BIOL342 L14
Final Report
Cameron Carvalho, Chesley Chow, Jonathan Kraft, Morgan Strohan

Growth Rate of *Tetrahymena thermophila*: Does progressive increases in incubation temperature result in a greater ability for *T. thermophila* to adapt to temperatures outside literature ranges for tolerance

Abstract

As a result of climate change, global temperatures are rising which have the potential to notably impact all ecosystems and the future of biodiversity (Bellard et al, 2012). *Tetrahymena thermophila* is a ciliated protozoan that can be found in freshwater (Cassidy-Hanley, 2012) and is one of the many species that may be impacted by increasing water temperatures. This investigated whether incremental increases in temperature could result in a *T. thermophila* culture surviving beyond the literatures cut-off of 41◦C (Frankel & Nelson, 2001). *T. thermophila* were incubated at 42◦C, 40◦C, and 35◦C over the course of four days, after the stock solution was initially diluted with SSP growth medium. After 44 hours of incubation, three samples from each temperature were collected and counted every two hours. A total of eight counts were made for each sample to generate a growth curve. Parallel to these controls that were in the same incubation temperature for the entirety of the experiment, a test group was moved step wise into warmer incubators each day with the growth curve collection being taken once the sample was in the 42◦C incubator. A two-way ANOVA test was performed, confirming temperature and time had a significant impact on the cell counts . A Tukey-Kramer post-hoc further revealed that the test group that had moved through the temperatures had no significant difference in cell counts from the control at the same temperature. The elevated temperatures were found to have a significantly adverse effect on growth rates and the incremental increase showed to have no significant advantage for the *T. thermophila*.

Introduction

Tetrahymena thermophila is a widely studied, model sexual organism due to its short life cycle and ease of handling with various laboratory techniques and procedures, as well as cost-effectiveness (Ruehle et al, 2016). They are unicellular, ciliated eukaryotes that can be found in freshwater over a wide range of conditions (Ruehle et al, 2016). Populations have a relatively fast doubling time, typically 2-3 hours under optimal conditions, and their large

30-50 µm size make them ideal for viewing under microscopes (Ruehle et al, 2016). As Tetrahymena reside in freshwater ecosystems, they undoubtedly come in contact with a variety of other organisms, from fish to other invertebrates and animals. Additionally, its ciliated morphology provide motility and *Tetrahymena* can exhibit parasitic qualities to varying degrees depending on the species (Pinheiro et al, 2015). Pinheiro et al (2015) were able to show that *T. thermophila,* when grown in co-culture with fish cells, are effective at destroying cell lines from a variety of fish species and tissues via a mechanism that seems to involve phagocytosis. Although these results are compelling in vitro, *T. thermophila* is not usually considered a fish pathogen. However, since *T. thermophila* feed on bacteria, which in turn are eaten by zooplankton, a food source for salmon, this illustrates a pathway by which the presence of Tetrahymena could influence the ability of salmon to find food.

T. thermophila exhibit optimal growth at a temperature around 35°C, with a doubling time of approximately 2 hours (Frankel & Nelsen, 2001). Increasing the temperature to ~39°C still results in an exponential population growth response, although slightly slower than at 35°C (Frankel & Nelsen, 2001). Most of the literature that deal with subjecting Tetrahymena to supraoptimal temperatures have shown that the upper limit for temperature that can still sustain population growth is around 40-41°C (Frankel & Nelsen, 2001).

Studies from previous BIOL342 classes, all of which can be found in The Expedition, have investigated different associations between *T. thermophila* and temperature. Acharya et al (2018) conducted their study on population growth rates directly relating to temperature, while Bhullar et al (2017) observed the effect of temperature on vacuole formation. As there are already a multitude of existing studies conducted within the optimal temperature range of *T. thermophila* at around 35°C*,* our study attempts to acclimate the Tetrahymena species to grow outside of this range. As *T. thermophila* seem to be the most resilient at higher temperature, they are prime candidates for an investigation of this nature. Frankel et al (2011) even discuss the presence of "heat shock proteins" *T. thermophila* produce when subjected to temperatures near the upper end of its heat tolerance range that help the organism adjust to these extremes.

For conducting the experiment there are three null hypotheses ($H_0$1, $H_0$2, $H_0$3) and three alternative hypotheses (H_A1, H_A2, H_A3). The three null hypotheses are as follows: the mean of the cell counts grouped by time are the same ($H_0$1), the mean of cell counts grouped by temperature are the same ($H_0$2), and there is no interaction between temperature and time for the cell counts ($H_0$3). The three alternative hypotheses are: the mean of the cell counts grouped by time are not the same (H_A1), the mean of the cell counts grouped by temperature are not the same

(H_A2), and there is an interaction between temperature and time for the cell counts (H_A3). Finally, the prediction is that for the incubation temperature of 42°C, the treatment group (TG) will survive better than the control group; this will be measured through cell counts.

Methodology

i) Culture Preparation

A previously cultured batch of *T. thermophila* (batch 2086) was acquired in a 50mL erlenmeyer sterile flask and brought to our work-space that had been prepared as a sterile environment. This environment was created through a complete wipedown of the work bench, with 70% ethanol, a flame was also maintained at all times to ensure the sterility of the field was maintained. All researchers wore appropriate lab equipment and sterile gloves ensuring washing with ethanol when contamination may have occurred. The culture was first "mixed" through successively drawing 200 microlitres of culture into a micropipette and ejecting it into the flask, creating currents in the flask that mixed the culture. A sample of 100 microlitres was then transferred into an eppendorf tube. Once more, sterile techniques were used throughout the duration of the experiment, where all glass necks were flamed once caps or seals were removed and flamed once more before the cap was put on. Ten microlitres of 3% glutaraldehyde was then micropipetted into the eppendorf as a fixative to immobilize and preserve the *T. thermophila* for examination. The eppendorf was mixed using the same procedure as above and was then plated onto a Fuchs-Rosenthal Hauser Scientific counting chamber. Using an AxioLab A1 Microscope, three counts were completed on this sample using separate counting-squares of the chamber and an average was used to make a concentration determination. This was repeated a second time and once more averaged with the prior count to give an average concentration determined from two different samples with three individual counts of the initial batch. A concentration of 76,799 cells/mL was calculated.

This concentration was input into our custom-made Microsoft excel program dubbed the "dilution program" which provided the dilution necessary to generate a 10mL sample at a concentration of 20,000 cells/mL. For the first dilution, 2.23mL of the stock *T. thermophila* was added into each of four 20mL test tubes. A further 7.77mL of SSP medium was added into each 20mL test tube. After being completed four separate times, the team had acquired four samples of 20,000 cells/mL of equal volumes. These samples were then labelled as C35, C40, C42 and TG along with group identification (note that the "C" identifies the test sample as a control at a specific

temperature and the “TG” is representative of the test group) and subsequently placed into incubators at 35℃ 40℃, 42℃ and 35℃ respectively.

ii) Sample concentration determinations and appropriate dilutions protocol

After 24 hours of incubation, the samples were removed from the incubator. In a sterile field and using sterile laboratory techniques, the samples were mixed using a micropipette and 100 microlitres were transferred to an eppendorf tube where a further 10 microlitres of 3% glutaraldehyde fixative was added. Twenty microlitres was then transferred onto the Fuchs-Rosenthal counting chamber where three concentration counts were made and input into the dilution program. The program averaged the counts, produced a concentration, and based on the desired final volume of 10mL with a concentration of 20,000 cells/mL outputted a volume of the stock to transfer to a new 10mL test tube along with the volume of SSP to makeup the balance. Transfers and dilutions were completed according to the program outputs into labelled test tubes and placed back into the appropriate incubators. Each time counts or dilutions were made, the samples were completed in the order of TG, C42, C40 and C35 to maintain consistency in incubation time. Of note, upon completion of the TG transfer and dilution, it was placed into the 40C incubator on day 2, unlike the controls which were placed into the same incubator they had previously been in. The previous days excess samples were disposed of according to UBC laboratory policies.

iii) Growth curve samples prepared

Following a further 20 hours of incubation the team returned and once more followed the above “Sample concentration determinations and appropriate dilutions protocol”. However, rather than diluting the entire sample into one 20mL test tube, the sample was broken into 3 samples each. Once more using the output from the dilution program (for this iteration, the parameters were set to produce a total volume of 3mL with a concentration of 20,000 cells/mL), the output volume was transferred from the 20mL stock sample into a 6mL test tube and mixed with SSP media that once more made the balance. This resulted in a total of three independent samples for each of the TG, C42, C40, and C35 samples. With all 12 samples procured and labelled, 100 microlitres of each sample was transferred to an appropriately labelled eppendorf tube with 10 microlitres of fixative. The nine control samples (three of each) were then placed in their respective temperature incubators while the TG samples were moved to the 42C incubator. The previously acquired eppendorfs were then mixed and analyzed for concentrations. The concentrations obtained are the initial concentrations of the growth curve.

iv) Establishing the growth curves of the controls and test group

Every two hours following the time the samples were initially placed into the incubators, 100 microlitres were taken from each of the 12 samples and combined with fixative. The group then performed counts on each of the collected samples and recorded. There was a total of six counts performed every two-hours on the first day and a further two counts the following day taken 26 and 28 hours after the initial count respectively. This culminated in eight reference counts for each of the twelve samples being recorded. These reference counts were then utilized as data points in the construction of each samples growth curve which were constructed using 'GraphPad Prism 8' software. The data collected was further run through a two-way ANOVA to determine the significance of our results and a post-hoc Tukey-Kramer test was used in order to further determine which groups when compared with one another were statistically significant.

v) Microsoft Excel Spreadsheet

As our in-lab procedure required the use of many formulae as well as the recording of multiple values for the cell counts, a spreadsheet was created using Microsoft Excel and then copied to Google Sheets so that all group members could access and modify it. The data table required some input values such as cell counts, but outputs for final cell count, volumes of solution or media etc. were programmed with the necessary formula to give the desired value once the required input values were entered. This prevented the need to do any actual math by hand. The spreadsheet was created to reduce the probability of human error as all the necessary formulae for dilutions, cell counts etc. were programmed into the table prior to coming into the lab. This also allowed all of our group members to focus on performing the lab procedure carefully and efficiently as the spreadsheet acted as a master copy, from which all the data could be collected and recorded in our lab notebooks post-lab.

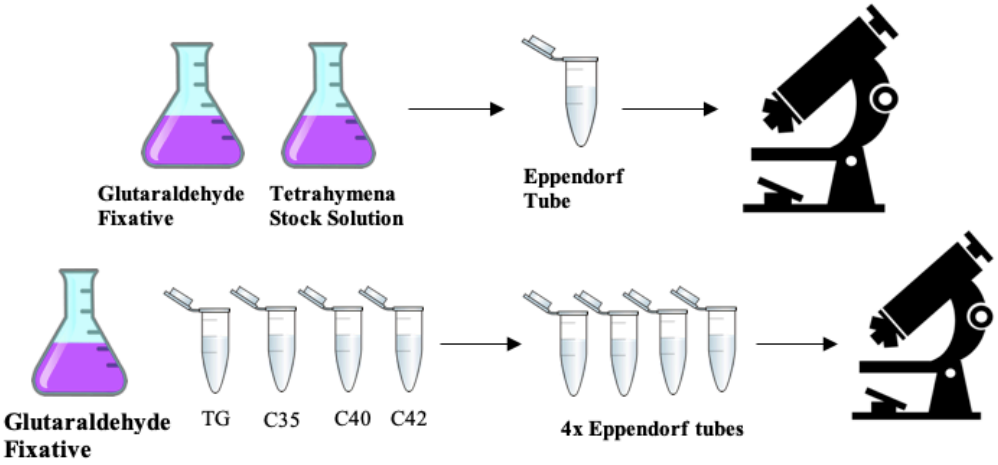

Figure 1. General experimental procedure to determine cell counts for Day 1 (top) and Day 2 to 4 (bottom). 10 microliters of glutaraldehyde fixative and 100 microliters of tetrahymena solution are added to Eppendorf tubes, mixed by pipetting up and down, and then transferred to haemocytometer grid for cell counts.

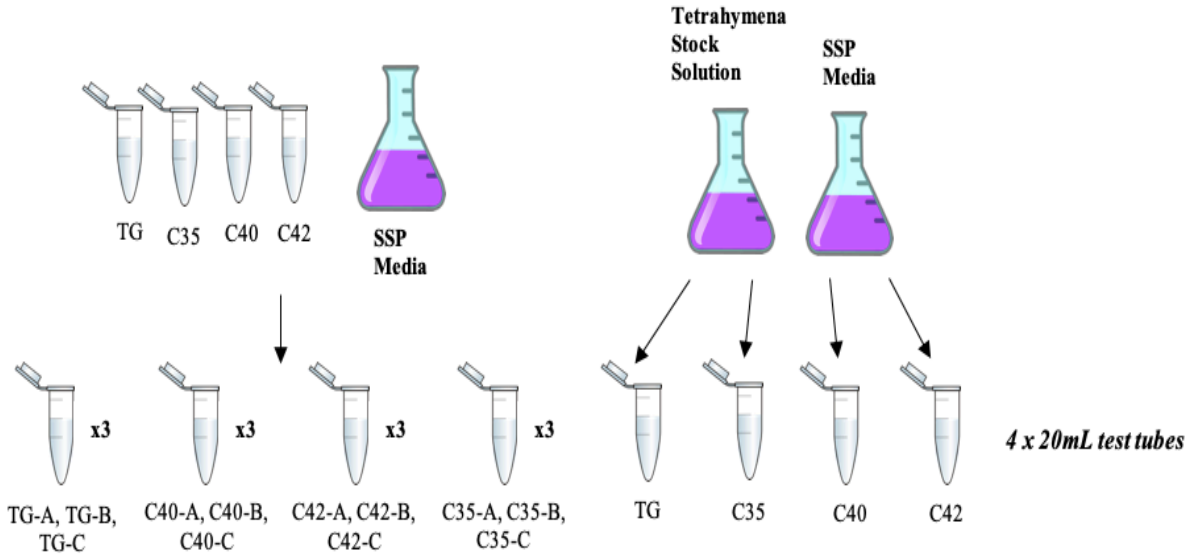

Figure 2. General experimental procedure for creating samples with 20'000 cells mL^{-1} in preparation for incubations. For Days 1 and 2 (right) the total sample volume is 10mL and for Day 2 and 4 (left) the total sample volume is 3mL.

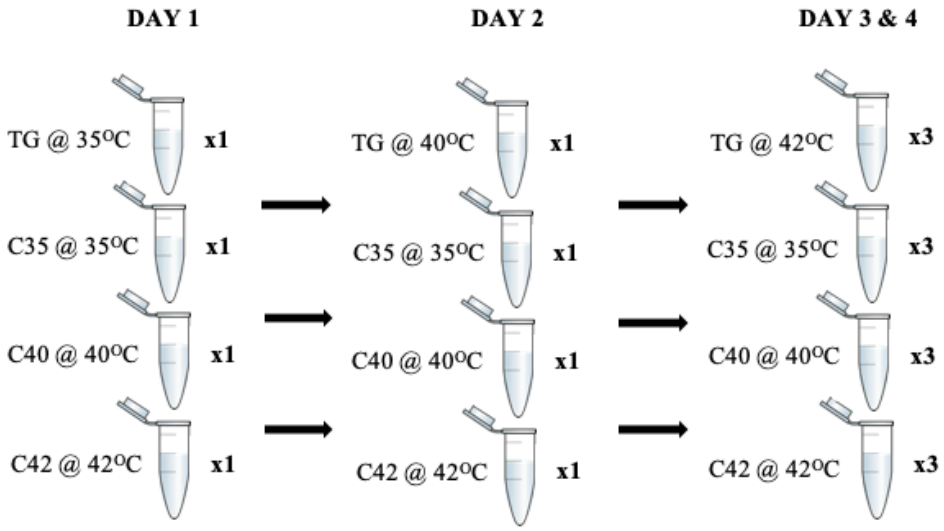

Figure 3. Incubation temperatures used for each day of experiment.

Results

Using 'Graphpad Prism 8' software, we plotted each control group's cell count at each temperature we looked at (35°C, 40°C, 42°C) and the treatment group's cell count over a period of 28 hours to generate a growth curve. Cell counts were taken in 2 hour increments for the first 10 hours, and two final counts made on day 4 at 26 and 28 hours respectively for further elucidation of growth trends (Figure 4). For all groups, there appeared to be a general positive trend upwards until the 26-hour mark. After the 26-hour mark, our TG and C40 and C42 saw a sudden drop in counts. The C35 however, continued its positive growth trend, increasing in cell count until the 28-hour mark when the experiment was concluded.

To test our three hypotheses regarding whether *T. thermophila* can survive outside of their optimal temperature range, we conducted a two-way ANOVA to test the significance of our data. The two variables that were utilized in the two-way ANOVA were temperature and time. Upon running the ANOVA a significant effect was seen when compared across time ($p<0.05$), treatment temperature ($p<0.01$) and further a significant interaction between time and treatment temperature was observed ($p<0.01$). A Tukey-Kramer post-hoc test was used to further elucidate the significant differences between the TG and the controls at the various times. The TG was found to differ significantly from C42 at ROW4 ($p=0.0072$), C35 at ROW5 ($p=0.0449$), C35 at ROW6 ($p=0.0492$) and C35

at ROW8 ($p<0.0001$). However, it did not have significant difference from any of the other groups specifically the C40 and C42 towards the end of the experiment.

Given the results of the ANOVA, all three of the null hypotheses are rejected and the alternative hypotheses are accepted as stated. The cell counts at different temperatures and times are not the same and there is an interaction between temperature and time. The teams prediction that the TG would survive beyond the literatures thermal limit was proven correct, however, there was no significant difference found between the control C42 and the TG.

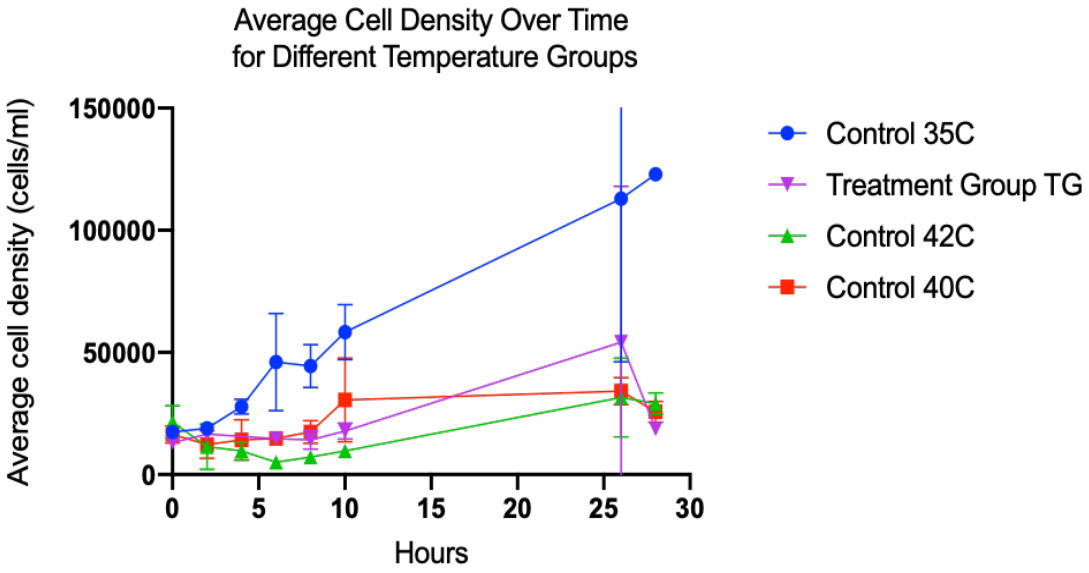

Figure 4. The average cell density (cells/mL) taken for the 3 control groups (35°C, 40°C, 42°C) and the Treatment Group (TG) over a period of 28 hours. Error bars on the data points represent 95% confidence intervals.

Discussion

This project was incepted on the idea of how our local ecosystem will respond to a changing global climate, this ultimately laid the groundwork for our preliminary research into the temperature ranges of *T. thermophila.* Many studies have been conducted on the growth rates of *T. thermophila*, but a knowledge deficit persists in a more realistic scenario where the temperature is incrementally increased with time. This notion formed the foundation of our research project and the team set out to determine whether incremental increases could result in a *T. thermophila* culture surviving beyond the literature cut-off of 41°C (Frankel & Nelsen, 2001). Further, the project sought to shed light on the effects of increasing temperature on the ability to survive when compared to other cultures that have been incubated at a constant temperature for the duration of the experiment. This model for experimentation offers

nuance to the experimentation and while the results speak directly to *T. thermophila*, they also offer the possibility of extrapolating trends on how a species with a limited number of generations will respond to changing environmental temperatures.

i) Results discussed

Upon initial data collection, the Treatment Groups which saw an incremental increase seemed to do slightly better than the Control that had been at 42℃ for the duration of the experiment, however, upon statistical analysis tit was only found to have a significantly higher count at the six hour mark but nowhere else in the observations. Compounding on these results was the control's (C42) survival at 42℃ for the duration of the experiment, which was initially predicted to be unlikely, as the temperature was beyond the ceiling of previously established literature ranges (Frankel & Nelsen, 2001). With the survival of the control (C42) and an insignificant difference between the growth rates and populations as shown by the post-hoc Tukey-Kramer test above, the progressive increases in temperature cannot be said to have a positive impact on the *T. thermophila*'s fitness or ability to survive or thrive in elevated temperatures.

The results do however lend support to the relevance of temperature and time as well as the interaction between the two in the growth rates of *T. thermophila*. The temperatures selected for the experiment (35℃, 40℃, and 42℃) were selected on the basis of available incubators which acted as limitations to the experiment. For the TG to start at 35℃ and increase environmental temperature by five degrees the following day is extreme and well beyond conditions that would be seen in a natural creek setting due to aquifer thermal insulation as discussed by hydrogeologists Anderson Jr., Storniolo, and Rice (2011). This leap in temperature would likely be too extreme for any micro-adaptations made by the *T. thermophila* to have much of a benefit. The following day with another increase of two degrees (though more manageable) was still greater than initially desired. The doubling time of *T. thermophila* being around two hours, allowed for ideally 12 doublings (12 generations) in each incubator before being moved into a warmer incubator (Cassidy-Hanley, 2012). The conceptual idea would be that *T. thermophila* ill-equipped for higher temperatures would preferentially die-off, while the ones better equipped would have any genetic variance owing to this improved fitness passed to the next generation. The following day going into a hotter temperature would repeat the process further refining the selection to the organisms once more equipped for hotter temperatures. However, admittedly the temperature jumps of five and two degrees on day two and three respectively seem too extreme to culture true selection. Also, when the control survives in the upper-most temperature the

question of whether this would provide a better environment for thermal-fitness-selection to occur must be asked. The duration of the experiment also limited the findings, as the growth rate of the TG and C42 never reached the exponential phase, and so it is fair to question whether any traits that allow for success at high temperatures were present in the population.

The results and statistical analysis certainly add further support to the state of knowledge in regards to *T. thermophila*'s optimal temperature range with 35℃ being well within it, allowing for proliferation and exponential growth. While, 40℃ and 42℃ from our experiment are on the threshold of survival. From our results it appeared that at the upper two temperatures tested, the exponential growth phase is no longer a part of the population's life cycle and the culture moves to a more meager survivalist lifestyle holding onto resources opposed to spending the energy and resources to reproduce (Hellung-Larsen, 2005).

ii) Physiological observations of treatments

Following the conclusion of the experiment photographs of individual *T. thermophila* organisms were taken as well as measurements gathered. The results of this showed noticeable changes to the shape, size and features of the microorganisms as seen in *Figure 2*. It was found that on average the C35 organisms were larger with an average size of 52.5 x 25 micrometers, with a distinctly elongated tear shape and visible cilia at a magnification of 1000. Contrastingly, both C42 and the TG had distinct circular shapes and were between 30-42.5 x 30-40 micrometers in size and with variation in whether cilia was visible or not. There are three hypotheses for these differences, first being that the *T. thermophila* in the warmer temperatures were in a "shock" response, contracting from a larger elongated tear into a smaller sphere (Frankel & Nelsen, 2001). Spheres have the highest surface area of all shapes with equal volume, but as previously established, the size and hence volume were significantly less in the spherical organisms. These organisms may have also withdrawn cilia to further limit the exposure to the higher temperatures in the environment surrounding it. The questions that arise from this hypothesis however, is why the organism did not elongate further rather than contracting to attain a greater thermal limiting shapes, rather it seems to have adopted the largest surface area shape while obviously (as evidenced by the growth curves) struggling to manage population growth due to the temperature.

A second hypothesis is that the slower growth of the organisms possibly suggests a slower life-cycle in every aspect including eating, reproduction, movement (as evidenced by reduced or absent cilia) and growth (Hellung-Larsen, 2005). The temperature may have slowed the growth curve so much, that what was seen was a

large number of cells in the post-binary fission phase, where they have split (which could explain the circular shape as well as being just over half the size of the C35) and are in the process of growing, but are much slower in movement and eating and by extension slower in growing. However, once more this demands answers as to whether the increased temperatures are slowing down the life-cycle, or if the size and shape simply point to a less successful organism. A further study elucidating the effects of elevated temperatures on doubling time and specifically on the average amount of time *T. thermophila* cells are alive at different temperatures is critical in rejecting this hypothesis. Specifically research focussing on whether the *T. thermophila* enters a "hibernation" phase and live significantly longer, further lending support to the previously mentioned hypothesis on the shape and size.

The final hypothesis and most supported by our finding is that *T. thermophila* at the higher temperatures were simply outside their range of tolerance, and in an active state of slowing dying off, which would explain the noticeable drop-off in populations at the 28 hour mark (Frankel & Nelsen, 2001). The growth curves certainly were not "normal" for the species, from an observational standpoint they looked like a different species altogether, which certainly would be indicative of functional compromises to the unicellular organism. In summation, the cells observed were simply in a "last-ditch" effort to survive, with little energy or resources being spent on growing or reproducing (once more as evidenced by qualitative observations as well as concentration counts).

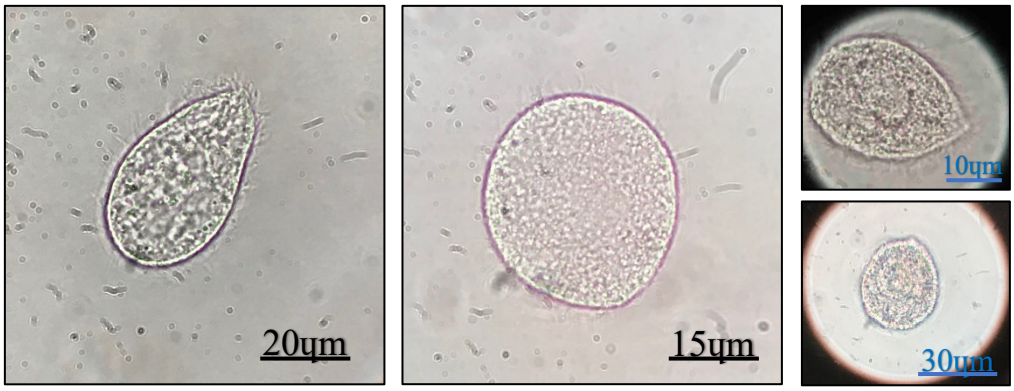

Figure 5. Left-most photo is a photo of the *T.thermophila* from the C35 group at the conclusion of the experiment, notice elongated tear shape and actual size was measured to be 52.5 x 25 micrometers. Middle photo was from the TG at the conclusion of the experiment, notice circular shape and size was 42.5 x 40 micrometers. Top-right is C40 at the conclusion of the experiment, it has a tear shape (medial shape between C35 and TG) and had a measured size of 35 x 30 micrometers. Bottom-right is C42, it had a circular shape, it also did not have noticeable cilia and had the smallest size at 30 x 30 micrometers.

iii) Concentration drop between hours 26 and 28

Another distinct feature of *Figure 4* that must be discussed is the steep drop off between the 26th and 28th hours of the experiment for C40, C42 and TG. This unfortunately is likely due to experimental design flaw. A focus of the setup had been to start all of the test groups at similar concentrations on day three to have a similar starting point for all of the growth curves. However, we also made this dilution on day one and day two, which resulted in a low concentration sample starting each day. This resulted in a situation where C35 which proliferated much quicker than the other test groups received a significantly larger amount of SSP media at the beginning of day three, while the other test groups were at a much lower concentration as their growth rates had been affected by temperature. Often, the concentration was hovering just above 20,000 cells/mL and so a small amount of SSP was added to the new batch made. This oversight may have ultimately led to a depletion of food or an accumulation of waste products from dead *T. thermophila* and the normal metabolic waste. While there was limited growth and it would appear that C40, C42 and TG did not require as much food, it is now impossible to distinguish whether this decision played a role in the results of the experiment. For future studies, pelleting the culture from the old media via centrifugation and adding to fresh media, followed by the dilution would be better, or using a larger batch and adding an appropriate ratio of food to volume each day. However, under the circumstances that this experiment was run under, it is possible that upon reaching the 26th or 28th hour, food became scarce and a decline in population is seen from starvation (Christensen et al, 2001).

A second possibility was that *T. thermophila* could no longer tolerate the temperature following four days of immersion which may have resulted in compromises to cellular function on a micro and macro scale, ultimately resulting in the organism failing to meet the demands required for life in the environment (Christensen et al, 2001). One glaring issue with this explanation is that TG was in the 35°C incubator for the entirety of day one, and was only in the hotter incubators for three days at the time of observed drop-off. The coinciding drop-off event that occurred synchronously with both C40 and C42 does not make sense in the aforementioned explanation as it should have seen the drop-off on day five.

The final possibility is that there was human error in the counts for this hour, while this certainly is the least ideal of the possibilities as it holds no useful information, it remains a possibility no less.

iv) Sources of Error and Variation

One of the sources of variation we encountered were non-uniform hours of counting. In order to attain our growth curve, we conducted cell counts every two hours consecutively for a total of 10 hours. Due to restricted hours in the lab, we could not continue counting and had to come in the following day (16 hours later). During this time, a lot of changes may have occurred with the samples and lent insight into what led to the drop-off in three of the groups between the 26th and 28th hour. However, all was done to minimize this and such limitations will persist in a non- 24 hour lab.

One of the largest sources of error briefly mentioned above was the reality of four different members of the group making counts throughout the experiment. The team's composition of aspiring yet novice scientists results in an inconsistency in the standard. Though strict counting procedures were established, in the interest of time, there was no accountability measures in place, such as having two different members collecting a count on each sample. When abnormal counts were identified that simply did not make sense given a doubling time of two hours, a second count was taken and kept every time (ie. the numbers were not cherry-picked, once a recount was decided, the numbers were kept regardless of fit within the data). Special care was taken to ensure a good mix during recounts as often the count was far too high or far too low if the eppendorf containing the fixative and sample was not well mixed. The research teams suggestion on future studies is to have a second count made by a second member, which could be averaged, further ensuring more accurate results.

v) Impact of research and meaning for BC salmon populations

As *T. thermophila* utilizes the same food source as salmon, their growth or decline will undoubtedly have an impact on salmon populations (Pinheiro & Bols, 2018). As an increase in temperature requires more metabolic activity from *T. thermophila,* that in turn requires them to have more food (Cassidy-Hanley, 2012). *T. thermophila* require bacteria/zooplankton as their main source of food (Cassidy-Hanley, 2012). This is the same for salmon. If temperatures were to increase it would require *T. thermophila* to have more food which could mean less food for salmon (Cassidy-Hanley, 2012). Another point to note is that in our experiment, although *T. thermophila* are 'heat loving' they do best at around 37.5°C (Frankel & Nelsen, 2001). With current climate change occurring, waters can reach abnormal temperatures-either too hot or too cold from the norm. It is unlikely today that waters where *T. thermophila* grow along with salmon will ever reach above 37.5°C but waters could also drop to temperatures colder than normal due to climate change. This could undoubtedly affect both *T. thermophila* and Salmon as previous

studies have shown that at temperatures around 20°C and lower *T. thermophila* does not grow well and just dies off (Acharya et al, 2018) This could result in a lack of *T. thermophila* which could affect bacterial diversity and diversity of zooplankton that Salmon feed on (Pinheiro & Bols, 2018). Specifically *T. thermophila* can act as 'clean-up' organisms and ingest cell debris through phagocytosis and even potential viruses (Pinheiro & Bols, 2018). If there is a lack of *T. thermophila,* we can predict that more cell debris and viruses could be picked up by other organisms such as Salmon and this could impact the livelihood of the species.

vi) Looking forward

The results from this experiment though conclusive in the parameters they were run in leave many questions to be further explored. The jump as previously mentioned from 35ºC to 40ºC and then finally to 42ºC was too large to give the TG any significantly better chance to adapt over more generations than the controls. Future experiments of similar design should look to find a more meaningful change in temperature than what is easily accessible; one such idea would be to find the hypothesized changes in global temperatures over the next 50 years and use a rate that better mimics this.

Further, the fitness of the TG (through progressive thermal increases) remains an important question, the control survived the temperature that had initially been predicted outside of the range of tolerance, so it remains unclear if the thermal increases gave the TG a better or worse chance, they simply had no significant difference throughout a majority of the experiment. This however, does not speak to robustness or ability of the remaining cells. A way to explore this would be to set the maximum temperature higher than 42ºC to ensure the upper control is killed, and to observe whether the successive thermal increases permit the *T. thermophila* to survive beyond the control that died off. Studies of similar design should also reach beyond twenty-eight hours to perhaps elucidate the phenomena that is seen in the twilight data points. Another study would be to test whether decreasing the temperature would have similar effects to this experiment. Research is also needed to further connect these findings and the anatomical changes observed with salmon eating habits and whether strains that are subjected to extreme heat such as the TG would be any different in regard to their interactions in the wild, both eating and living practices.

Conclusion

The results found in our experiment confirms that *T. thermophila* are able to survive outside of the literatures limit of 41°C. The null hypotheses were rejected, which supports the alternative hypotheses that temperature and time have an effect on the cell counts of *T. thermophila* and that there is a relationship between temperature and time in this experiment. Upon completion of the growth curves and further statistical analysis, the control at 35°C was found to significantly differ in concentration of organisms from the other treatment groups, specifically the TG. Meanwhile, the TG was found to have only one point where it differed significantly from the C40 and C42, while being significantly different from the C35 at three of the later counts (8, 10, 28 hours respectively), notably when the growth curve of C35 enters its exponential growth phase. Our results agree with similar research experiments found within the literature, however limitations in our study permits further analysis and show a significant knowledge deficit in studies built upon variation in temperature, unfortunately, this variable is becoming increasingly destabilized due to climate change and studies that mirror this reality must increase.

Acknowledgements

Our team would like to acknowledge that we have had the opportunity to conduct our research on the traditional, ancestral, and unceded territory of the Musqueam people. The team would also like to acknowledge UBC and all the figures that have contributed to the creation of the learning experience that is encapsulated within BIOL 342. We sincerely thank Jordan Hamden, our BIOL 342 instructor for his constant support during our experiment. Jordan helped us tune our experiment in order to make it the best it could be and helped us minimize potential errors and setbacks we could have encountered. Also, we would like to thank Mindy Chow for growing our culture of *T. thermophila* and for her constant help in lab regarding equipment and experimental techniques. Lastly, but certainly not least, our TA Carol Ayumi Sato for her constant oversight on our experiment and helpful lab technique tips to ensure that we would get accurate results.

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Appendix

Equipment List : Axiostar Compound Microscopes, 4-20ml test tubes with caps, 4 test-tube holders, 36 eppendorf tubes, incubators, various sizes of micropipettes (Thermo Scientific brand) with tips (Fisherbrand), click-counters, coverslips (Fisherbrand), Kimtech Kimwipes, Haemocytometer, Erlenmeyer flask, Bunsen Burner.

Chemical List : Glutaraldehyde fixative, cultured wild-type *Tetrahymena Thermophila* in stock solution, SSP growth medium, ethanol (70%), distilled water.

Table 1. Tukey HSD test

Row 1					
C35 vs. C40	993.1	-12846 to 14833	No	ns	0.9592
C35 vs. C42	-4062	-31120 to 22995	No	ns	0.7576
C35 vs. TG	4316	-3237 to 11869	No	ns	0.1571
C40 vs. C42	-5055	-26464 to 16353	No	ns	0.6991
C40 vs. TG	3323	-7776 to 14421	No	ns	0.5807
C42 vs. TG	8378	-15688 to 32445	No	ns	0.3758
Row 2					
C35 vs. C40	6646	-11101 to 24393	No	ns	0.4170
C35 vs. C42	7448	-24690 to 39586	No	ns	0.6176
C35 vs. TG	2368	-6870 to 11606	No	ns	0.6604
C40 vs. C42	802.1	-27430 to 29034	No	ns	0.9991
C40 vs. TG	-4278	-24484 to 15928	No	ns	0.6514
C42 vs. TG	-5080	-40212 to 30052	No	ns	0.7937
Row 3					
C35 vs. C40	13524	-14585 to 41633	No	ns	0.2350
C35 vs. C42	18028	6635 to 29420	Yes	*	0.0107
C35 vs. TG	-24674	-194015 to 144668	No	ns	0.7688
C40 vs. C42	4504	-22437 to 31445	No	ns	0.8263
C40 vs. TG	-38197	-199108 to 122714	No	ns	0.5491
C42 vs. TG	-42701	-211389 to 125986	No	ns	0.4838
Row 4					
C35 vs. C40	37232	-31727 to 106191	No	ns	0.1581
C35 vs. C42	46972	-22553 to 116496	No	ns	0.1049
C35 vs. TG	37423	-31592 to 106438	No	ns	0.1568
C40 vs. C42	9740	4326 to 15153	Yes	**	0.0074
C40 vs. TG	191.0	-5645 to 6027	No	ns	0.9990
C42 vs. TG	-9549	-14842 to -4255	Yes	**	0.0072
Row 5					
C35 vs. C40	21847	-8891 to 52585	No	ns	0.1368
C35 vs. C42	25552	-19226 to 70330	No	ns	0.2066
C35 vs. TG	30250	1306 to 59194	Yes	*	0.0449
C40 vs. C42	3705	-40794 to 48203	No	ns	0.9805
C40 vs. TG	8403	-23661 to 40467	No	ns	0.5775
C42 vs. TG	4698	-48435 to 57831	No	ns	0.9422
Row 6					
C35 vs. C40	29437	-14579 to 73454	No	ns	0.1563
C35 vs. C42	40819	-1321 to 82960	No	ns	0.0552
C35 vs. TG	40514	305.0 to 80723	Yes	*	0.0492
C40 vs. C42	11382	-35016 to 57780	No	ns	0.7586
C40 vs. TG	11076	-41894 to 64046	No	ns	0.6322
C42 vs. TG	-305.6	-50098 to 49487	No	ns	>0.9999
Row 7					
C35 vs. C40	78780	-185116 to 342676	No	ns	0.3946
C35 vs. C42	81377	-163111 to 325865	No	ns	0.3772
C35 vs. TG	58741	-158299 to 275780	No	ns	0.7071
C40 vs. C42	2597	-53068 to 58263	No	ns	0.9921
C40 vs. TG	-20039	-271517 to 231438	No	ns	0.9407
C42 vs. TG	-22637	-254194 to 208921	No	ns	0.9254
Row 8					
C35 vs. C40	97358	82402 to 112313	Yes	****	<0.0001
C35 vs. C42	104271	99715 to 108827	Yes	****	<0.0001
C35 vs. TG	104271	99715 to 108827	Yes	****	<0.0001
C40 vs. C42	6913	-9482 to 23308	No	ns	0.2522
C40 vs. TG	6913	-9482 to 23308	No	ns	0.2522
C42 vs. TG	0.000	-2755 to 2755	No	ns	>0.9999

Table 2. two-way ANOVA table

Table Analyzed	Data 1				
Two-way RM ANOVA	Matching: Stacked				
Assume sphericity?	No				
Alpha	0.05				
Source of Variation	**% of total variation**	**P value**	**P value summary**	**Significant?**	**Geisser-Greenhouse's epsilon**
Time x Group	26.96	0.0005	***	Yes	
Time	20.58	0.0108	*	Yes	0.2391
Group	26.00	0.0002	***	Yes	
Block	2.833	0.5720	ns	No	
ANOVA table	**SS**	**DF**	**MS**	**F (DFn, DFd)**	**P value**
Time x Group	24812409082	21	1181543290	F (21, 56) = 3.044	P=0.0005
Time	18940789030	7	2705827004	F (1.674, 13.39) = 6.972	P=0.0108
Group	23928717002	3	7976239001	F (3, 8) = 24.48	P=0.0002
Block	2606935596	8	325866950	F (8, 56) = 0.8396	P=0.5720
Residual	21735056055	56	388126001		
Data summary					
Number of columns (Group)	4				
Number of rows (Time)	8				
Number of subjects (Block)	12				
Number of missing values	0				

Table 3. Cell Counts for each group along with hours elapsed.

CONTROL 35C

Hours	Count A	Count B	Count C
0	17302	16843	17989
2	16500	22229	17959
4	30937	24864	27500
6	31968	60133	
8	53166	35530	44687
10	50666	53000	71270
26	153541	149416	35933
28	121916	122375	124666

CONTROL 40C

Hours	Count A	Count B	Count C
0	17416	19364	12375
2	15125	15927	5729
4	22564	14208	5958
6	13082	16614	14895
8	20739	14093	
10	49916	24291	17416
26	40104	33229	29218
28	28416	27843	20625

CONTROL 42C

Hours	Count A	Count B	Count C
0	26430	24256	13835
2	5958	22114	6302
4	9739	13406	6072
6	4010	4812	6531
8			7218
10	8822	10541	
26	39875	41937	12947
28	31968	31281	24062

TREATMENT GROUP (TG)

Hours	Count A	Count B	Count C
0	14552	13979	10656
2	17531	17416	14666
4			
6	14093	13291	16614
8	10083	15259	17302
10	17416	21197	14781
26	127722	15812	19135
28	19250	19135	17760

Table 4. Section of Spreadsheets in Google sheets used for Day 1 of experiment

						**NOTE	Haemocytometer dilution factors -->				Smallest Square (green)		80000				
Initial Dilution Factor				1							Medium Square (red)		5000				
Volume Fixative (µL)				10							Largest Square (blue)		312.5				
Volume Stock Solution used for cell count (µL)				100			**NOTE										
Dilution Factor for Haemocytometer				833	*6xred squares		If didn't dilute initial solution (stock) for count, put "Initial Dilution Factor" value = 1										
					833 (5000/6)	--> 1st round of counts											
Correction for Fixative		1.1															
Cell counts	Square 1	64				**had to do second round of counts:			Square 1	55							
	Square 2	147	Cell Count Average		98	Dilution Factor for Haemocytometer:			Square 2	56	Cell Count Average		58				
	Square 3	83				**5xred squares		1000	Square 3	63							
Final Cell Count		89797.4				**Final Cell Count**		63800									
for Working Solution (20'000 cells/mL):				For average of 2 counts: Final Cell Count			76798.7	**average of C14 and I14									
Volume Stock Solution		2.227235978			**Volume Stock Solution**		**2.604210748**	--> ***these were are final volumes used***									
Volume SSP Medium		7.772764022			**Volume SSP Medium**		**7.395789252**										

Table 5. Section of Spreadsheets in Google sheets used for Day 2 of experiment

Volume Fixative (µL)				10													
Volume Initial Solution used for cell count (µL)				100													
Correction for Fixative				1.1													
C35	Microscope 10175			**C40**	Microscope #R1517			**C42**	Microscope 10175			**TG**	Microscope 10175				
Initial Dilution Factor		1		Initial Dilution Factor		1		Initial Dilution Factor		1		Initial Dilution Factor		1			
Dilution factor for Haemocytometer		1666.666667		Dilution factor for Haemocytometer		312.5		Dilution factor for Haemocytometer		500		Dilution factor for Haemocytometer		1666.666667			
Cell counts	Sqaure 1	77	*3 red sq.	Cell counts	Square 1	76	*blue sq	Cell counts	Square 1	56	*10 red sq.	Cell counts	Square 1	59	*3 red sq.		
	Square 2	59			Square 2	52			Square 2	57			Square 2	66			
	Square 3	63			Square 3	65			Square 3	58			Square 3	52			
Cell count average		66.33333333		Cell count average		64.33333333		Cell count average		57		Cell count average		59			
Final cell count		121611.1111		**Final cell count**		22114.58333		**Final cell count**		31350		**Final cell count**		108166.6667			
if diluting, enter "Final cell count"		121611.1111		*if diluting, enter "Final cell count"*		22114.58333		*if diluting, enter "Final cell count"*		31350		*if diluting, enter "Final cell count"*		108166.6667			
Vol. Stock Solution (mL)		1.644586569	1644.586569	**Volume Stock Solution**		8.139425341	8139.425341	**Volume Stock Solution**		6.379585327		**Volume Stock Solution**		1.848998459	1848.998459		
Vol. SSP Medium (mL)		8.355413431	8355.413431	**Volume SSP Medium**		0.8605746585	860.5746585	**Volume SSP Medium**		3.620414673		**Volume SSP Medium**		8.151001541			
			(microliter)	***changed total volume*		0.0894253415	89.4253415										
				from 10ml --> 9ml (ran out of stock solution from previous day)													
								**calculating dilution factor for Haemocytometer --> (Dilution factor / # of squares)									
				C35	C40	C42	TG										
if saturation needed, enter "Final cell count" value																	
	Volume Media (mL)			0	0	0	0										
	Volume Stock Solution (mL)			10	10	10	10										

Table 6. Section of Spreadsheets in Google sheets used for Day 3 of experiment. Showing portion of counts done at 9:00am and 10:20am (T0). Not pictured are counts for 12:20pm (T1), 2:20pm (T2), 4:20pm (T3), 6:20pm (T4) and 8:20pm (T5)

Volume Fixative (µL)			10											
Volumt Initial Solution used for cell count (µL)				100		**for distributing into smaller test tubes for growth rate, total volume = 3mL per tube for C35, C42 and TG								
Correction for Fixative			1.1							total volume = 2mL per tube for C40				
	C35					**C40**				**C42**				**TG**
9:00am	Initial Dilution Factor		1			Initial Dilution Factor		1		Initial Dilution Factor		1		Initial Dilution
	Dilution Factor for Haemocytometer			1250		Dillution factor for Haemocytometer			1250	Dilution factor for Haemocytometer			555.5555556	Dilution Factor
	Cell counts	Square 1	79	*4 red sq.		Cell counts	Square 1	51	*4 red sq.	Cell counts	Square 1	54	*9 red sq.	Cell counts
		Square 2	52				Square 2	51			Square 2	55		
		Square 3	70				Square 3	60			Square 3	57		
	Cell count average		67			Cell count average		54		Cell count average		55.33333333		Cell count aver
	Final cell count		92125			**Final cell count**		74250		**Final cell count**		33814.81481		**Final cell coun**
	if diluting, enter final cell count			92125		*if diluting, enter final cell count*			74250	*if diluting, enter final cell count*			33814.81481	*if diluting, ente*
	Vol. Stock Solution (mL)		0.6512890095	651.2890095		**Volume Stock Solution**		0.5387205387	538.7205387	**Volume Stock Solution**		1.774370208	1774.370208	**Volume Stock**
	Vol. SSP Medium (mL)		2.348710991	2348.710991		**Volume SSP Medium**		1.461279461	1461.279461	**Volume SSP Medium**		1.225629792	1225.629792	**Volume SSP M**
						C35	C40	C42	TG		***Microscope #***	10173		
	if saturation needed, enter "Final cell count" value											10176		
			Volume Media (mL)			0	0	0	0					
			Vol. Stock Solution (mL)			15	15	15	15					
T0														
10:20 AM	**C35**	**A**				**B**					**C**			
		Initial Dilution Factor		1		Initial Dilution Factor			1		Initial Dilution Factor		1	
		Dilution Factor for Haemocytometer			312.5	Dilution Factor for Haemocytometer				312.5	Dilution Factor for Haemocytometer			312.5
		Cell counts	Square 1	50	*blue square	Cell counts	Square 1	50	*blue sq.		Cell counts	Square 1	42	*blue sq.
			Square 2	51			Square 2	47				Square 2	57	
			Square 3	50			Square 3	50				Square 3	58	
		Cell count average		50.33333333		Cell count average		49			Cell count average		52.33333333	
		Final cell count		17302.08333		**Final cell count**		16843.75			**Final cell count**		17989.58333	
	C40	**A**				**B**					**C**			
		Initial Dilution Factor		1		Initial Dilution Factor			1		Initial Dilution Factor		1	
		Dilution Factor for Haemocytometer			312.5	Dilution Factor for Haemocytometer				312.5	Dilution Factor for Haemocytometer			312.5
		Cell counts	Square 1	51	*blue sq.	Cell counts	Square 1	62	*blue sq.		Cell counts	Square 1	37	*blue sq.
			Square 2	51			Square 2	59				Square 2	36	
			Square 3	50			Square 3	48				Square 3	35	
		Cell count average		50.66666667		Cell count average		56.33333333			Cell count average		36	
		Final cell count		17416.66667		**Final cell count**		19364.58333			**Final cell count**		12375	