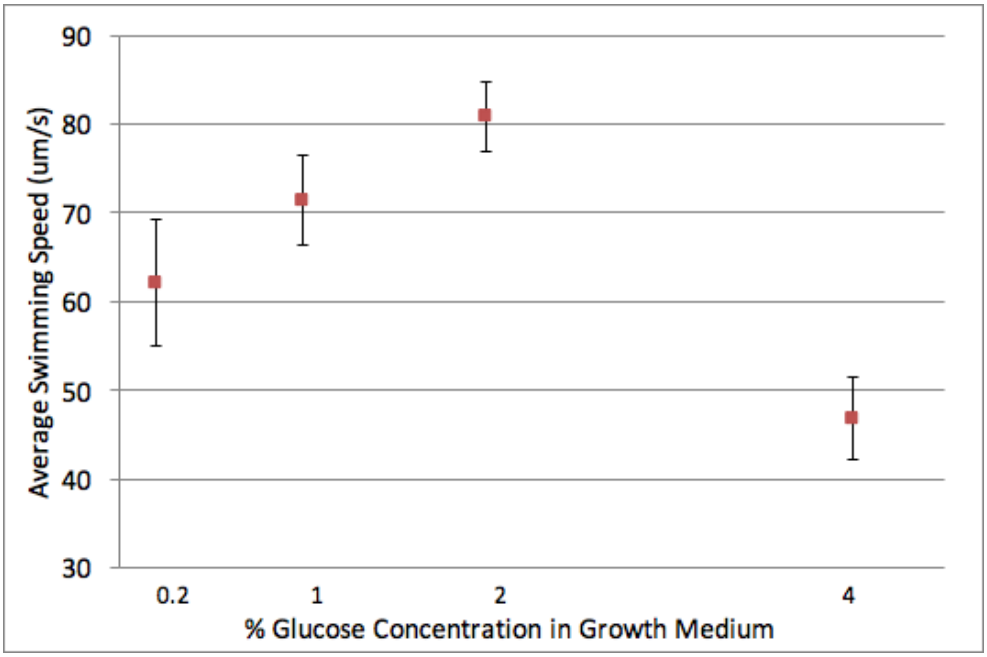

Figure 6. Average speed of *T. thermophila* in treatments with concentrations of glucose in growth media at 0.2%, 1%, 2% and 4%. The error bars show 95% confidence intervals, n=4, *p*-value=1.12 x 10^{-3}.

In order to identify whether the change in speed in different glucose media are statistically significant, a one-way ANOVA statistical test was performed. A *p*-value of 1.12 x 10^{-3} was obtained from the test, indicating a significant difference between the treatments. In Figure 6, the confidence intervals for 0.2% and 1% glucose concentration overlap but the 2% and 4% treatments do not overlap.

Discussion

We reject our null hypothesis as the *p*-value was 1.12×10^{-3} which is smaller than the critical value of 0.05. The results of this experiment support the alternative hypothesis, that increasing the concentration of glucose available to *T. thermophila* has an effect on the swimming speed of the organism. A statistically significant difference in the swimming speeds for treatments with 2% and 4% glucose concentrations was found, but the treatment with 1% glucose concentration was not significantly different from the control (Figure 6).

We observed an increase of swimming speed of *T. thermophila* from concentrations of 0.2% glucose to 2% glucose in growth media. This supports our prediction that increasing glucose availability to the organisms will lead to a faster swimming speed. Glycolysis is part of the process of catabolism where food molecules are broken down to obtain energy for the cell. This pathway starts with a molecule of glucose, which is converted into pyruvate and then oxidized to produce ATP (Alberts *et al*. 2010). Free ATP is then used to power dyneins which are motor proteins that cause the microtubules of cilia to slide past each other, creating the beating motion that propels the organism forward (Lodish *et al*. 2000).

From Kiy and Tiedtke (1992) we know that *T. thermophila* have a preference for glucose in chemically defined media as it was consumed completely over other nutrients during their experiment. Since the cells in 2% glucose concentration were exposed to more glucose than the 0.2% and 1% glucose concentration treatments, they had more opportunity to ingest and utilize the glucose. In addition, as glucose was the main energy source available, breakdown of other food materials to form glucose likely did not take place, possibly making ATP production more efficient and yielding more free energy for the organisms to use towards locomotion.

However, it was found that the swimming speed of *T. thermophila* in the treatment with 4% glucose concentration decreased to a speed that was below the one found for the 0.2% glucose treatment. It is possible that when the organisms were initially added to the growth medium, with 4% glucose concentration, they started to metabolize the readily available glucose very efficiently. The rate of their metabolism may have caused a rapid depletion of oxygen in the test tube, negatively impacting their subsequent metabolic rate (Blum 1970). In addition, with excess amounts of glucose available to *T. thermophila* in treatments with higher concentration of glucose, Blum (1970) found that glycogen synthetase does not get repressed in *Tetrahymena* and glycogen, a form of energy storage, accumulates. This was observed in our experiment as cell size increased in treatments with higher glucose concentration, shown in Figure 5. We also observed cells become less oblong and rounder in higher concentrations (Figure 5) but we believe it did not contribute to the swimming speed of *T. thermophila*.

The catabolism of glucose to form ATP may have caused the oxygen levels in the test tubes to fall as oxygen was consumed to carry out the processes (Alberts *et al.* 2010). This could have led to the switch from aerobic respiration, where glucose was converted to pyruvate and then oxidized to make ATP, to anaerobic respiration, in which glycogen is used to produce energy (Blum 1970). Consequently, less free energy would have been available to be used for locomotion, as anaerobic respiration produces significantly less ATP than aerobic respiration (Blum 1970). This corroborates with Kiy and Tiedtke's (1993) experiment, during which they observed longer stationary phases of *T. thermophila* as the concentration of glucose increased from 0% to 6% glucose concentration. In our experiment, cells in the 4% glucose concentration, often stopped moving for periods of time and then continued on swimming (Figure 5d). This

pattern of movement caused the average calculated swimming speeds of the cells to decrease, as speeds were taken by the distance each cell travelled in the time they were present on screen.

The large confidence interval observed in the control (0.2%) glucose concentration, can be attributed to by an error in timing (Figure 6). When taking the videos for the first replicate of this treatment, we left the organisms in the growth medium for longer than the other replicates and the samples were not taken immediately when they were removed from the water bath. The temperature of the replicate decreased and may have affected swimming speed readings. Since cilia movement has a positive relationship with temperature, when temperature decreases, the transport of ATP to dyneins and its subsequent hydrolysis decreases as well, leading to a slower cilia beat frequency (Humphries 2013). This resulted in a speed reading that was inconsistent with the other replicates in the treatment, contributing to the large confidence interval observed. This timing error may have been instrumental in classifying the effect, 1% glucose concentration has on swimming speed, statistically not significant when it may be

Another source of error that could have impacted our findings is the omission of shaking the cultures during the duration of the experiment. It was observed that live *T. thermophila* usually swim near the surface of the growth medium. When the replicates were placed in the water bath, they were left untouched as a standing culture. Since most of the organisms were at the top of the growth medium, the glucose may have become depleted in that area. *T. thermophila* are known to completely consume glucose over other nutrients and the lack of available glucose may have affected our experiment (Kiy and Tiedtke 1992). If we had shaken our cultures at intervals throughout the incubation period, glucose would have been distributed to the depleted areas and the organisms would have had more opportunity to ingest the glucose for energy production. Shaking would have also helped aerate the culture, distributing oxygen

throughout the growth medium (Hellung-Larsen and Anderson 1989). Since both glucose and oxygen are limiting factors in producing ATP, increasing their availability could lead to faster swimming speed readings.

Additionally, inaccuracies when calculating speed could be a source of error. When finding the speeds of the cells, the distances they swam were calculated by measuring the distance they travelled between each frame of the video on Tracker. The points that were very close together were hard to measure accurately and may have been overestimated. This would have affected the speed readings for the treatment with 4% glucose, as cells in those treatments had location points that were close together and overlapped. The average speed for the 4% glucose concentration treatment is not as accurate as the others and may have contributed to the variation observed in the confidence interval for that treatment.

Conclusion

We reject our null hypothesis and provide support for our alternative, that increasing the glucose concentration in the growth medium of *T. thermophila* has an effect on its swimming speed. Swimming speed increased when glucose concentration rose from 0.2% to 1% and from 1% to 2%, with the 2% concentration of glucose resulting in statistically significantly different swimming speeds. However, we found that when the glucose concentration increased to 4% the swimming speed decreased significantly.

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