Starvation of adult C*aenorhabditis elegans* and its effect on health and reproduction

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ABSTRACT

The effect of varying the amount of food on the health of *Caenorhabditis elegans* was examined. Our alternate hypothesis stated that a decreased initial quantity of *Escherichia coli* would increase the number of starved *C. elegans* after nine days. Plates were prepared with three different amounts of *E. coli*: 400 μL, 200 μL, and 100 μL. Counts of adults starved and living, juveniles and eggs were done on the seventh and ninth day after transfer onto the treatment plates. There were no starved *C. elegans* observed on day seven. On day nine the mean count of live *C. elegans* for the three treatments 400 μL, 200 μL and 100 μL were 4.78 ± 1.53, 0.78 ± 0.67, and 0.00 ± 0.00 respectively. The mean count of starved *C. elegans* for the three treatments 400 μL, 200 μL and 100 μL were 4.11 ± 2.35, 11.78 ± 6.25 and 19.00 ± 9.23. The results showed a significant difference among all three treatments when considering the live *C. elegans*, and a significant difference in starved *C. elegans* counts between the 100 μL and 400 μL treatments. We rejected the null hypothesis and our alternate hypothesis was supported by our data. The literature has supporting arguments for our data stating that a decrease in food availability has a negative effect whether or not the organism was healthy or showed signs of being starved. Behavioural effects are shown in the literature as causing further problems in obtaining nutrition.

INTRODUCTION

Caenorhabditis elegans is a free-living soil nematode commonly used in laboratories, with adults growing to approximately one millimetre in length. *C. elegans* has many properties that make it an ideal model organism: it is inexpensive to maintain and it reproduces quickly, having a life span of approximately 55 – 70 hours (Byerly *et. al* 1976). In addition, each life cycle produces a large number of new progeny: 250 – 300

for hermaphrodite-hermaphrodite mating and 1000 for male-hermaphrodite mating (Félix and Braendle 2010). Finally, *C. elegans* is transparent. This transparency has been a stepping-stone, which has led to the derivation of *C. elegans'* entire cell lineage and genome (Sulsten and Horvitz 1976). Given the biological importance of this organism and the frequency in which it is studied in the laboratory, it is important for it to be as efficient and cost-effective as possible to investigate. A logical variable that could be modified in order to optimize costs would be to vary the quantity of the food source added to the organisms' agar.

With the objective to contribute new data in an attempt to optimize the amount of *E. coli* supplied to a nutrient agar, we observed the change in the quantity of starved worms over time on agar dishes containing three different initial amounts of *E. coli* (100 µL, 200 µL, and 400 µL).

It is logical to deduce that a decrease in the amount of available food would result in an increase in starved *C. elegans*. Work done by Boyel *et al.* (2003) on the feeding rate and movement habits of *C. elegans* relative to the availability of their food source led to the development of our alternate hypothesis, which states that decreasing the initial quantity of *Escherichia coli* will increase the number of starved *Caenorhabditis elegans* after nine days of cultivation (Figure 1). The null hypothesis states that decreasing the initial quantity of *Escherichia coli* will decrease or have no effect on the number of starved *Caenorhabditis elegans* present after nine days of cultivation.

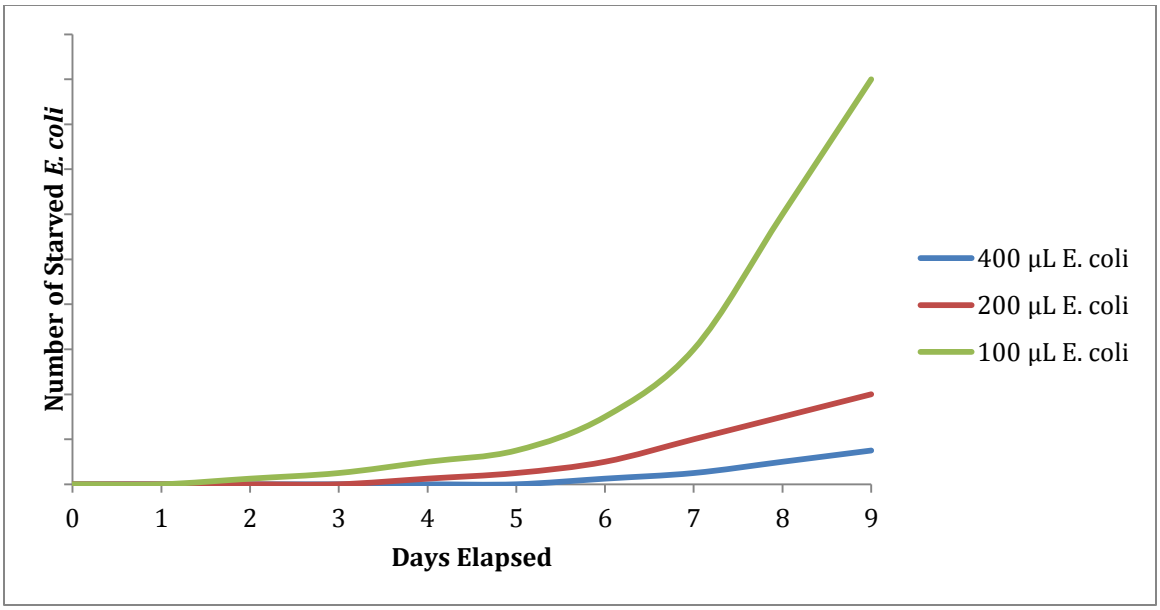


Figure 1: The projected trend of the number of starved *C. elegans* after nine days of cultivation with a varying amount of food (*E. coli*) provided.

MATERIALS AND METHODS

Obtaining Specimens

The *C. elegans* were provided by Dr. Don Moerman's genetics lab. All specimens were wild-type N2 strain.

Methods

Our three treatment levels consisted of plates with *E. coli* in volumes of 100 µl, 200 µl, and 400 µl. Each treatment level had three replicates, for a total of nine plates. Using worm picks and dissecting microscopes we transferred 10 adult *C. elegans* to each of the nine plates. A diagram of this is shown below (Figure 2). Once the *C. elegans* were transferred onto their respective plates, they were stored at 20^{o}C for seven days. After seven days, we counted the total number of adults, juveniles, and eggs in each plate. In

order to be as accurate as possible, we placed a grid on top of each plate. This grid consisted of 100 squares each 0.5 cm by 0.5 cm. We used a random number generator (1-100), to determine which 3 squares we would look at for each respective plate. We recorded the number of adults, juveniles and eggs for each of the three squares and then averaged these three numbers. This gave us the average density of a square, which we assumed was a good representation of the population density of that entire plate. In order to have a clear view of each square we used the DinosScope and took pictures which we later used to conduct the count. Figure 3 displays the differences among *C. elegans* adults, juveniles and eggs.

Two days after our first count, day nine of the incubation period, we did another count using the same method as described above. In this second count we also recorded the number of starved and dead *C. elegans.* This was not necessary in the first count because all adult *C. elegans* showed healthy behaviour. No other food source other than *E. coli* was present on the plates. All plates were sealed immediately following the transfer of the 10 initial adults, and were not opened for the counts. The control for this experiment was Treatment #3, which consisted of 3 plates each containing 400 µL of *E. coli*. This amount of *E. coli* is the standard amount used when growing *C. elegans* in a 60-mm diameter petri dish.

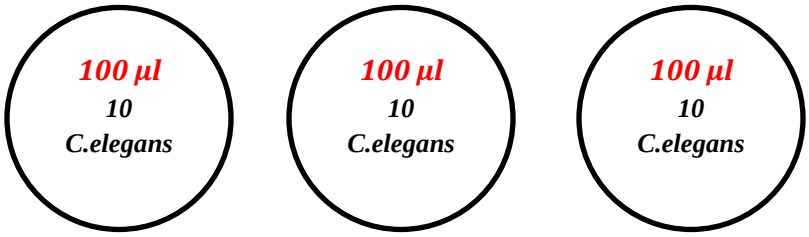
RESULTS AND DATA ANALYSIS

Using Microsoft® Excel® Version 14.3.8, data were organized into three columns, each representing one of the three treatments: 400 µL, 200 µL and 100 µL. Means,

standard deviations and confidence intervals were calculated and graphs were generated in Microsoft Excel. The 95% confidence intervals were calculated with α=0.05. Results were deemed significant if confidence intervals between treatments did not overlap.

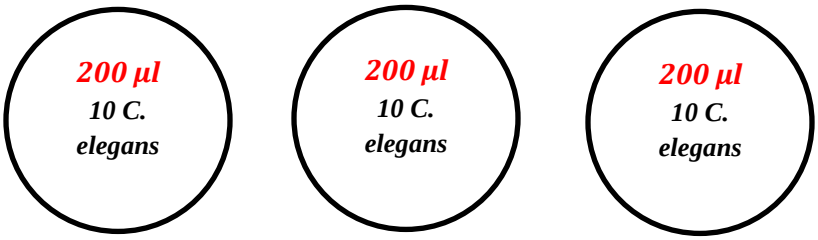
TREATMENT #1: 100 µl of *E. coli*

- 3 replicates



TREATMENT #2: 200 µl of *E. coli*

- 3 replicates



TREATMENT #3: 400 µl of *E. coli*

- 3 replicates

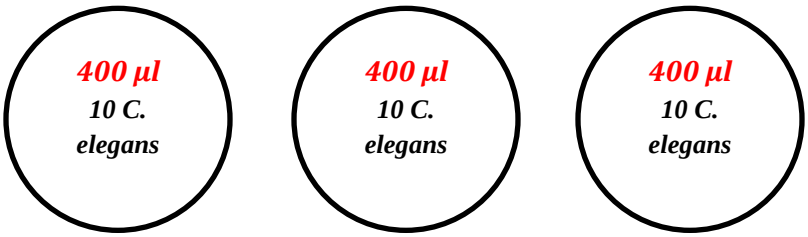


Figure 2: A diagram showing 9 plates. Each row represents one of the three treatment levels. Each plate started with 10 *C. elegans* adults.

Figure 3: A picture taken with a Dino-Eye Microscope Eye-piece Camera that shows one adult *C. elegans* (orange arrow), many juveniles (green arrow), and some eggs (blue arrow).

RESULTS

Example Calculation for Mean, Standard Deviation and Confidence Interval

$\overline{x} = \frac{\sum_{i=1}^{n} x_i}{n}$ Mean: 11+22+21+2+3+5+19+10+20/9=12.56

$s = \sqrt{\frac{\sum(x-\overline{x})^2}{n-1}}$ Standard Deviation: √ ([(11-12.56)^2+(22-12.56)^2+(21-12.56)^2+…]/8)= 7.65

$\overline{x} \pm t \frac{s}{\sqrt{n}}$ Confidence Interval: 12.56 ± 2*7.65/√(9)= 12.56 ± 5.00

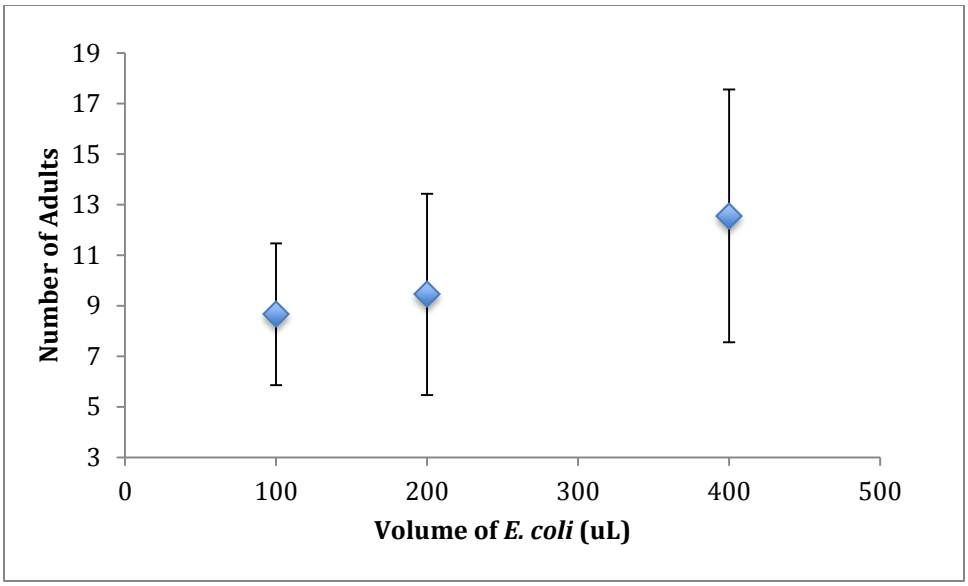


Figure 4. The average count of adult *C. elegans* on various amounts of *E. coli* (100 µL, 200 µL and 400 µL) after seven days. 95% confidence intervals are shown for all means. N=3 for each treatment.

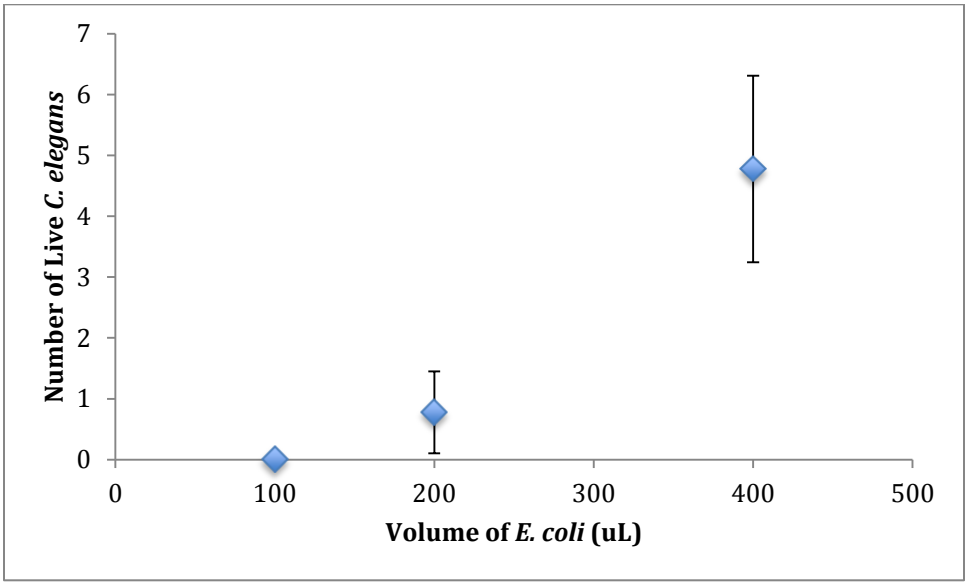


Figure 5. The average count of live adult *C. elegans* on various amounts of *E. coli* (100 µL, 200 µL and 400 µL) after nine days. 95% confidence intervals are shown for 200 µL and 400 µL means. An average count of zero for the 100 µL treatment has no confidence interval. N=3 for each treatment.

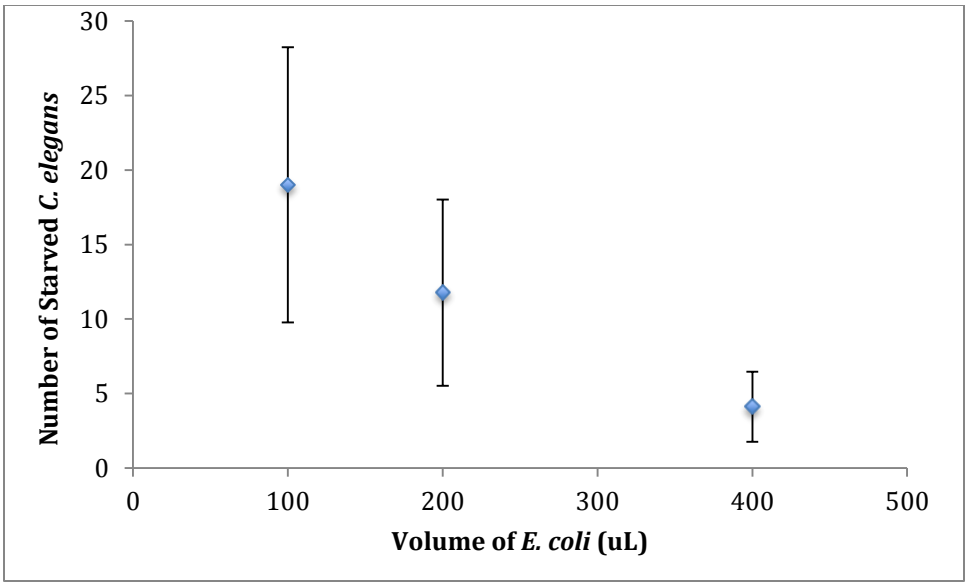


Figure 6. The average count of starved adult *C. elegans* on various amounts of *E. coli* (100 µL, 200 µL and 400 µL) after nine days. 95% confidence intervals are shown for all means. N=3 for each treatment.

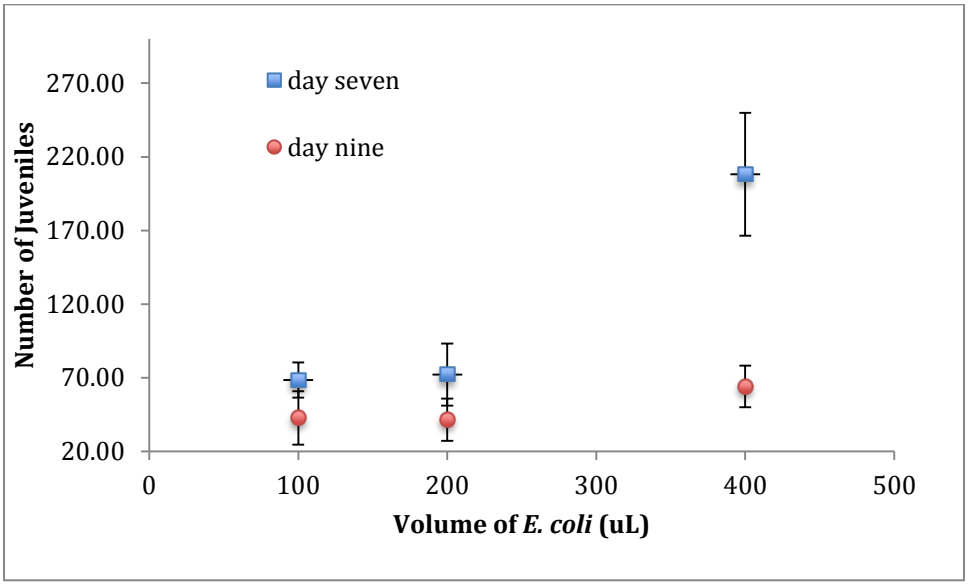


Figure 7. The average count of juvenile *C. elegans* on various amounts of *E. coli* (100 µL, 200 µL and 400 µL) measured after seven days and nine days. 95% confidence intervals are shown for all means. N=3 for each treatment.

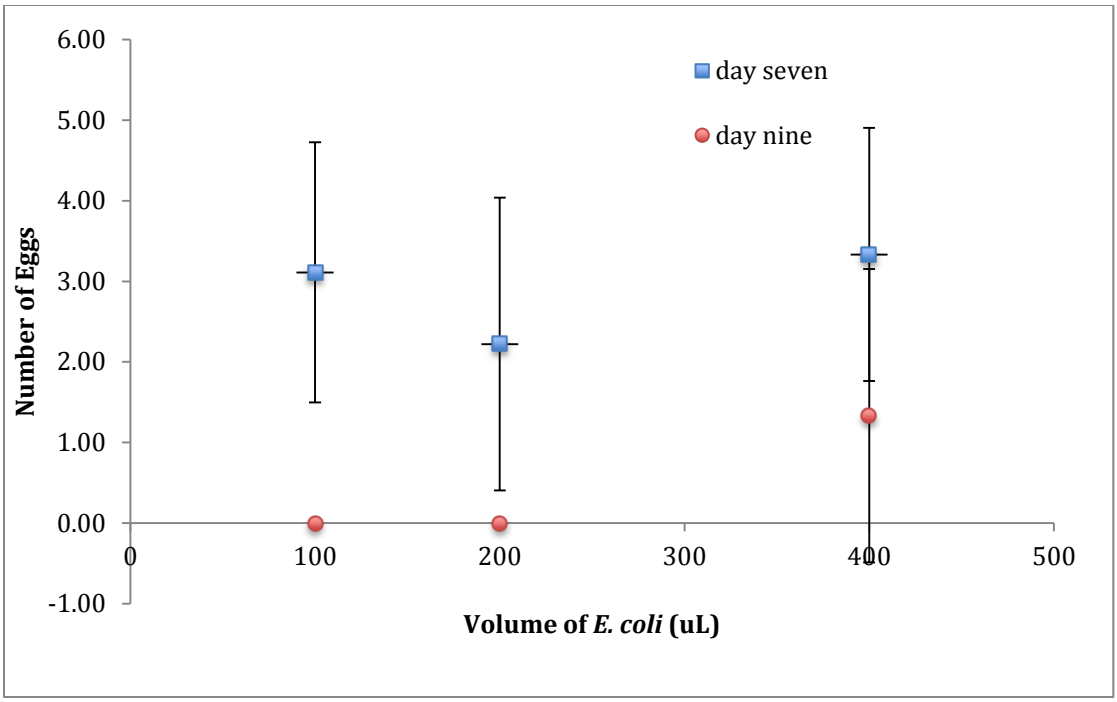


Figure 8. The average count of *C. elegans* eggs on various amounts of *E. coli* (100 µL, 200 µL and 400 µL) measured after seven days and nine days. 95% confidence intervals are shown for all day seven means and the 400 µL treatment on day nine. An average count of zero for the 100 µL and 200 µL treatment have no confidence intervals. N=3 for each treatment.

Description of Results

There were no observed starved *C. elegans* on the day seven plates. The means of adult *C. elegans* in 400 µL, 200 µL, and 100 µL of *E. coli* treatments were as follows: 12.56 ± 5, 9.44 ± 3.98, and 8.67 ± 2.81 respectively (Figure 4). There was no significant difference in the number of adults among all treatments on day seven. On day nine the mean count of live *C. elegans* in the 400 µL, 200 µL, and 100 µL treatments were 4.78 ± 1.53, 0.78 ± 0.67, and 0.00 ± 0.00 respectively (Figure 5). There was a significant difference among all three treatments when considering the live counts of *C. elegans* on the ninth day. On day nine the mean count of starved *C. elegans* in the 400 µL, 200 µL, and 100 µL treatments was 4.11 ± 2.35, 11.78 ± 6.25 and 19.00 ± 9.23 (Figure 6). There

was a significant difference between the 100 µL and 400 µL treatments in starved *C. elegans.* There was no significant difference between the 100 µL and 200 µL or 200 µL and 400 µL.

The mean counts of juvenile *C. elegans* in 400 µL, 200 µL, and 100 µL treatments for day seven were 208.11 ± 41.74, 72.22 ± 21.10 and, 68.44 ± 11.90, respectively. There was no significant difference between the 100 µL and 200 µL treatments but there was a significant difference seen between the 100 µL and 400 µL treatments as well as the 200 µL and 400 µL treatments (Figure 7). For the ninth day the means of juvenile *C. elegans* in 400 µL, 200 µL, and 100 µL treatments were 64.11 ± 14.19, 41.56 ± 14.29, and 42.78 ± 18.15, respectively (Figure 7). There was no significant difference in the count of juvenile *C. elegans* for the three treatments on day nine.

Egg count means were compared on both day seven and day nine. For the 400 µL, 200 µL, and 100 µL treatment, day seven means were calculated at 3.33 ± 1.57, 2.22 ± 1.82, and 3.11 ± 1.61 (Figure 8). The three treatments were not significantly different for the day seven measurement. On day nine the three treatments (400 µL, 200 µL and 100 µL) had mean egg counts of 1.33 ± 1.82, 0.00 ± 0.00 and 0.00 ± 0.00. There was no significant difference between the three treatments.

DISCUSSION

The varying amount of *E. coli* present in three treatments produced different effects on the number of healthy *C. elegans,* starved *C. elegans*, juveniles, and eggs found after a nine-day time interval. Based on our results and statistical analysis of our

experiment, we rejected the null hypothesis and therefore supported our alternate hypothesis that decreasing the quantity of *E. coli* will increase the number of starved *C. elegans* after nine days of cultivation. Although we found differing results between *Caenorhabditis elegans* at various life stages, there was a significant difference when dealing specifically with starved adults between the plates with 400 μL and 100 μL of *E. coli*, which could be either due to a number of biological reasons or possible sources of error in our procedure.

As illustrated by Figure 4, which represents seven days after the transfer of *C. elegans* into the agar plates with different amounts of *E. coli* present, we found there to be no starved adult worms at any of the treatment levels, distinguishing the starved from the healthy organisms by their decreased body length and fat quantity that is evident in starving worms (Mörck and Pilon 2006). However, after nine days, we found entirely opposite trends in the amount of healthy *C. elegans* (as shown in Figure 5) compared to the amount of starved *C. elegans* (as shown in Figure 6) between the different *E. coli* quantities. There was a significantly greater amount of healthy adults at 400 μL of *E. coli* than at either of the lower amounts, while there was a significantly greater amount of starved adults at 100 μL of bacteria than 400 μL. This increase in starved *C. elegans* could be caused simply by the decreased amount of food made available, or because of a decrease in movement that accompanies starvation (Boyd *et al.* 2003). In a study conducted by Boyd *et al.* (2003), the effects of food availability on adult *C. elegans* were observed, and it was discovered that both movement and feeding increased with increased concentrations of *E. coli;* Boyd *et al.* (2003) further state that

movement requires a higher amount of energy, which is negatively impacted by a decrease in food source. Therefore, in our experiment, the number of starved *C. elegans* could have increased due to an inability to move and attain more nutrients.

In contrast to the adult *C. elegans,* there were no significant differences between the numbers of eggs produced at the different *E. coli* amounts, as illustrated in Figure 8. However, there was overall a lower amount of eggs present after nine days than after seven. According to Crawford *et al.* (2007), limited food availability has a negative effect on the reproductive system of *C. elegans*, by inhibiting reproduction through germ line removal, and potentially influencing the longevity of the species. This could possibly explain the fewer number of eggs present in our results over a longer interval of time, which also correlates to the overall lower number of juvenile *C. elegans* present after nine days than after a week, as shown in Figure 7.

Although we cannot conclude further information based solely on our results, several studies have been conducted where food limitation and restriction have been determined to lead to an increase, rather than a decrease, in lifespan of *Caenorhabditis elegans*. Kaeberlein *et al.* (2006) performed an experiment where they tested the effect of complete removal of bacteria on adult *C. elegans*, and found that the absence of a food source results in a greater increase in lifespan compared with a reduction in food. Furthermore, because pharyngeal pumping, which is used by *C. elegans* to concentrate bacteria during uptake, is affected by the amount of bacteria present, Avery and Horvitz (2005) found that starved worms that had been exposed to no food for four hours were better able to pump bacteria than healthy worms, which also contributed to a longer

lifespan, particularly when exposed to limited amounts of food. Therefore, if we had conducted an experiment over a longer period of time, our results may have been different, as we would most likely find a greater number of dead *C. elegans* at 400 μL than at lower levels of bacteria. However, as our experiment only lasted a period of nine days, our results simply showed an increase in the number of starved adults at 100 μL of *E. coli*; if we extended our study time, we might eventually see that the adult *C. elegans* at 100 μL would respond better to a lower nutrient environment than the adults at 400 μL, resulting in an extended lifespan.

Apart from our experiment being too short to study the long-term effects of reduced food quantities on *C. elegans*, there were other aspects and possible sources of error in our procedure that may have influenced our results. Amongst these are possibilities of human error, particularly when counting the number of *C. elegans* present on the agar plate, and misuse of sterile technique, resulting in mold growing on two of the replicates, that could have killed the organisms on that plate. Furthermore, there is the possibility that some adults could have been killed in the initial transfer into the treatment dishes, which would have affected the reproduction and amount of eggs produced.

CONCLUSION

Based on our results for *Caenorhabditis elegans* adults, we rejected our null hypothesis and supported our alternate hypothesis that decreasing the initial quantity of *Escherichia coli* will increase the number of starved *Caenorhabditis elegans* after nine

days of cultivation. There were a significantly greater number of starved adults present in 100 μL of *E. coli* than in 400 μL of *E. coli*, as a decrease in the amount of food available not only decreased the length and size of the organism but also decreases its energy and ability to move and attain more food. However, as mentioned in the Discussion, an experiment testing longer-term effects on limited food quantities would prove more beneficial to determining how lifespan is affected by a decrease in food availability, and how *Caenorhabditis elegans* respond and adapt to real-life environments with lower nutrient sources.

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Your figures are generally well-done. Your biological explanation for your results needs some clarification. Good literature cited section.