Effect of Ammonium Ion Concentrations on *Tetrahymena thermophila* Growth

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Abstract

Following action against an ammonia spill into a tributary of the Fraser River by CIMCO Refrigeration and the University of British Columbia, this study looks to characterize the effect of varying ammonium ion concentrations on the growth rate of the protist, *Tetrahymena thermophila. T. thermophila* populations were placed in 4 cell cultures of differing ammonium ion concentrations (0, 5, 50, and 500 mg/L) and allowed to incubate for 24 hours at 35^{o}C, respectively. These treatments were fixed and the cells were counted at intervals of 0, 2, 4, 6, 8 and 24 hours, in order to determine their growth rate. Kruskal-Wallis rank sum test showed that ammonium ions do not impact *T. thermophila* growth rate ($p = 0.07487$), with post-hoc tests all showing a p-value > 0.05. Our results were inconclusive on whether the growth rate of *T. thermophila* is directly impacted by differing ammonium ion concentrations, thus more research must be done in order to come to a definite conclusion.

Introduction

Ammonia is commonly found in the aquatic environment as it is naturally excreted by many plants and animals, however many human processes such as industrial emissions and the run-off of fertilizers can significantly increase concentrations in the water that are above the natural background level (Randall & Tsui, 2002). This can be a major concern as high concentrations of ammonia in the water has shown to be toxic for organisms such as fish (Randall & Tsui, 2002). Recently, the University of British Columbia (UBC) and CIMCO Refrigeration were fined 1.2 million dollars for leaking ammonia into a storm drain leading to

Booming Ground Creek, a tributary of the Fraser River (Climate Change Canada, 2019). Approximately 70 fish were found dead in the creek after two days and as a consequence, UBC and CIMCO have been placed on the Environmental Offenders Registry (Climate Change Canada, 2019). This event further confirms that ammonia can have major impacts on aquatic organisms such as fish, however studies on its exact effects are limited, especially for protists such as *Tetrahymena thermophila* that inhabit the salmon-containing ecosystems of British Columbia (Cheng et al., 2019). Protists such as *T. thermophila* are integral parts of freshwater ecosystems, therefore studying the effects of chemical spills such as the one that occurred at Booming Ground Creek would help us understand the environmental impact of chemicals on a variety of organisms, and subsequently allow us to better maintain our local freshwater ecosystems (Pratt & Cairns, 1985).

This study is designed to determine the effects of varying ammonium concentrations on the growth rate of *T. thermophila*. Previous studies have implied that ammonium ions can impact the growth rates of *T. thermophila*, thus we hypothesized that ammonium ions will have an effect on *T. thermophila* growth rates (Larsen et al., 1988). Based on the outcomes of the ammonia spill mentioned above, we predict that ammonium ions will be toxic to *T. thermophila* and decrease their growth rates, especially at higher concentrations. Mortimer et al. (2007) and Klimek et al. (2012) also used *T. thermophila* to test for toxicity and our study was based on their findings, in regards to the procedures used to test for ammonium ion toxicity.

Methods

Varying Ammonium Concentrations and Control

While we were unable to find any studies on ammonia's effect specifically on *T. thermophila*, we did find studies on its effects on similar protists; showing that concentrations ranging from 5-500 mg/L were significantly detrimental to protist growth (Klimek et. al., 2012). Therefore, we used concentrations of 5, 50, and 500 mg/L to use in our population samples.

Tetrahymena Culture

Tetrahymena culture media used was an SSP medium consisting of 2% Proteose Peptone, 0.1% yeast extract, 0.2% glucose, and 33 μM $FeCl_3$ (Gorovsky et al., 1975). Our ammonium ion media was created by adding NH_4Cl to the 20 mL of culture, such that we would have 1000 mg/L of NH_4^+ in that 20 mL of culture.

Diluting Tetrahymena Cultures

T. thermophila populations were cultured to stationary phase and provided to us by lab technicians here at UBC. Populations were measured in 2 hour intervals, as this is the doubling time of *T. thermophila* in ideal conditions (Cassidy-Hanley, 2012). The *T. thermophila* cultures obtained from the lab technicians were diluted to 1/8th concentration using the formula $C_1V_1 = C_2V_2$, in order to ensure *T. thermophila* populations were in exponential growth phase during the experiment.

The 1/8th concentration *T. thermophila* solution was then diluted again by placing 2 mL into 4 test tubes (4 treatments, 5 replicates each, total 20 tubes), with 2 mL of standard culture media/ammonium-containing culture media added for a total volume of 4 mL (refer to Fig. 1).

Thus, the initial concentrations of *T. thermophila* are 1/16th that of the stationary phase we received.

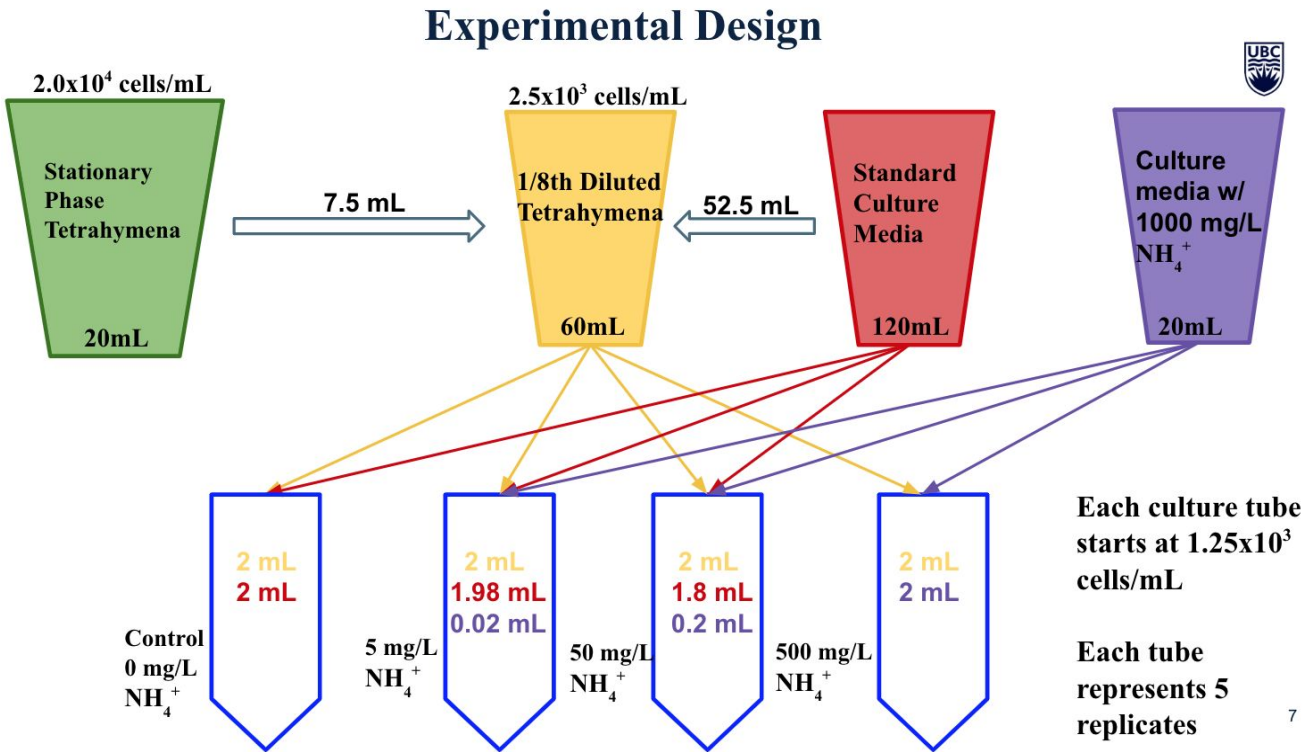

Figure 1. Diagram of initial setup before starting to incubate *T. Thermophila* at 35°C

Growing *T. thermophila* Cultures

T. thermophila samples were put into an incubator at 35°C, as optimal doubling occurs at this temperature (Cassidy-Hanley, 2012).

Cell Counts

To count cells, 100 uL from each test tube was fixed with 10 uL of fixative solution. Cell counts were done with a haemocytometer and a compound microscope. 20 uL of the fixed solution was put onto a haemocytometer 4 times (4 measurements of each replicate) and an average was taken to determine the cell count of each replicate.

Data Analysis

Using R for all our data analysis, we took the logarithm (base 10) of our cell counts and used linear regression on each replicate to determine their growth rate. Once we obtained the growth rates of each replicate, we compared the means of the treatments by using a Kruskal-Wallis rank sum test against our hypothesis.

Results

In figure 2, the mean growth rate of each treatment level was plotted on a bar graph with error bars representing each treatment standard error. The dots on the graph are the calculated values of each replicate in the treatment. The spread of our individual data points within treatment levels varied and did not seem to follow a normal distribution. For this reason, we used the Kruskal-Wallis test instead of a One-Way ANOVA as it does not require our sample to be normally distributed. Testing our null hypothesis that ammonium ions do not affect the growth rate of *T. thermophila*, our Kruskal-Wallis analysis run using R gave us $\chi^2 = 6.9086$, df = 3 and p-value = $0.07487 > 0.05 = \alpha$. With our p-value larger than α, we are unable to reject our null hypothesis.

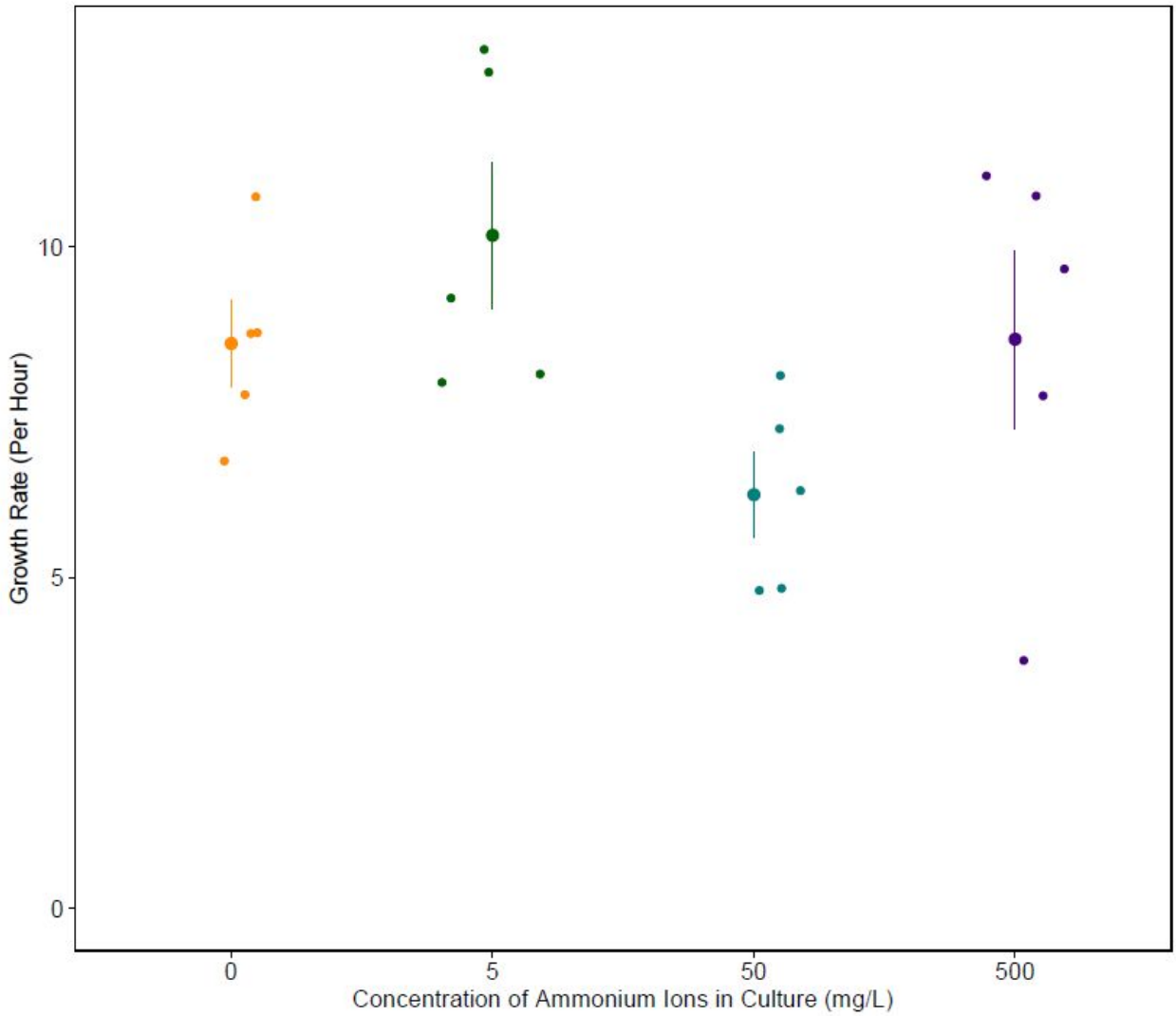

Figure 2. Error plot of mean growth rates per treatment level (n = 5). Error bars show standard error from the mean and the dot is the calculated growth rate of each replicate in that treatment level. Control mean was 8.541 hr^{-1} (SE = 0.66 hr^{-1}), at 5 mg/L mean was 10.178 hr^{-1} (SE = 1.10 hr^{-1}), 50 mg/L mean was 6.256 hr^{-1} (SE = 0.64 hr^{-1}) and 500 mg/L mean was 8.606 hr^{-1} (SE = 1.34 hr^{-1}). Kruskal-Wallis sum test gave us a p-value = 0.07487 ($\chi^2 = 6.9086$, df = 3).

Discussion

It was found that varying concentrations of ammonium ions had no effect on *T. thermophila* growth rates, which was further confirmed by the Kruskal-Wallis test that gave us a p-value = 0.07487 > 0.05 = α. Thus, we failed to reject the null hypothesis which stated that

ammonium ions has no effect on the growth of *T. thermophila* populations. Our results are not consistent with our predictions, as we predicted that ammonium ions would be toxic to *T. thermophila* and decrease their growth rates. This prediction was based on a previous study that showed the toxic effects of ammonium ions on other protists, however this literature does not seem to support our findings (Klimek et al., 2012). There was a previous study which had similar results to ours testing *T. thermophila* growth in the presence of 17mM ammonium, which found no significant difference in growth rate (Larsen et. al., 1988). However, ammonium concentration in relation to growth wasn't the focus of that study, and 17mM is a relatively low concentration, so our study collected better data on the subject. The similar results could suggest validity in our data. However, final populations negatively correlating with ammonia levels 0 > 5 > 50 > 500 mg/L suggests ammonium does have an effect on growth in some capacity; further research would likely confirm. Despite this, it is difficult to come to a definite conclusion based off our results as there were many experimental limitations and sources of error during our study, which could have significantly impacted our results.

The most obvious source of error in our experiment is experimental fatigue. Our experiment was conducted in two sessions, with the first session lasting approximately 10 hours. Fatigue acquired during the experiment cannot be ignored, as it likely increased the chance of human error when performing our experiment. To account for this, three researchers took turns observing and recording data. During cell counting, some contamination was observed in the form of fibrous plant matter, which could have affected cell growth and cell counts.

In addition, while using the haemocytometer with our small initial cell counts, it was difficult to obtain consistent counts for each fixed samples. This was mitigated by taking more

than one measurement per sampling, however we think this may have contributed to some error.

Future experiments could utilize a greater sample size, increasing the number of replicates from 5 to 30. This would lower the standard error of the means of our graph in figure 2, which would provide better statistics and allow us to be more confident with our results. Furthermore, increasing the ammonium ion concentrations from 0 - 500 mg/L to 0 - 1000 mg/L would likely have a more significant impact on the growth rate of *T. thermophila*. Finally, our experiment had cell counts done in 2 hour intervals from 0 to 8 hours, and a final count at 24 hours. Redoing a similar experiment utilizing cell counts in 2 hour intervals from 0 to 24 hours would yield more consistent data and better showcase patterns in the data.

Other sources of ammonia or ammonium ions, such as refrigerant anhydrous ammonia could also be used to directly test how *T. thermophila* cope with common environmental disasters (Climate Change Canada, 2019).

Conclusion

Our results show that increases in ammonium ion concentrations had no significant effect on the growth rate of *T. thermophila*, which is shown by the obtained a p-value ($p = 0.07487$). Thus, we were unable to reject our null hypothesis which stated that ammonium ions will not have an effect on *T. thermophila* growth. Despite this, more research must be conducted on the effects of ammonium ions on different aquatic organisms, as it is clear that it can have a big impact on organisms such as fish, which is demonstrated by the death of 70 fish as a direct consequence to the ammonia spilled by UBC and CIMCO refrigeration. Future research should include increasing the number of replicates measured, the number of measurements and ammonium ion concentration as well as utilizing different kinds of ammonium ion sources such

as refrigerant anhydrous ammonia.

Acknowledgements

We would first like to thank our instructor Jordan Hamden and our teaching assistant Tessa Blanchard for supervising this study and providing us with crucial feedback throughout our research project. We would also like to thank the lab technicians Mindy Chow and Chanelle Chow for growing our *T. thermophila* cultures and providing us with all the equipment needed to perform our experiment. Finally, we would like to thank the University of British Columbia for funding our research.

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