The effect of 15, 20, and 25 degrees Celsius environments on population growth in *Caenorhabditis Elegans*

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Research Question: What are the effects of varying temperatures (specifically, 15, 20, and 25 degrees Celsius environments) on population growth in cultured Caenorhabditis Elegans?

1: Introduction

Caenorhabditis elegans, also known as C. Elegans, is a microscopic, free-living organism found all around the world (*Corsi*, 2015). C. Elegans is a nematode, or roundworm, that is commonly found feeding on the bacteria (Escherichia Coli) of rotting vegetables (*Corsi*, 2015). Most C. Elegans are hermaphroditic, meaning they have both male and female reproductive organs and are able to reproduce through self-fertilization (*Palikaras*, 2013). Additionally, C. Elegans have a relatively rapid life cycle, with a living period of approximately 2 weeks, producing more C. Elegans daily post-maturity (*Corsi*, 2015).

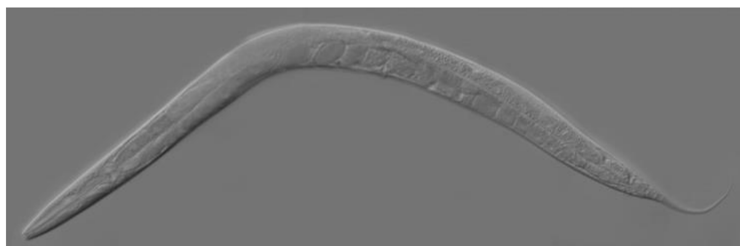


Figure 1: Adult C. Elegans from electron microscope (*Your Genome*, 2015)

Unusually, these nematodes are perfect for biological studies cross-applicable to humans. For one, C. Elegans can be cheaply grown in large quantities, making them easy to obtain (*Your Genome*, 2015). They are also easily visible under a microscope and are relatively simple organisms, which is useful for developmental studies (*Your Genome*, 2015). However, a key component of C. Elegans is that they are genetically similar to humans, although anatomically distinct. Since genes in C. Elegans have functional counterparts in humans, pathological lab scientists utilize them as models for human disease (*Your Genome*, 2015).

I chose to study C. Elegans because I wanted to take the opportunity to study in a lab environment, something I had never done. Having minimal experience in the lab, I was able to learn how to use equipment and gain knowledge about lab-work, such as using microscopes and growing cultures on petri dishes. Furthermore, I wanted to conduct a study that would be applicable to human biology, with a growing interest in treating diseases such as HIV and cancers. By testing the effects of temperature on population growth in C.

Elegans, I would discover the kind of environments that human diseases need to cultivate, and perhaps potential thermal-therapeutic hypotheses on handling human disease.

2: Investigation

2.1: Background Knowledge

Fortunately, this isn't the first time a curious scientist decided to conduct a thermal experiment on C. Elegans. Temperatures have been adjusted on the C. Elegans microbiomes for a number of reasons. For one, because C. Elegans is a well-established model for mammalian aging and neuronal health, experiments on these easy-access, quick-living nematodes have been an efficient strategy in developing therapeutic drugs for humans (*Clovis*, 2019).

In one study, scientists concluded that Caenorhabditis Elegans, along with other nematodes, have shorter life spans with higher temperatures (*Lee*, 2009). The reason for this is the increasing rate of chemical reactions occurring within the C. Elegans (*Lee*, 2009). Another study supports this conclusion, revealing that the C. Elegans behavioral locomotion displays frequent turns and increased velocity with exposure to higher temperature environment (*Parida*, 2014).

Although population size is not directly measured here, one potential assumption is that with shorter life spans (at higher temperatures), populations will decrease as time in adulthood (and thus opportunity for reproduction) is reduced. However, because of the aforementioned "increasing rate of chemical reactions," it is uncertain whether the C. Elegans will just reproduce faster in a shorter timespan when exposed to higher temperatures, remaining at a similar population as other microbiomes.

2.2: Hypothesis

H_1: If temperature is related to population growth in C. Elegans, then as temperature is decreased in livable conditions per C. Elegans environment, the population size will increase accordingly.

H_0 (NULL): There will not be a significant difference in the population growth of C. Elegans between temperatures ranging from 15, 20, and 25 degrees Celsius.

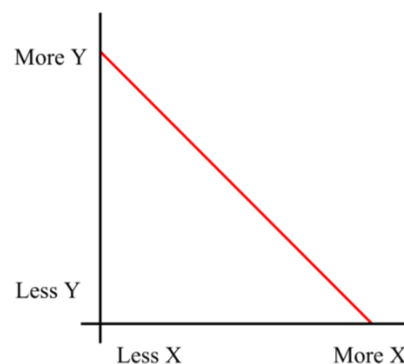


Figure 2: Graph displaying inverse variable relationship,
where Y is population size and X is temperature.
(H_1)

2.3: Variables

<u>Independent Variables:</u>

Temperature of C. Elegans environment, measured in degrees Celsius. The temperatures chosen were 15, 20, and 25 degrees Celsius.

Dependent Variables:

Population size of C. Elegans environment, measured in C. Elegans per microscopic field view.

C. Elegans were hand counted through microscopic photography. Microscopic field views were kept at 40X magnification.

Controlled Variables:

To isolate the independent variable, I kept all other parts of the experiment constant throughout each sample. When plating onto cultures, I pulled C. Elegans from the same source (to ensure similar health status of C. Elegans) and put them into dishes with equal amounts of E. Coli (to avoid the food source from impacting the experiment). The size of the C. Elegans environment extracted was equal, with roughly similar population densities, resulting in approximate equivalency in the initial number of C. Elegans. Additionally, when collecting data, samples were counted at constant time intervals including initial population, 48 hours, and 96 hours.

2.4: Ethical Issues

This experiment completely adheres to IB's animal experimentation policy guide. IB's policy states that "...experimentation involving animals must be subject to approval following a discussion between teacher and student," which I have already done. Additionally, it states that "experiments involving animals must be based on observing and measuring aspects of natural animal behavior. Any experimentation should not result in any pain or undue stress..." This experiment is indeed based on observing the natural behavior of C. Elegans and does not cause any pain or stress to the animals. This is supported by the studies mentioned in Section 2.1 (background knowledge), where temperatures of 15 to 25 degrees Celsius were used and recommended as they are most preferred by the species.

3: Procedure

3.1: Apparatus

To set up microbiomes:

Styrofoam insulators [x2] - to regulate temperatures in microbiomes
60-Watt light bulb [x2] – to heat Styrofoam incubator
Ice Pack [x4] – to refrigerate Styrofoam cooler
Thermometer – to measure temperatures inside incubator and cooler

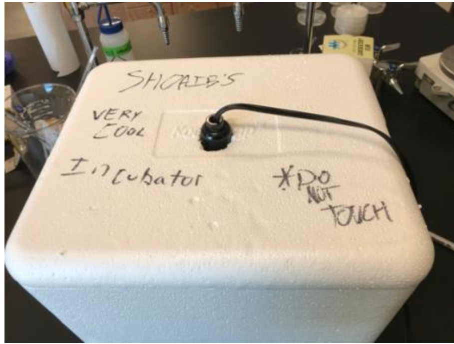
To set up cultures:

Sample C. Elegans and Petri dishes with E. Coli (separately)
Spatula – for extracting C. Elegans and placing on petri dishes
Ethanol, distilled water, lighter, alcohol burner – to sterilize tools (spatula)
Scissors and sharpie – labeling purposes
Parafilm – to wrap samples and prevent contamination
Sterile gloves - safety purposes

To measure data:

Microscope (20X to 40X magnification)
Phone – to take pictures of C. Elegans
Microscope phone holder – to attach phone to microscope lens

3.2: Photograph of Set-up



Picture taken on my iPhone, 2/21/20, displaying incubator; light bulb is fixed onto socket, then put through top; lid will be lifted to edge of Styrofoam insulator to keep incubator at 25 degrees Celsius (it reaches upwards of 60 degrees Celsius with a closed top).

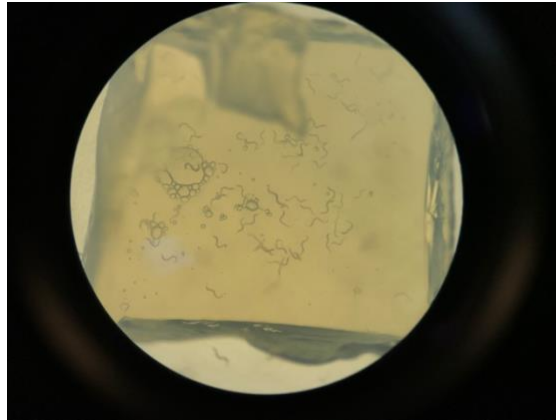
The same is done for the cooler, where ice packs are placed inside of the insulator (top closed).

3.3: Methodology

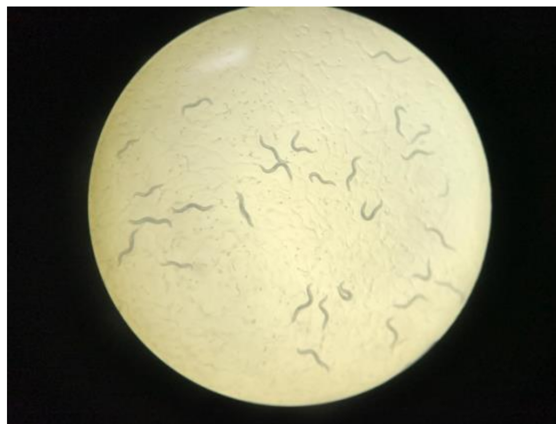
The first step is to set up the microbiomes. After acquiring all materials, I chose a storage room in my school with an outlet and empty space. For the incubator, I cut a hole in the center of the lid and fixed my light bulb through it. For the cooler, I used a smaller Styrofoam box and placed two ice packs inside to keep it near 15 degrees Celsius. I switched out the incubator's light bulb and the cooler's two ice packs every morning to keep the temperature regulated. For 20 degrees Celsius, I placed the C. Elegans wrapped on a counter in the storage room (room temperature remains near or at 20 degrees Celsius).

The next step is to set up the cultures. To set up the cultures, I first gathered all materials on a work bench, including C. Elegans, E. Coli, tools, and solutions. With sterile gloves on, I created the solution (to sterilize the spatula) is created with 40 mL of ethanol and 10 mL of distilled water. After lighting the alcohol burner, I dipped the spatula into the ethanol solution, then held it horizontally over the flame to burn any contaminants on the spatula. After waiting 30-40 seconds for the spatula to cool down, I cut a square with side lengths the size of the spatula's tip into the sample of C. Elegans, then transferred the extract into a blank dish with E. Coli. To avoid contamination, I would avoid speaking or breathing directly onto the extracts or tools, including when I took the lids off C. Elegans and bacteria plates. I would ensure the transfer was complete by placing the petri dish under a microscope and seeing whether C. Elegans were on the E. Coli plate. Re-sterilizing after every culture, I repeated these steps until I had 15 cultures of C. Elegans.

Once I had 15 cultures, I labeled each plate with my name, date, which microbiome it belonged to (rT for room temperature, C for cooler, Inc for incubator), and which sample it was (#1-5). I used the parafilm to wrap all of the ones belonging in the same environment together, then placed them in their microbiomes. I collected data by taking pictures (on my iPhone with the microscope and phone-holder) of each sample the day they were plated, 48 hours after, and 48 more hours after that. The microscopic field view was kept at 40X magnification (with 10X objective lens and a 4X eyepiece) to keep the sample area the same for each picture. Denser areas of populations were photographed in each sample to provide sufficient data.



Sample picture of plated C. Elegans taken from my iPhone, 2/17/20



Same picture (zoomed in 40X magnification), 2/17/20

3.4: Justification

I used 5 samples per microbiome so that I would have enough data to draw conclusions, improving the accuracy and reliability of my data. I did this to ensure that trends can be determined from my data.

Although only three temperatures were chosen, previous studies have that this 10-degree difference in temperature is enough to see substantial shifts the behavior of C. Elegans, therefore 15, 20, and 25 degrees Celsius environments were sufficient for this experiment. Additionally, without electric incubators and refrigerators, 2.5-degree intervals would have been difficult to regulate thoroughly in the experiment.

The dependent variable (population size) was chosen because population is a prominent indicator of the health of a species. Animals are often more populated in environments they prefer, thus, manipulating the independent variable (temperature) would reveal which temperature Caenorhabditis Elegans prefer.

3.5: Risk Assessment

Safety Issues: Experimentation was handled with care. Ethanol solution was created carefully to prevent any spillage, and tools were held horizontally to prevent flames from alcohol burner from spreading. The solution and burner were placed on opposite sides of workbench. Moreover, gloves were worn throughout the process, including when creating cultures and placing them in microbiomes. Light bulbs were switched out daily to prevent overheating, and the incubator was kept at a temperature which would not create damage.

Ethical Issues: See Section 2.4.

Environmental Issues: There were no environmental issues to be taken into account.

4: Raw Data

Table 1: A table showing the populations of the Caenorhabditis Elegans in 15 degrees Celsius environments at 0, 48, and 96 hours of growth.

Sample	0 Hours Number of C. Elegans	48 Hours Number of C. Elegans	96 Hours Number of C. Elegans
A	9	24	40
B	6	19	31
C	8	25	33
D	9	26	34
E	10	28	48

Table 2: A table showing the populations of the Caenorhabditis Elegans in 20 degrees Celsius environments at 0, 48, and 96 hours of growth.

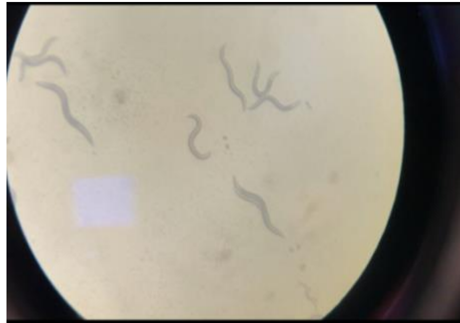
Sample	0 Hours Number of C. Elegans	48 Hours Number of C. Elegans	96 Hours Number of C. Elegans
A	8	20	29
B	11	23	34
C	8	17	25
D	7	16	26
E	9	21	31

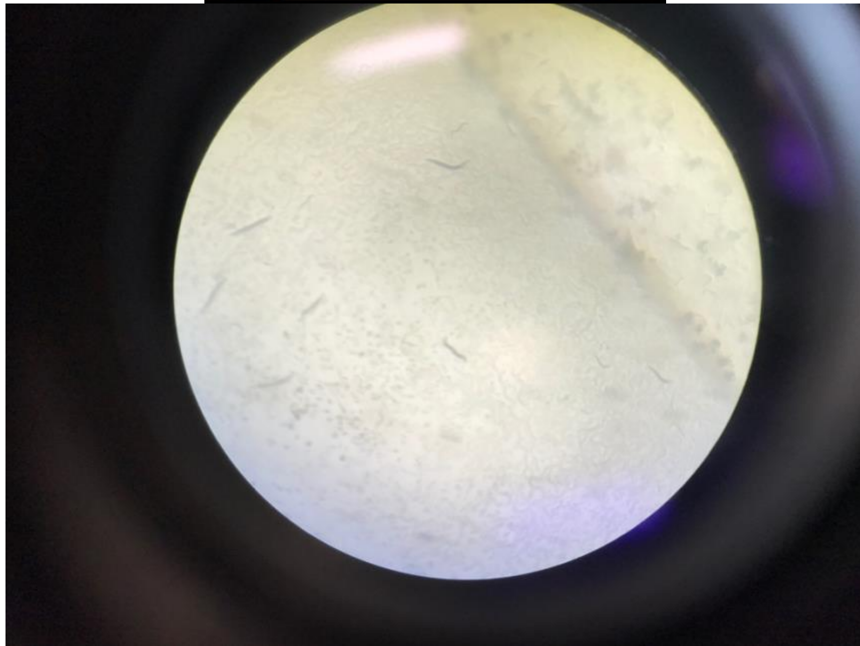
Table 3: A table showing the populations of the Caenorhabditis Elegans in 25 degrees Celsius environments at 0, 48, and 96 hours of growth.

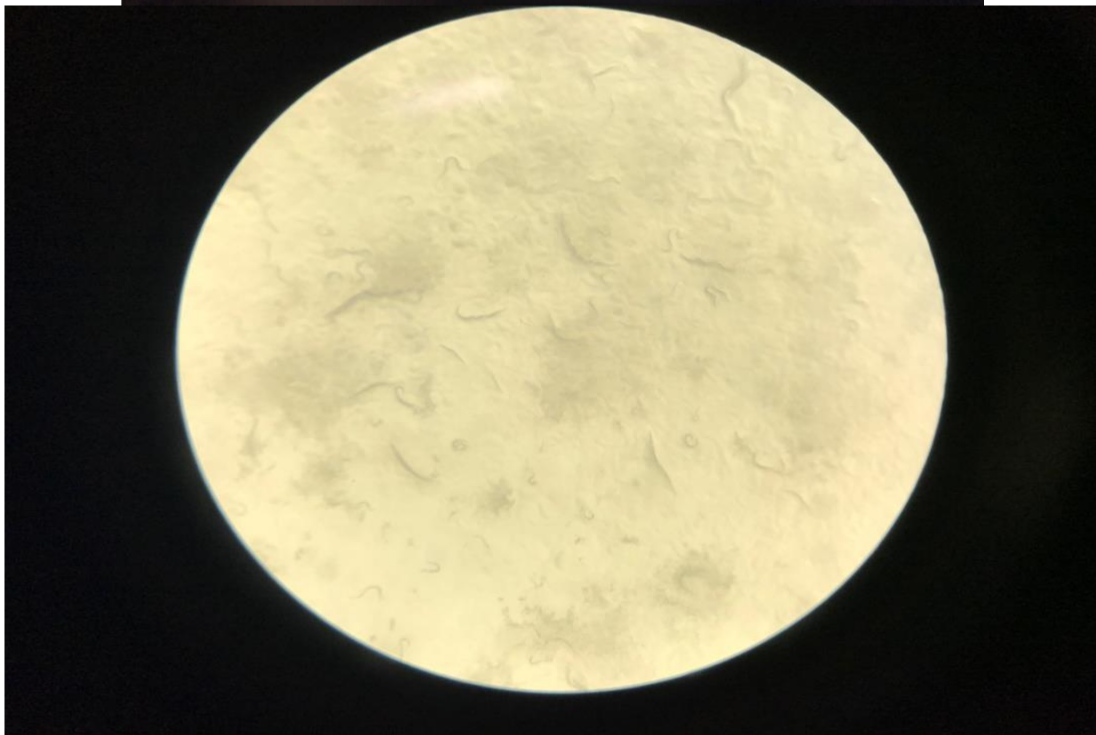
Sample	0 Hours Number of C. Elegans	48 Hours Number of C. Elegans	96 Hours Number of C. Elegans
A	9	16	25
B	9	18	22
C	7	11	18
D	10	15	24
E	9	15	25

4.1: Notes and Qualitative Observations

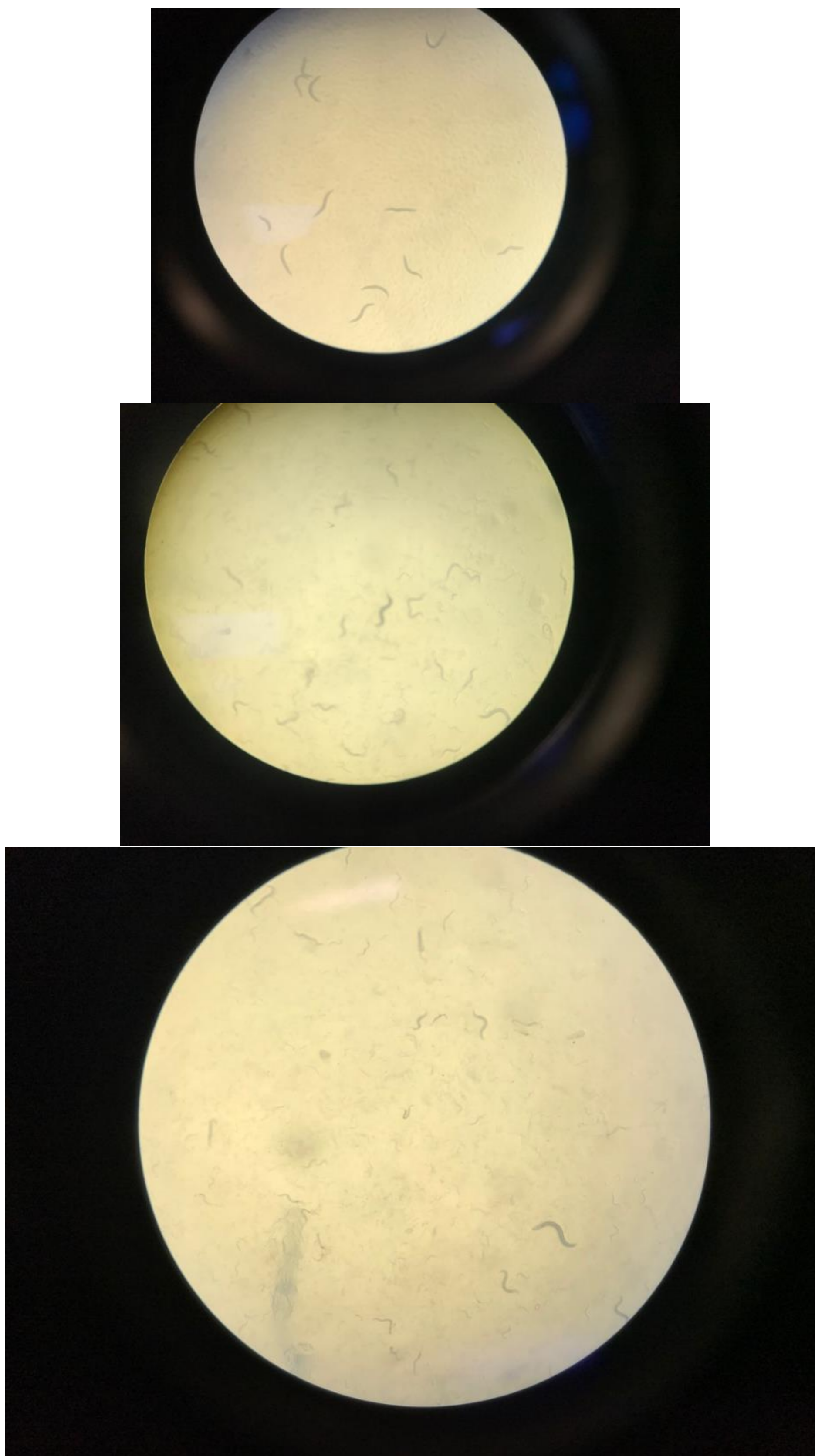
Thus far, it seems that the raw data seems to match H_1, that as temperature is increased in the C. Elegans’ environment, population will decrease accordingly. Not only are populations higher in colder environments, but the C. Elegans in these environments also seem to be healthier than ones exposed to warmer temperatures (in body size and movement).



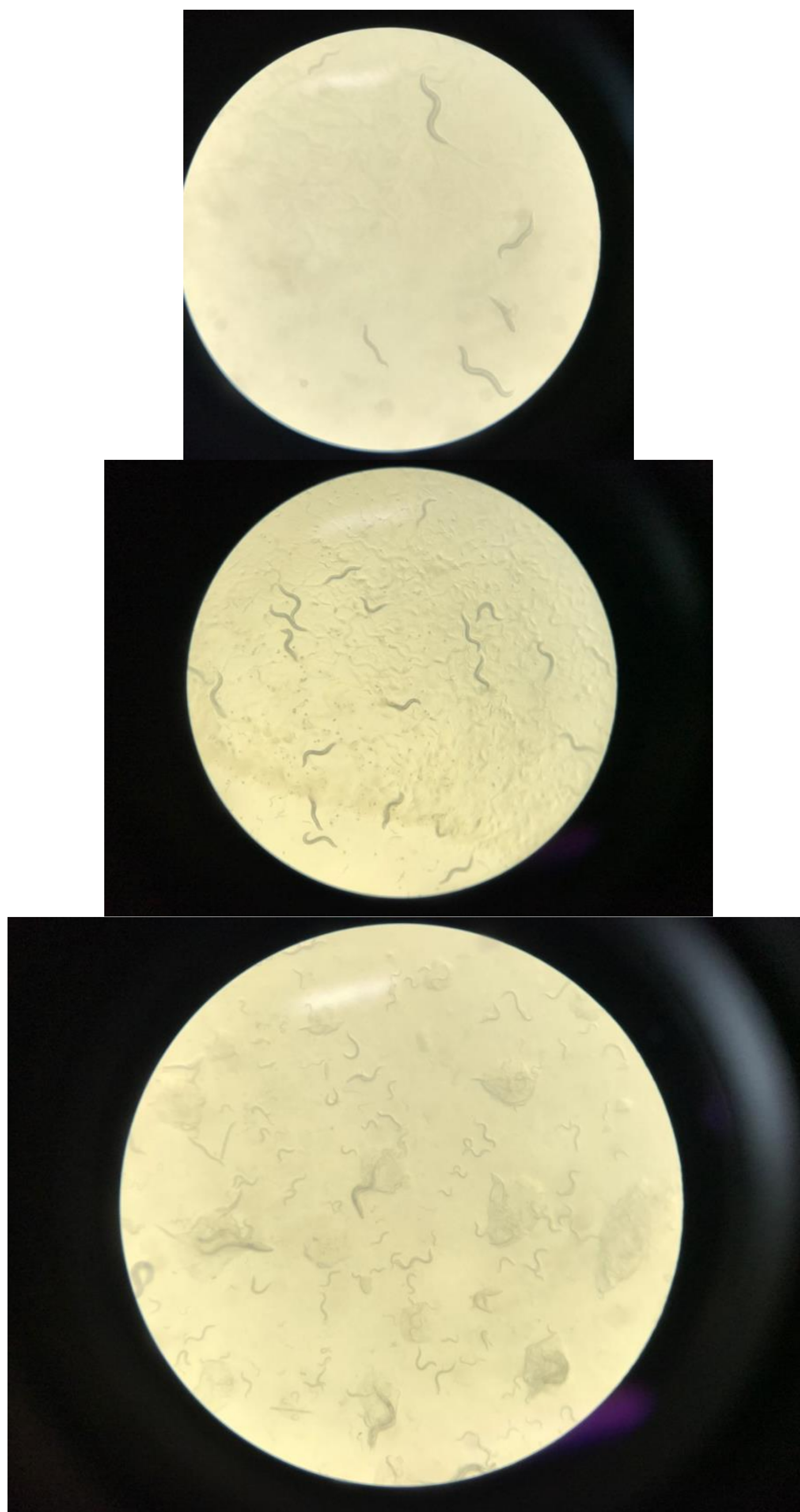




<u>C. Elegans population in 25 degrees Celsius</u>
Top: Sample A at 0 hours (9 C. Elegans)
Middle: Sample D at 48 hours (15 C. Elegans)
Bottom: Sample B at 96 hours (22 C. Elegans)



C. Elegans population in 20 degrees Celsius
Top: Sample B at 0 hours (11 C. Elegans)
Middle: Sample B at 48 hours (23 C. Elegans)
Bottom: Sample D at 96 hours (26 C. Elegans)



C Elegans population in 15 degrees Celsius
Top: Sample B at 0 hours (6 C. Elegans)
Middle: Sample A at 48 hours (24 C. Elegans
Bottom: Sample E at 96 hours (48 C. Elegans)

Uncertainty: Due to the fact that data was measured by hand, data uncertainty is relatively high in this experiment. This is because of the possibility for human error while counting, which may happen at any moment. To reduce this, pictures were counted multiple times over to ensure reliability of data. However, with small C. Elegans here and there, the exact number for multiple data points could be slightly off. Regardless, the impact of this uncertainty is not devastative because the trend is seen even if the data is adjusted by 1 or 2 marks.

5: Processed Data

Sample	0 Hours Number of C. Elegans	48 Hours Number of C. Elegans	96 Hours Number of C. Elegans
A	9	24	40
B	6	19	31
C	8	25	33
D	9	26	34
E	10	28	48
AVG=	8.4	24.4	37.2

Sample	0 Hours Number of C. Elegans	48 Hours Number of C. Elegans	96 Hours Number of C. Elegans
A	8	20	29
B	11	23	34
C	8	17	25
D	7	16	26
E	9	21	31
	8.6	19.4	29

Sample	0 Hours Number of C. Elegans	48 Hours Number of C. Elegans	96 Hours Number of C. Elegans
A	9	16	25
B	9	18	22
C	7	11	18
D	10	15	24
E	9	15	25
	8.8	15	22.8

Figure 3: Screenshot of data from Excel, with averages added at bottom
From left to right: 15, 20, and 25 degrees Celsius, respectively

Averages graphed accordingly, displaying each microbiome's average population per sample at 0, 48, and 96 hours:

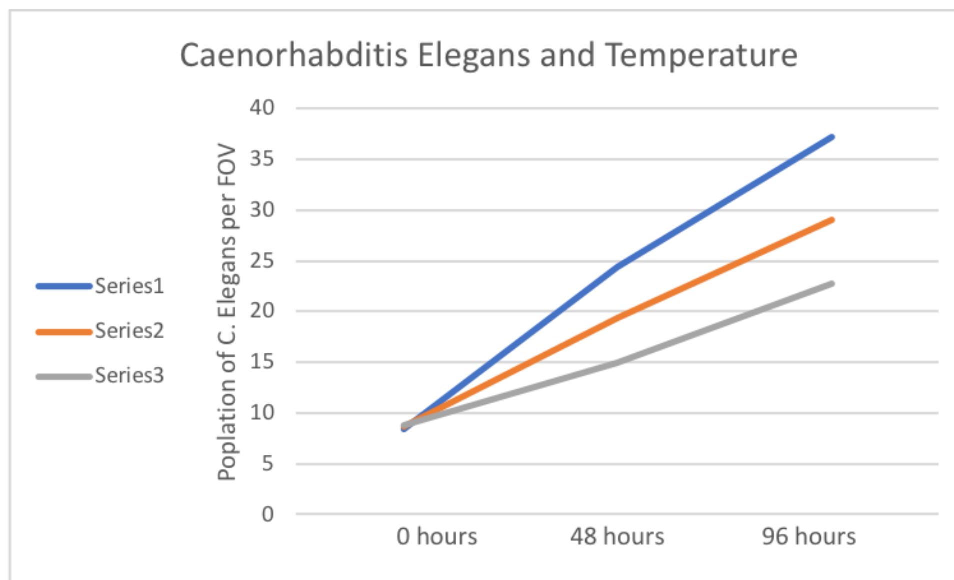


Figure 4:
Series 1, 2, and 3 being 15, 20, and 25 degrees Celsius, respectively.

5.1: Statistical Test

Using an online calculator, a one-way Anova test was conducted to determine two things: whether the variance of initial values in the experiment was significant (enough to interrupt data credibility) and whether the result of the data measured varied significantly.

The calculator takes the data and retrieves a p value. If the p-value is less than .05, the result is significant. If it is greater than .05, the result is insignificant.

Left panel (0 hours)

Data Entry

Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
9	8	9		
6	11	9		
8	8	7		
9	7	10		
10	9	9		

Reset Calculate

Data Summary

	Samples					
	1	2	3	4	5	Total
N	5	5	5			15
ΣX	42	43	44			129
Mean	8.4	8.6	8.8			8.6
ΣX^2	362	379	392			1133
Variance	2.3	2.3	1.2			1.6857
Std.Dev.	1.5166	1.5166	1.0954			1.2984
Std.Err.	0.6782	0.6782	0.4899			0.3352

standard weighted-means analysis

ANOVA Summary Independent Samples k=3

Source	SS	df	MS	F	P
Treatment [between groups]	0.4	2	0.2	0.1	0.905583

Middle panel (48 hours)

Data Entry

Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
24	20	16		
19	23	18		
25	17	11		
26	16	15		
28	21	15		

Reset Calculate

Data Summary

	Samples					
	1	2	3	4	5	Total
N	5	5				10
ΣX	122	97				219
Mean	24.4	19.4				21.9
ΣX^2	3022	1915				4937
Variance	11.3	8.3				15.6556
Std.Dev.	3.3615	2.881	1.0954			3.9567
Std.Err.	1.5033	1.2884	0.4899			1.2512

standard weighted-means analysis

ANOVA Summary Independent Samples k=3

Source	SS	df	MS	F	P
Treatment [between groups]	62.5	-1	-62.5	-7.97	<.0001

Right panel (96 hours)

Data Entry

Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
40	29	25		
31	34	22		
33	25	18		
34	26	24		
48	31	25		

Reset Calculate

Data Summary

	Samples					
	1	2	3	4	5	Total
N	5	5				10
ΣX	186	145				331
Mean	37.2	29				33.1
ΣX^2	7110	4259				11369
Variance	47.7	13.5				45.8778
Std.Dev.	6.9065	3.6742	1.0954			6.7733
Std.Err.	3.0887	1.6432	0.4899			2.1419

standard weighted-means analysis

ANOVA Summary Independent Samples k=3

Source	SS	df	MS	F	P
Treatment [between groups]	168.1	-1	-168.1	-6.87	<.0001

Figure 5: Anova tests
Left: data from 0 hours, middle: data from 48 hours, right: data from 96 hours

The Anova tests show that the p value (seen in the bottom right) is insignificant for the initial values (data at 0 hours), but extremely significant for the other two sets of data (data at 48 and 6 hours).

6: Evaluation

6.1: Conclusion

The results of the experiment reveal that as temperature is decreased in livable conditions for cultured Caenorhabditis Elegans from 25 to 15 degrees Celsius, population growth and size increase accordingly. Therefore, H_0 (the null hypothesis given in Section 2.2) is rejected, as the data measured was seen to be significant through the use of Anova tests in Figure 5, and H_1 (hypothesizing an inverse relationship between temperature and population growth) is accepted. This hypothesis is supported through multiple pieces of evidence. I first began to suspect H_1 would be accepted after seeing the qualitative observations in Section 4.1, with healthier and noticeably more C. Elegans in cooler environments. Additionally, after processing the raw data in Tables 1-3, Figure 4 showed that the average C. Elegans population per sample grew quicker at lower temperatures, further substantiating H_1.

The significance of these results is that it reveals the thermal preference of human diseases. Not only this, but it opens up the possibility of future thermal therapeutic treatments. One application I can extrapolate from this investigation is that since C. Elegans reproduce at significantly lower rates at higher temperatures, given that they are accepted models for how human cells with diseases would act, if precision technology could one day warm human cells at a microscopic level, the rate of growth for the human disease could potentially be controlled or even manipulated. This, of course, would be very difficult, but with a number of diseases which surpass our current medical knowledge (such as HIV or cancer), biotechnology and thermal therapeutics may have vast potential in treating millions of patients across the world.

6.2: Strengths

While the experiment is not perfect, it does have its strengths. I believe the data from the experiment is reliable because it isolated the dependent variable (temperature) across 15 different samples, so that a relationship between temperature and growth could be determined. Plus, when the data was collected, although the method was prone to human

error (hand-counting C. Elegans), it was repeated to ensure a constant measure was acquired. Given that budget and timeframe were heavily restricted, I believe this experiment did the best it could creating reliable incubators and coolers, developing sound data, and reaching a grounded conclusion.

6.2: Weaknesses

On the other hand, one limitation of the experiment was that I could only test three temperatures, and the temperatures were restrained to abide to IB's animal experimentation guidelines. More samples from a variety of temperatures would have helped solidify the inverse relationship proposed by hypothesis one, rather than relying on assumptions that the trend will carry onto livable temperatures colder than 15 degrees Celsius.

Another limitation of the experiment was that for counting the C. Elegans, I could only count grown, adult C. Elegans (since smaller, developing C. Elegans would have appeared in the hundreds in miniscule forms or have not appeared at all). It is uncertain whether it is the reproduction rate that is slowed by heat, or the individual growth of the C. Elegans. It is only known that as temperature is decreased, more and healthier adult C. Elegans are seen in the culture.

Other weaknesses in the experiment came unexpectedly, from imperfections in them methodology. Some unexpected variables that were not kept as controls were culture damage and water contamination.

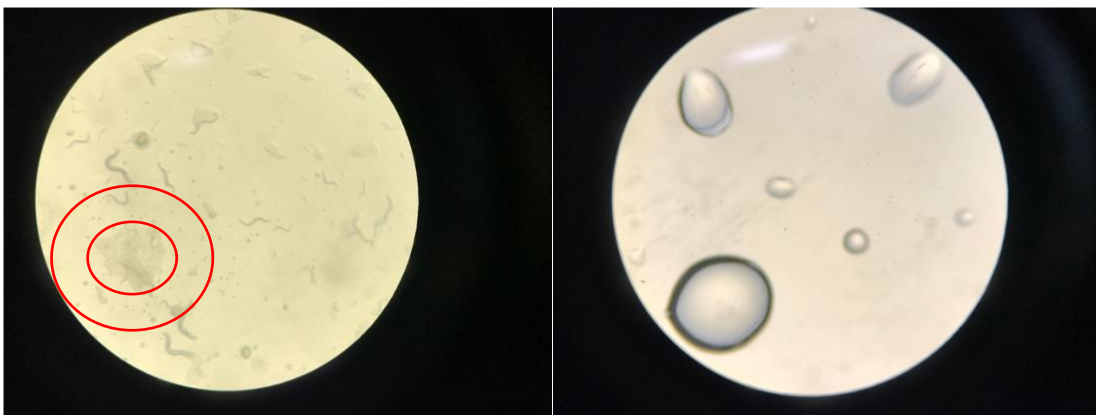


Figure 6: Pictures displaying culture damage (left) and water contamination (right)

The culture damage likely came from a defected bacteria plate which was used without notice. The water contamination was seen inside the wrapped cultures from condensation within the closed petri dishes, since more water was found in the warmer samples. To avoid unreliable data, C. Elegans were counted further from defects in dishes.

6.3: Extensions

Further experimentation on this subject is highly recommended. To obtain certain results, I would recommend the use of electric incubators, to avoid inconsistency in the regulation of incubator and cooler temperatures. Additionally, I would test hypothesis one for a larger time span, as well as smaller temperature intervals (including incubators at 17.5 and 22.5 degrees Celsius). If possible, data collection would become more effective and reliable from tagging and staining C. Elegans electronically, then using machinery to count C. Elegans instead of doing it by hand. Such enhancements would ensure and truly determine whether there is a significant effect of temperature on the population growth of C. Elegans.

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