Population growth of *Tetrahymena thermophila* under various $FeCl_3$ concentrations in SSP medium

Brian Koo, Jasdeep Sekhon, Sandy Cheng, Sangjoon Park, Younghee Lim

Abstract

The growth of *Tetrahymena* depends heavily on the iron concentration present in the growth medium. The objective of this study was to further examine how *Tetrahymena thermophila* respond to an increase in iron concentration in the growth medium. *T. thermophila* cells were suspended in SSP growth mediums with three varying concentrations of $FeCl_3$ that were prepared (0.024 μM $FeCl_3$, 0.072 μM $FeCl_3$, and 0.14 μM $FeCl_3$) while all other components remained constant. The cells were counted at increments of 3 hours, 5 times, over 24 hours using a haemocytometer. The results showed that *T. thermophila* inherited the same pattern of growth, starting off slow and resulting in significant growth at the 24 hour mark in all treatments. However, the 0.14 μM $FeCl_3$ medium showed the most significant growth while the 0.072 μM $FeCl_3$ medium showed the least growth. Further analysis resulted in failure to reject the null hypothesis as the errors were substantial and the confidence intervals overlapped significantly. Further studies must be performed in order to establish a correlation between varying iron concentrations and abundance of *T. thermophila*.

Introduction

Tetrahymena are eukaryotic, unicellular free-living protozoa, with a rapid doubling rate of two hours (Hanley 2013). There have been numerous studies about various factors that could affect the growth rate of *Tetrahymena* (Hoffman and Cleffman 1981, and Wolfe et al. 2005). Among the various influential factors, iron was the focus of this study as it is frequently referred to as a limiting factor of growth (Hanley 2013). In other words, the presence of iron is essential whether it be for the rapid growth of *Tetrahymena* or for the cell's enzymatic reactions (Kludt and Rasmussen 1970, and Conner and Cline 2007).

Because these reactions are responsible for protein synthesis, carbohydrate dissimilation and energy production for rapid cell replication, it can be said that iron plays a crucial role in rapid cell replication (Conner and Cline 2007).

Many studies spotlighted how the presence or absence of iron in the growth media affects the abundance of *Tetrahymena* (Nozawa *et al.* 1981, Asai and Forney 2000). One experiment discovered a remarkable gap between the abundance of *Tetrahymena* in the presence and absence of iron, as *Tetrahymena* in medium lacking iron showed lower growth rate (Elson et al. 1969). Elson et al. (1969) discovered that in the presence of iron, *Tetrahymena* reached their maximum growth rate within three hours. In addition, the formation rate of protein and glycogen, which are necessary for cell growth, increased significantly (Elson et al. 1969). On the other hand, *Tetrahymena* produced considerably less protein and glycogen in a growth medium lacking iron, resulting in a much slower growth rate. With the support of additional experiments it can be said that iron is in fact a limiting growth factor for *Tetrahymena* (Nozawa *et al.* 1981).

However, there are not enough scientific articles that directly establish a relationship between the abundance of *Tetrahymena* and varying iron concentrations in the growth medium. Therefore, the goal of this experiment is to examine how *Tetrahymena* respond to an increase in iron concentration in the growth medium. We chose to study an increase in iron as it has been shown that *Tetrahymena* are able to adapt well to higher concentrations (Rasmussen *et al.* 1984). By investigating the cell abundance in three different iron concentrations, we were able to effectively study the relationship between the iron concentration and *Tetrahymena* abundance. In addition, we can investigate further as to what iron concentration maximizes cell abundance. The hypotheses for this study are as follows:

H_a: An increase in the concentration of $FeCl_3$ in SSP medium will increase the abundance of *Tetrahymena thermophila*.

H_o: An increase in the concentration of $FeCl_3$ in SSP medium will decrease or have no effect on the abundance of *Tetrahymena thermophila.*

Iron plays an integral role as part of the growth medium (Conner and Cline 2007). Moreover, it has been shown that an increase in iron concentration results in an increase in growth rate. An increase in growth rate, in turn, suggests an increase in the abundance of *Tetrahymena*. This relationship is what we would like to investigate during the course of this experiment.

Methods

We started with three different concentrations of $FeCl_3$ in SSP Media containing no live cells as depicted in Figure 1. The control treatment was the optimal SSP growth medium for *T. thermophila*: 2% protease peptone, 0.1% yeast extract, 0.2% glucose, 0.725 mL/L of 33 µM $FeCl_3$ (0.024 µM $FeCl_3$). The first treatment differed as it contained 3 times as much $FeCl_3$ at 2.175 mL/L of 33 µM $FeCl_3$ (0.072 µM $FeCl_3$). The second treatment contained 6 times as much $FeCl_3$ at 4.35 mL/L of 33 µM $FeCl_3$ (0.14 µM $FeCl_3$). All other ingredients, apart from the $FeCl_3$ concentrations, remained the same in all three media. The 50 mL stock cell culture had the same makeup as our control treatment and contained all of the live *T. thermophila* cells.

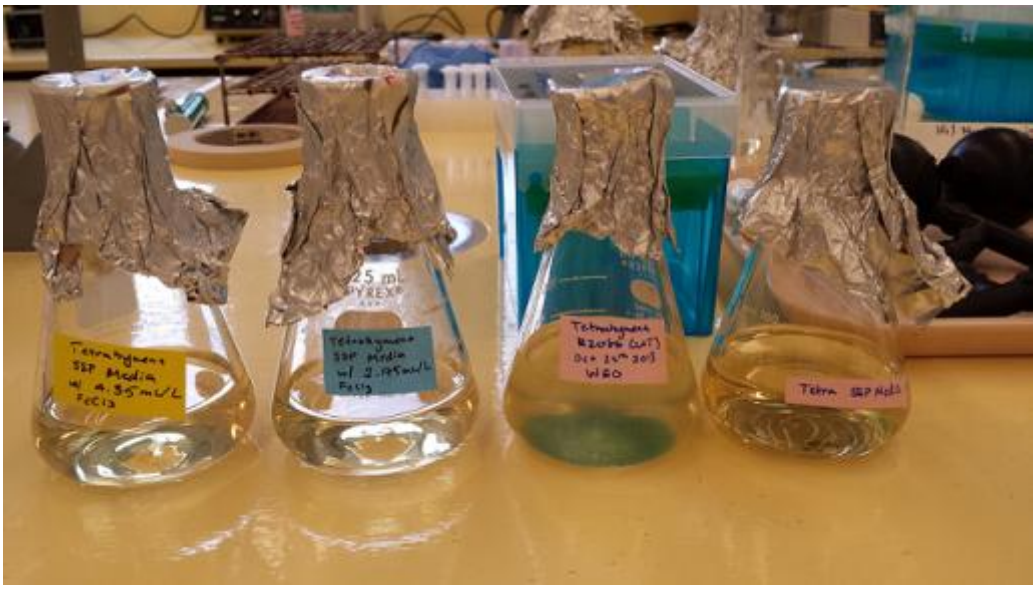

Figure 1. Three treatments with varying $FeCl_3$ concentrations in SSP media and the 50 mL stock cell culture. (Left to right: 4.35mL/L $FeCl_3$, 2.175mL/L $FeCl_3$, stock culture, 0.725mL/L $FeCl_3$ control).

We conducted the initial cell count on 50 µL of the stock cell culture and 5L of gluteraldehyde which served as our fixative. Of the total 55 µL, we analyzed 15 µL using a haemocytometer. For the initial count, we counted one 4x4mm box on the haemocytometer grid three times and calculated the average to be 6.3 cells. Accounting for the 1:10 ratio of fixative to cells, we estimated the initial cell count to be 34,833.3 cell/mL. In order to achieve our desired initial population of 5000 cells/mL in each treatment, we added 1.4 mL of the stock cell culture to each of the three 15 mL test tubes and centrifuged each test tube for a total of 10 minutes at full speed. The supernatant in each test tube was removed by pouring on the opposite side of the pellet. The pellets containing the live *T. thermophila* cells were then suspended in the new growth media up to the 10 mL mark on the15 mL test tubes as shown in Figure 2 to achieve the desired initial population. Each of these 3 treatments was then divided into 4 replicates, each containing 2 mL in 6 mL tubes. A total of 12 replicates were prepared.

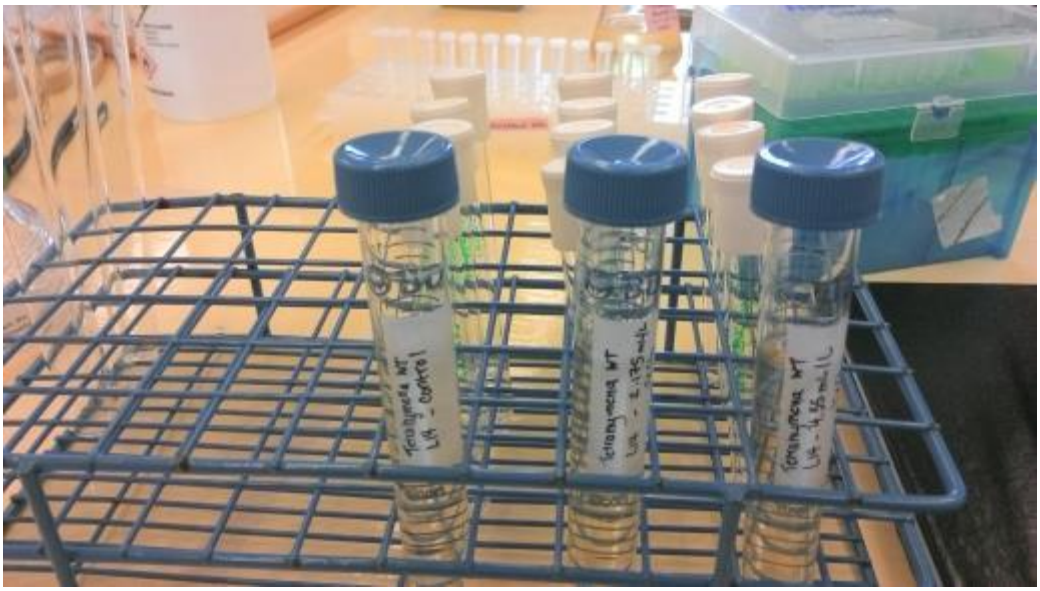

Figure 2. 10 mL of each of the three treatments in 15 mL test tubes. (Left to right: control, 2.175mL/L $FeCl_3$, 4.35mL/L $FeCl_3$).

A total of 5 counts, including the initial and final cell counts, were conducted during a 24 hour period. The initial count was conducted at 11:35am on October 30th, and each following count was conducted 3 hours apart with the fourth count conducted at 8:35pm. The final count was performed on October 31st at 11:35am, 16 hours following the previous days count. The counts were all conducted following the same procedure. For every count, a 50 µL sample of cell culture was pipetted from each replicate of each treatment (total 12 replicates) into 500 µL centrifuge tubes and were fixed with 5 µL of fixative as depicted in Figure 3. We then pipetted 15 µL of each of the 12 samples into the haemocytometer and counted all cells that were present, not including the cells that were outside of the grid range. We recorded the cell count from each sample and in order to statistically evaluate these results, we used 95% confidence intervals and percentage differences (details under results). In addition, we noted any observations pertaining to each count including any differences in appearance and size in cells. After each count was complete, all replicates were placed in an incubator maintained at 29

degrees Celsius until the next count was to take place. We assumed the pH of all replicates was relatively constant and did not monitor the pH specifically during every count. *T. thermophila* do not rely on light for any vital processes, so light intensity was not monitored. However, all replicates were exposed to the same light intensity while counts were being conducted as well as during the incubation periods.

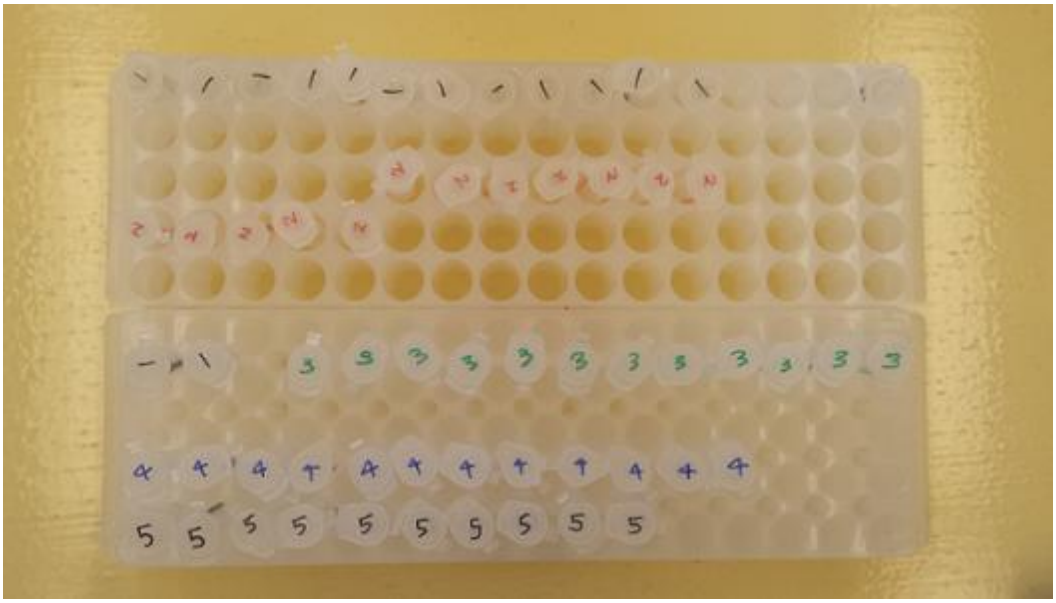

Figure 3. 12 samples (one sample per replicate) for each of the 5 counts in 500µL centrifuge tubes.

Results

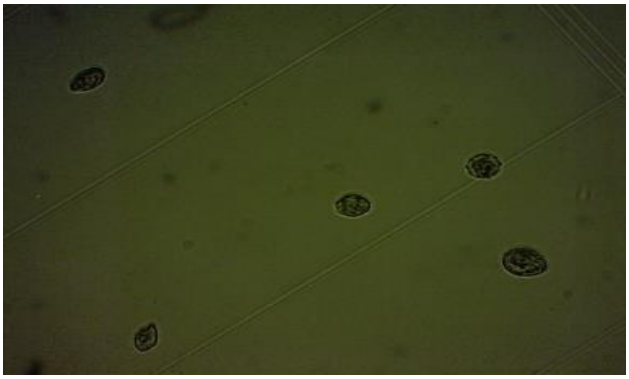

Figure 4. *Tetrahymena* in 0.072 µM $FeCl_3$ after 3 hours

Figure 5. *Tetrahymena* in 0.14 µM $FeCl_3$ after 3 hours

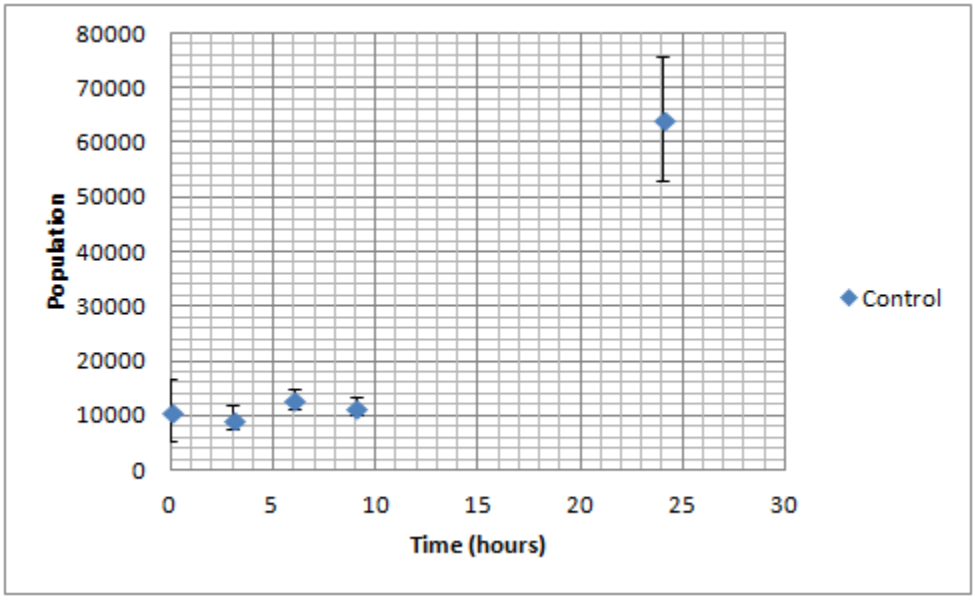

Figure 6. Population of *T. thermophila* in control medium at times 0, 3, 6, 9 and 24 hours. 4 replicates (n = 4) were used in each count. 95% confident intervals represented as the error bars.

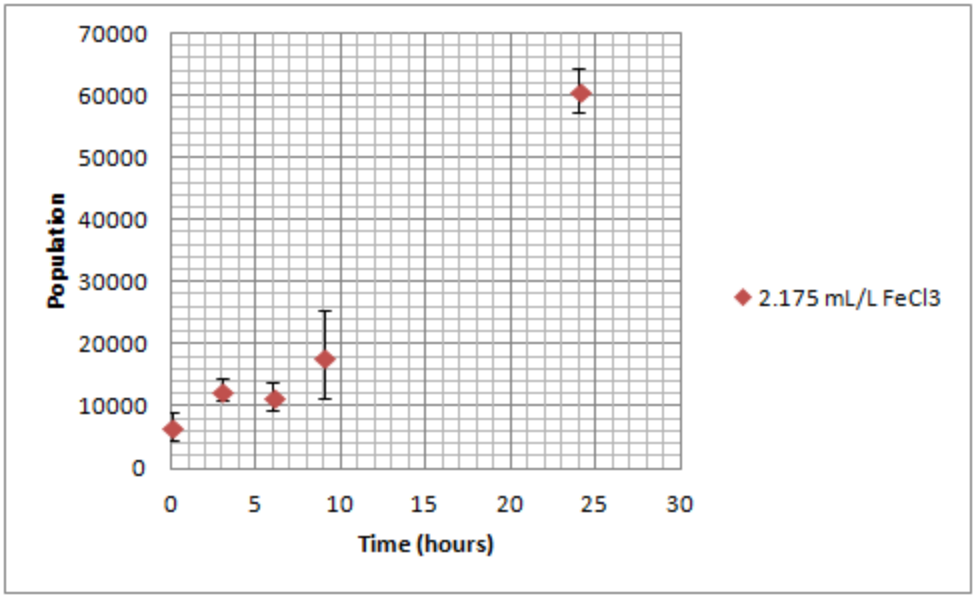

Figure 7. Population of *T. thermophila* in 2.175 mL/L $FeCl_3$ medium at times 0, 3, 6, 9 and 24 hours. 4 replicates (n = 4) were used in each count. 95% confident intervals represented as the error bars.

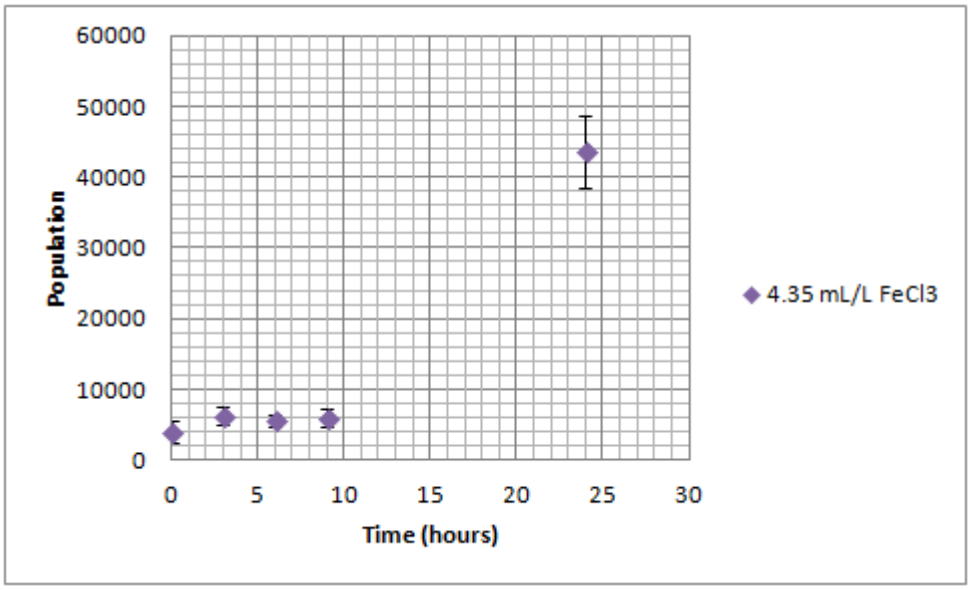

Figure 8. Population of *T. thermophila* in 4.35 mL/L $FeCl_3$ medium at times 0, 3, 6, 9 and 24 hours. 4 replicates (n = 4) were used in each count. 95% confident intervals represented as the error bars.

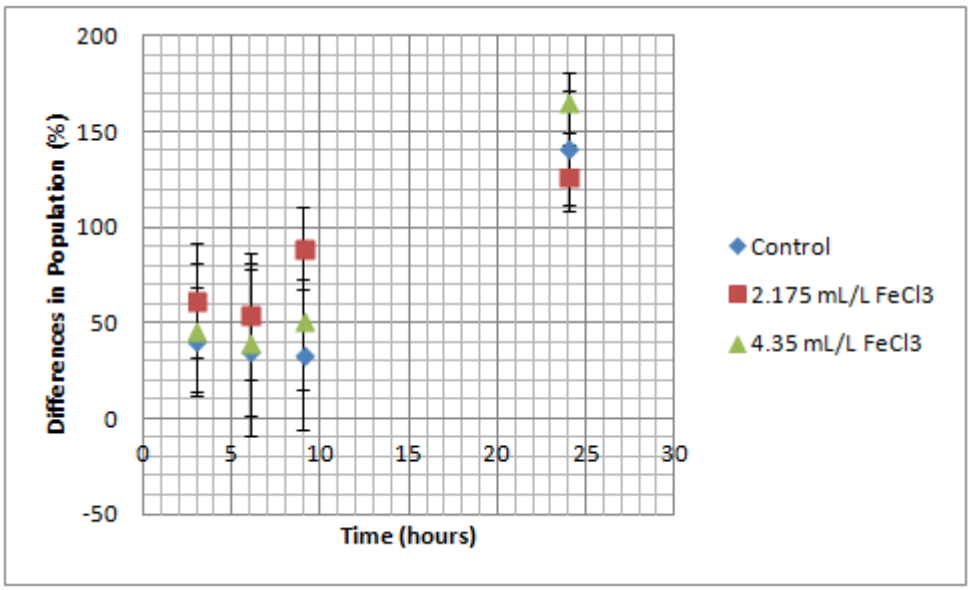

Figure 9. Differences in population of *T. thermophila* in three different media at times 0, 3, 6, 9 and 24 hours. 4 replicates (n = 4) were used in each count. 95% confidence intervals are represented as the error bars.

Sample Calculation

Control group from count #1 (11:35-11:40 AM Wednesday)

Replicate	Cells Counted	Factor	Total Cells
#1	50	343.75	17187.5
#2	29	343.75	9968.75
#3	11	343.75	3781.25
#4	39	343.75	13406.25
Average	32.25	343.75	11085.9375

Control group from count #5 (11:35-11:40 AM Thursday)

Replicate	Cells Counted	Factor	Total Cells
#1	183	343.75	62906.25
#2	233	343.75	80093.75
#3	181	343.75	62218.75
#4	151	343.75	51906.25
Average	187	343.75	64281.25

Total Cells = Cells counted * Factor (Constant) * Fixative factor
Ex) Replicate #1
62906.25 = 183 * 343.75 * 1.1

Confidence Interval Calculation
Variance = $[(62906.25)^2 + (80093.75)^2 + (62218.75)^2 + (51906.25)^2] / 64281.25 = 136440107.5$
Standard deviation = $\text{Variance}^{½} = 11680.76$
CI = Average +/- 1.96*SD/2 = 11447.14
Highest value = 64281.25 + 11447.14 = 75728.39
Lowest value = 64281.25 - 11447.14 = 52834.11

% Difference in Population Compared to the Initial Population
Taking Control Count 1 and Count 5 for example,
There were 4 replicates in each count.
For each replicate the % difference in population from the initial population count was calculated:

% Difference in Population = [|Population in Count 5 - Population in Count 1| / (Average Population Between Count 1 and Count 5)] * 100%
For Replicate 1:
% Difference in Population = [|62906.25 - 17181.5|] / ([62906.25 + 17181.5] / 2) * 100%
% Difference in Population = 114.16%

Using the same calculation method,
Replicate 2 % Difference in Population = 155.73%
Replicate 3 % Difference in Population = 177.08%
Replicate 4 % Difference in Population = 117.89%

Average % Difference in Population = (Replicate 1 % Difference in Population + Replicate 2 % Difference in Population + Replicate 3 % Difference in Population + Replicate 4 % Difference in Population) / 4
Average % Difference in Population = 141.22%

For the error calculation of average % difference in population, same calculation method was used as “Confidence Interval Calculation”.

Description

From the data obtained, we observed an increase in the population of *T. thermophila* for all three treatments over the 24 hour time span. Figure 6 shows the *T. thermophila* control

population grown in the 0.024 µM $FeCl_3$ medium. Although increasing population trend was observed in first 6 hours of growth, overlap in 95% confident interval indicate that the changes were not significant. However, a significant population change was observed after 24 hours of growth. Figures 7 and 8 show *T. thermophila* population growth when they were cultured in 0.072 µM $FeCl_3$ and 0.14 µM $FeCl_3$ respectively. The results of all three treatments depicted an increase in population for the first 6 hours, and a significant increase in the population at the 24 hour mark. Figure 9 compares percent differences for all three populations. After 24 hours, the 0.14 µM $FeCl_3$ treatment showed the largest population growth and the 0.072 µM $FeCl_3$ showed the smallest population growth.

Discussion

In our experiment, we failed to reject the null hypothesis of an increase in $FeCl_3$ concentration decreasing or having no effect on the abundance of *Tetrahymena*. This is due to all of the confidence intervals overlapping when analyzing the percentage differences between different treatments at each time count. There is still, however, a general trend of increasing average population difference with increased $FeCl_3$ concentration when compared to the control treatment after 24 hours have passed.

Overall, no significant growth was seen in the samples during the initial 8 hours, most likely due to the fact that added iron supplements show little to no effect during the initial 24 hours of growth (Shug *et al.* 1969). This is further supported by the idea that *Tetrahymena* grow in a particular pattern, where there is a short lag phase followed by a sudden exponential

growth (Peng and Elson 1971). This lag phase is most likely what accounted for the slow growth and what appeared to be an insignificant difference in population counts.

The control utilized SSP media at its regular stock concentration of 0.024 μM $FeCl_3$. Although the treatment with 0.14 μM $FeCl_3$ is six times the normal concentration, we do not see any poisoning of cells to the point of death. Although the exact mechanism is not fully understood yet, this is possibly through the work of a protein (e.g. metallothionein) that is able to bind to heavy metals like iron and aid in the detoxification process, much like what we see in the human body (Rasmussen *et al.* 1984).

Even though the treatment with 0.14 μM $FeCl_3$ had the highest average percentage difference from its initial value, the 0.072 μM $FeCl_3$ treatment showed the most progress in terms of growth in its population during the first 9 hours. This is most likely due to the phenomena displayed by *Tetrahymena* in which they are able to adapt physiologically to different media and different concentrations of iron that they are grown in (Rasmussen *et al.* 1984). This means that despite the fact the initial population of cells were taken from a culture grown in SSP media, there is nothing limiting *Tetrahymena* in growing to its full potential when transferred to a new and different medium. It should be expected that the more drastic the change in iron is however, the longer the cells should take to adapt.

The experiment was naturally limited by the errors performed during its execution. A major error was that the rack of samples was not returned to the incubator between Count 3 and 4. Typical cultures grown in the laboratory are kept at temperatures between 27 and 32°C for optimal growth (Cassidy-Hanley 2012). After being left out for 3 hours however, samples were cool to the touch, which meant a significant temperature drop from the samples kept in

the incubator. This could have impacted the results as an approximate 5-10°C drop in temperature occurred and in turn could have decreased the doubling rate of the organism.

The isolation process of cells for transfer into new media was also a major source of error. We originally planned to have the same initial population for all three treatments; instead, we saw a wide range of numbers during our initial count. For one, *Tetrahymena* are strong swimmers, and centrifugation of the samples for 10 minutes was just barely enough to hold the cells into a pellet (Cassidy-Hanley 2012). We did not centrifuge the samples for a third cycle to avoid unnecessary cell death, and this could have resulted in a considerable amount of cells being lost from the tubes when the supernatant was discarded. Different initial counts could have impacted the growth rate of the cells because depending on the number of cells present, it could be in the middle of a lag phase, a growth phase, or a plateau region where growth has ceased (Peng and Elson 1971). This could be why we saw population counts stay unchanging between two time counts of one treatment and increasing for another treatment.

Tetrahymena are also quite sensitive to their environment, especially when left in a pellet for longer periods of time (a few minutes), and prolonged exposure can result in cell death (Cassidy-Hanley 2012). When we re-suspended the cells into their new media, each treatment took relatively 5 minutes to prepare. While preparing the control treatment, the other two treatments were left exposed, and hence could have resulted in some cell death. While preparing the first treatment, the second treatment was exposed for a longer duration of time, and hence could have suffered even greater losses. This could explain why the number of cells that we started off with in each treatment steadily decreased from what we recorded in

the control, although each tube that came out of the centrifuge theoretically should have had the same number of cells.

Further studies which look at the organism over a 72 hour period would have to be conducted in the future to provide a more in-depth and accurate look at the trends in growth rate over its full growth cycle.

Conclusion

Iron has been shown to be an important aspect of the chemical media *Tetrahymena* are grown in, and the experiment strived to show how the abundance of *T. thermophila* would change given that its growth medium concentrations were changed as well. Although no statistically significant data arose from our findings, a general trend of increased population with increased $FeCl_3$ after 24 hours was observed.

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Literature Cited

Asai, D.J. and Forney, J.D. 2000. *Tetrahymena thermophilia*. Methods in Cell Biology **62**: 127-186

Cassidy-Hanley, D.M. 2012. Methods in Cell Biology. Academic Press, San Diego, CA.

Conner, R.L. and Cline, S.G. 2007. Iron deficiency and the metabolism of *Tetrahymena pyriformis*. The journal of eukaryotic microbiology, **11**(4): 486-491

Hanley, D.M. 2013. *Tetrahymena* in the Laboratory: Strain resources, methods for culture, maintenance, and storage. Method Cell Biology, **109**: 237-276

Hoffman, E. and Cleffman, G. 1981. External factors limiting the multiplication potential of *Tetrahymena*. Journal of Cell Science, **50**:407-418

Kludt, T.A. and Rasmussen, L. 1970. Particulate material as a prerequisite for rapid cell multiplication in *Tetrahymena* cultures. Biological Institute of the Carlsberg Foundation, **59**(3): 456-463

Nozawa, T. et al. 1981. Changes in hemoprotein content in *Tetrahymena pyriformis* (Strain NT-1) as studied by low temperature spectroscopy. Journal of Biochemistry, **91**(2): 487-496.

Peng, Y.M. and Elson, C.E. 1971. Effect of iron on lipid metabolism of *Tetrahymena pyriformis*. Journal of Nutrition **101**(9): 1177.

Rasmussen, L., Toftlund, H., and Suhr-Jessen, P. 1984. *Tetrahymena.* Adaptation to high iron. Experimental Cell Research **153**(1): 236-239.

Shug, A.L., Elson, C., and Shrago, E. 1969. Effect of iron on growth, cytochromes, glycogen and fatty acids of *Tetrahymena pyriformis*. Journal of Nutrition **99**(3): 379.

Wolfe, A., Meal, K.J., and Soiffer, R. 2005. Limiting conditions for conjugation in *Tetrahymena*: Cellular development and factor active in conjucation. Journal of Experimental Zoology, **212**(1): 37-46