The relationship between food quantity and the social feeding behaviour of *Caenorhabditis elegans.*

Malak Abdelhamid, Hyang Im Lee, Soomin Lee, YoungJu Lee, Justin Sidhu

ABSTRACT

This experiment aimed to investigate the social feeding behaviour of *Caenorhabditis elegans* under varying food concentrations. The finding that the nociceptive neuron, a neuron that detects aversive stimuli, induces social feeding led us to observe if limitation in food amount is also an aversive stimulus. In selection of the nematode's food source *Escherichia coli* was chosen as it is one of its common foods. The nematodes were grown in an incubator at 20 degrees Celsius in different *E. coli* amounts: $0\mu L$, $25\mu L$, $50\mu L$, $75\mu L$ and $100\mu L$ where the $0\mu L$ and $100\mu L$ treatments were negative and positive controls, respectively. The surface area of clusters in a $1cm^2$ grid were measured daily for four days. We hypothesized the surface area of *Caenorhabditis elegans* clumps will be greater for a smaller amount of *Escherichia coli*. However, the results show no significant statistical difference among the values of average surface area from different treatments levels. The average of the clump size in order of increasing food amount is: 0.85 ± 1.02 %, 5.89 ± 4.12 %, 19.17 ± 13.08 %, 22.88 ± 26.94 % and 15.34 ± 14.89%. Thus we fail to reject our null hypothesis. It is our understanding that overpopulation was another stressor along with the limitation in food amount, interfering with causing the cluster sizes in relation to food amount.

INTRODUCTION

Virtually all organisms adapt to changes in order to overcome introduced stresses in their environment. Odum (1985) observed that stresses are constantly experienced and inevitable incidents that significantly affect the survival of every organism, which makes *Caenorhabditis elegans* (roundworm) not an exception. When the food resource is limited, *C. elegans* must change its behaviour and adapt in order to endure the stress because food consumption is vital for survival. Therefore, the behaviour of forming social feeding clusters was selected to relieve the stress (Boender *et al*. 2011).

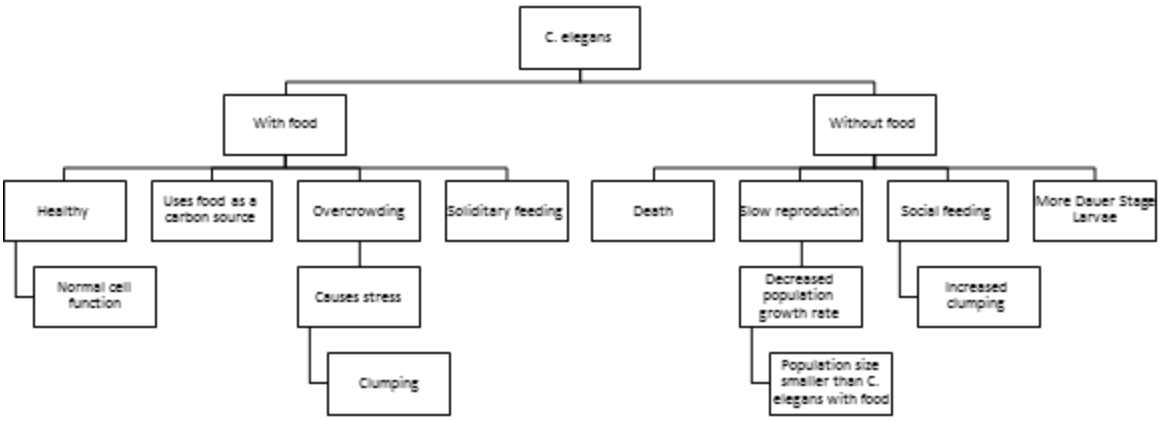

Figure 1. Flow chart of *C. elegans* responses to conditions that have food and that have not. The factor tested was the clumping without food.

C. elegans is a free-living nematode that is transparent and has an elongated cylindrical body with no segmentation or appendages (Wood 1988). It has a simple nervous system that includes nociceptive neurons which work as detectors that react to adverse or stressful conditions (de Bono and Bargmann 1998). The neurons have the receptors encoded by *NPR*-1 gene that is homologous to neuropeptide Y (NPY) receptors in mammals. NPY works as a stimulant that activates the nociceptive neurons which induces the aggregation of *C. elegans* to decrease the level of stress (de Bono *et al.* 2002). They regulate food consumption and sensitivity to stressful signals and allow the neurons to play a key role in the induction of social feeding to reduce the stress caused by the limited food resource (Coates and de Bono 2002).

The objective of this experiment was to investigate the effect of the amount of food resource on the formation of social feeding clusters of *C. elegans*. The study of the nociceptive neurons with the receptors that are involved in the occurrence of the behaviour being

homologous to that of mammals makes this investigation important for providing useful information to further studies of NPY receptors in mammals. In addition, the existence of the neurotransmitter, NPY, in both *C. elegans* and mammals allows this study to be applicable to humans. Therefore, learning more about the *NPR*-1 gene may help to understand the differences in human behaviour (de Bono *et al*. 2002).

One of the common factors used to test social feeding of *C. elegans* is starvation. Also, food availability is an important environmental variable and because of that, we used a study done by Cheung *et al.* (2006) as our model. The study was looking for the effect of the combined factors, O_2 and food source, on social feeding behaviour. However, instead of using both factors in our study, only food source was chosen as a factor to find the effect of starvation on *C. elegans*.

Our alternative hypothesis (H_A) states that exposing *Caenorhabditis elegans* to a smaller amount of *Escherichia coli* will yield larger social feeding clusters. The null hypothesis (H_O) is that exposing *Caenorhabditis elegans* to a smaller amount of *Escherichia coli* will yield smaller or equal social feeding clusters.

METHODS

Design

In this experiment, we divided the *C. elegans* into five treatment groups of varying food quantity to observe the variation in social feeding behavior between the groups: $0\mu L$, $25\mu L$, $50\mu L$, $75\mu L$ and $100\mu L$, where the food source was *Escherichia coli*. The $0\mu L$ treatment group was the positive control; as the complete lack of food represents extreme starvation and thus, we expected clumping to occur. Correspondingly, the $100\mu L$ treatment group was the negative

control, for which we did not expect clumping to occur, as 100μL is a sufficient amount of food to ensure a non-stress environment. 100μL of *E.coli* was the standard amount of food used in many experiments studying *C. elegans,* such as So *et al.*'s 2011 experiment. For each treatment group, we set up five replicates, yielding a total of 25 replicates. The replicate plates were 60mm plates of food, which contained nothing but the amount of *E. coli* appropriate to its respective treatment group (i.e. 5 plates contained 25μL of *E. coli*, etc.).

The experiment was conducted over a period of two weeks. Data was collected once every 24 hours following the experimental set-up. Each day after data collection, all replicate plates were kept in the incubator at a temperature of 20ºC (Stiernagle 2006). However, during the weekends, no observations were made and the worms were kept in the incubator at a lower temperature of 15ºC to slow down reproduction; as "*C. elegans* grows 1.3 times faster at 20°C than at 16°C" according to Stiernagle (2006).

Procedure

Experimental set-up

Using worm-picks and sterile technique, we added three adult nematodes to each replicate plate from an initial 60mm plate of wild-type *C. elegans* (with *E. coli*). However, it was critical that we transfer the nematodes to an empty 60mm plate (with *E. coli*) first before transferring them to the replicate plates, to make sure the organisms were healthy and that the transfer was successful. Once three adult nematodes were in each replicate plate, we sealed the plates with parafilm for the remainder of the experiment. Therefore, no additional food was added for the duration of the experiment to simulate starvation conditions for all treatment levels. Overall, all organisms were starved for a period of 12 days.

Data collection

For the first week of the experiment, we observed the reproduction and population growth in all replicates using a dissecting microscope (Kyowa) at 4.5x objective magnification. These observations were made to ensure the adult nematodes have reproduced and successfully started a population in each plate. We recorded our observations and took pictures of each replicate using a DinoScope, as seen in Figure 2.

Figure 2. Dinoscope picture of healthy population of *C. elegans* including a healthy adult, multiple larvae, eggs and visible tracks taken in replicate 5 of the 0μL treatment on day 3 of the first week of the experiment, under 45x magnification.

During the second week, we focused on observing social feeding behavior by locating and identifying clumps of *C. elegans* using a dissecting microscope (Kyowa) at 1.5x objective magnification. The 'clumps' appeared as dark grey spots of highly concentrated gatherings of nematodes in one area, as seen in Figures 3 and 4.

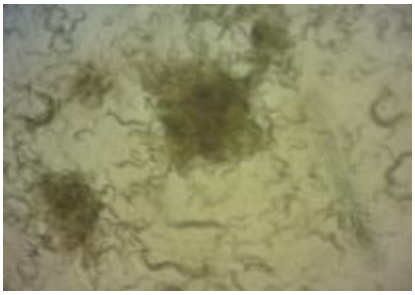

Figure 3. Dinoscope picture of multiple *C. elegans* clusters in replicate 5 of the 50μL treatment on day 2 observed under 15x magnification.

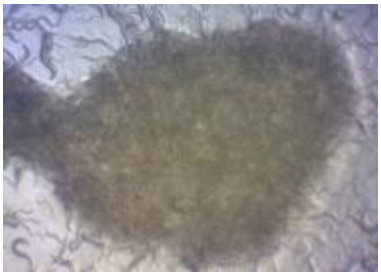

Figure 4. Dinoscope picture of a large *C. elegans* cluster in replicate 2 of the 75μL treatment on day 4 of clump size observation; taken under 45x magnification.

To quantify the size of the clumps, we then measured the surface area occupied by each clump using a grid divided in $1cm^2$ squares. All measurements were taken at a 1.5x objective magnification. We set up $1cm^2$ on the grid to represent 100% coverage (i.e. if the clump was measured to cover $0.25cm^2$ of the plate, it would represent 25% coverage). We started to record data the first day we observed a clump (i.e. day 1 is the day of first clump appearance). In this experiment, day 1 was 8 days after the day of the experimental set-up. Therefore, the first clump we observed was after 8 days of starvation.

Data analysis

If a replicate contained several clumps, we summed the clump sizes to obtain a total percent coverage per replicate. We then calculated the average clump size of those replicates, to yield a mean clump size per treatment; and calculated the 95% confidence intervals for each treatment to test for significance. Then, we presented these results in four scatter plots that would illustrate the relationship between food quantity and average clump size for each day clump size measurements were made. We created an additional scatter plot to show the overall average clump size, where each data point represented the average clump size for all four days of each treatment.

RESULTS

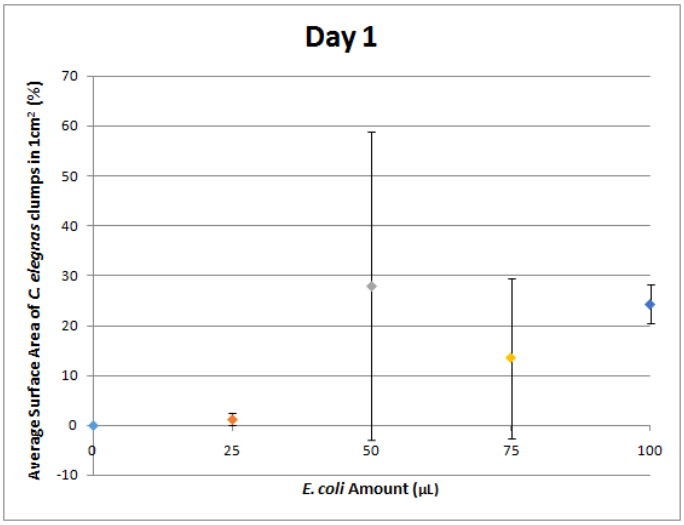

Figure 5. Day 1 average surface area of *C. elegans* clumps in percentage where 1cm^2 surface area represents 100% coverage for different amounts of *E. coli*. At 0 (n=4), 25 (n=4), 50 (n=3), 75 (n=3), 100 (n=3) µL the respective average values are 0.00, 1.20, 27.86, 13.40 and 24.31 percent. The 95% confidence intervals are represented by the error bars. Day 1 refers to 9 days after the start of the experiment.

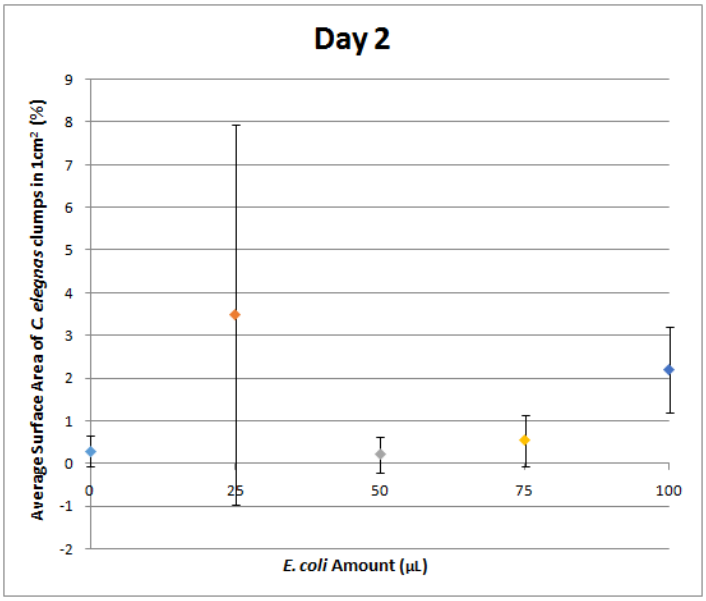

Figure 6. Day 2 average surface area of *C. elegans* clumps in percentage where 1cm^2 surface area represents 100% coverage for different amounts of *E. coli*. At 0 (n=4), 25 (n=4), 50 (n=3), 75 (n=3), 100 µL (n=3) the respective average values are 0.29, 3.48, 0.21, 0.53 and 2.20 percent. The 95% confidence intervals are represented by the error bars. Day 2 refers to 10 days after the start of the experiment.

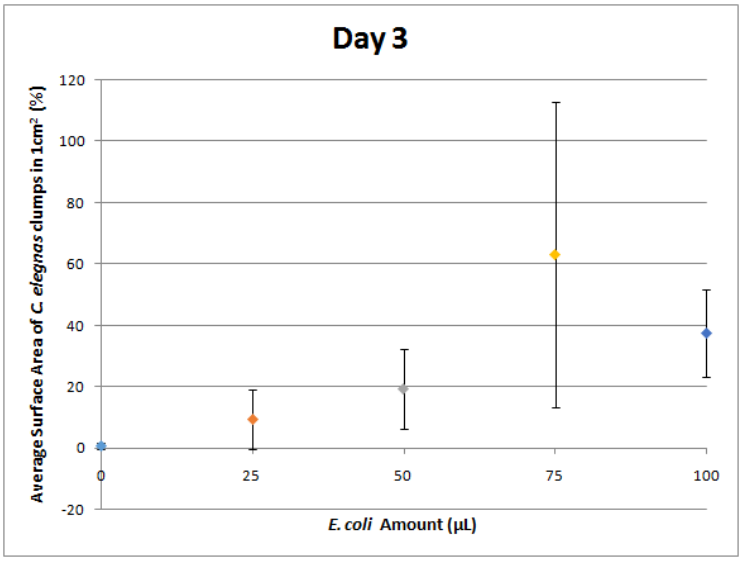

Figure 7. Day 3 mean surface area of *C. elegans* clumps in percentage where 1cm^2 surface area represents 100% coverage for different amounts of *E. coli*. At 0 (n=4), 25 (n=4), 50 (n=3), 75 (n=3), 100 µL (n=3) the respective average values are 0.75, 9.38, 1.73, 63 and 37.5 percent. The 95% confidence intervals are represented by the error bars. Day 3 refers to 11 days after the start of the experiment.

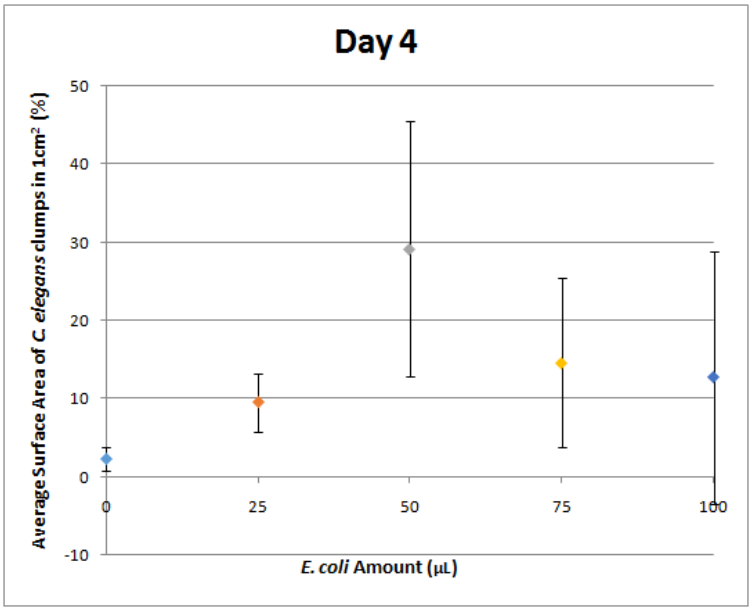

Figure 8. Day 4 mean surface area of *C. elegans* clumps in percentage where 1cm ^{2} surface area represents 100% coverage for different amounts of *E. coli*. At 0 (n=4), 25 (n=4), 50 (n=3), 75 (n=3), 100 µL (n=3) the respective average values are 2.34, 9.50, 29.17, 14.58, and 12.71 percent. The 95% confidence intervals are represented by the error bars. Day 4 refers to 12 days after the start of the experiment.

Below are the sample calculations for the average, standard deviation and 95% confidence intervals for day 1 for the 25µL treatment group:

Average = $\bar{x} = \frac{\sum x_i}{n} = \frac{0+3+1+2+0}{5} = \frac{6}{5} = 1.20$

Standard Deviation = $\sigma = \sqrt{\frac{\sum(x-\bar{x})^2}{n-1}}$

$$= \sqrt{\frac{((0-1.20)^2+(3-1.20)^2+(1-1.20)^2+(2-1.20)^2+(0-1.20)^2)}{5-1}} = 1.30$$

95% Confidence Interval = $\bar{x} \pm \frac{1.96 \times \sigma}{\sqrt{n}} = 1.20 \pm (\frac{1.96 \times 1.30}{\sqrt{5}}) = 1.20 \pm 1.14$ $\therefore (0.06, 2.34)$

Judging by the average of the values on each day, the *C. elegans* displayed no trend between quantity of *E. coli* and clump size; since the results are not significantly different due to the overlapping 95% confidence intervals in all the graphs (Figures 5-8).

DISCUSSION

We fail to reject the null hypothesis: we observed that exposing *Caenorhabditis elegans* to a smaller amount of *Escherichia coli* will yield smaller or equal social feeding clusters than a larger amount of *Escherichia coli.*

C. elegans use their nociceptive neurons to detect stressful conditions; which include high population density, limited food source, and increased temperature (Meléndez *et al.* 2003). In response to these conditions, the neurons induce social feeding behaviour as a defense mechanism to reduce their stress (de Bono *et al.* 2002). Among the listed conditions, we chose food limitation as our variable. With that limit, *C. elegans* have less resources to be shared for survival which will add stress, and eventually result in increased social feeding behaviour,

demonstrated by highly dense aggregations of worms surrounding the food source (i.e. clumps). For that reason, we predicted a negative relationship between *E. coli* quantity and *C. elegans* clump size; as smaller food amount implies higher degree of starvation, which then causes higher stress level and in consequence, larger clumps of socially feeding worms. The outcome of our experiment, however, did not coincide with our predictions.

First, our predictions led us to expect a negative slope in the graphs for all 4 days. However, the data in Figures 5-8 show no trend between the amount of *E. coli* and the cluster size of *C. elegans*. At day 1 of clump size measurements we expected all replicates to be starved. However, we made the assumption that the treatments that started off with more food (i.e., 75µL, 100µL) would be less starved and thus less stressed than those that started off with less food (i.e. 0µL and 25µL). Thus, we expected to observe clumps in all treatments; and that the clumps would be larger in the 'more starved' groups. However, due to large variations within the treatment levels, most of the 95% confidence intervals overlap and thus, we cannot say that there is a significant difference between the treatments. Additionally, each graph showed a different pattern, which means that no trend can be observed. Therefore, our results show no relationship between food amount and clumping, and thus no relationship between limiting food source and social feeding behavior can be made.

We believe that our results were affected by a third variable, which is supported by studies done by other literatures. Overcrowding is another stress factor that is known to enhance *C. elegans*' aggregation behaviour (de Bono *et al.* 2002). Decreasing space availability for *C. elegans*, as in increasing crowding, causes an increase in DAF-16 nuclear localization. DAF-16 is a transcription factor that regulates different stress responses (Du *et al.* 2013). Thus, crowding induces stress responses in *C. elegans*, just like starvation. In general, stress promotes mutualistic

behavior as a strategy for survival (Odum 1985). Thus, due to crowding stress, the worms feed in groups to assist in toxin protection (de Bono *et al*. 2002). In short, population density also influences the cluster formation of the nematodes. Thus, we come to conclude that there were two stress factors affecting the clustering behaviour: restricted food amount and high population density. This can be seen in the 100µL treatment, which was the treatment with the highest population density in our experiment. In Figures 6 and 8, the 100µL treatment had a mean clump size that was significantly larger than the mean clump size for the 0µL and 25µL treatments on day 1 and day 3. As for day 2, the 100µL treatment had a mean clump size that was significantly larger than all the treatments except the 25µL group (see Figure 7). Thus, we can conclude that we observed a higher clump size than we expected for the 100µL treatment due to the influence of both overpopulation and starvation as combined stress factors.

Furthermore, we expected the average cluster size to be the biggest at 0µL, which represented the 'most stressed' group. However, this was overturned by the minimal clustering behaviour of the nematodes observed for all four days in that treatment. Figures 5-8 show that the average cluster size for the 0µL treatment was always significantly lower than another treatment; and therefore never had the biggest cluster size. Nonetheless, this can be explained; wild type *C. elegans* of strain N2 are known to aggregate into swarms when there is a limited food source, yet they disperse when food is completely absent (de Bono and Bargmann 1998). Thus, the nematodes exhibiting little or no social feeding behaviour at the 0µL treatment correlate with the findings of de Bono and Bargmann (1998); the worms disperse and are not keen to form groups.

In this experiment there were multiple sources of error. First, overpopulation of nematodes is a probable source of error in our measurements since overpopulation created a stressful environment for the nematodes, even when there was plenty of food.

Also, we may have accidentally transferred unwanted elements from the initial plate to the replicate plates, thus gaining unexpected errors. We used the transfer plates to get rid of any residue such as eggs, larvae or food from the adult nematode's body, which can affect the population growth and the consistency of the treatment levels. Still, there was the chance of accidental transfer of *E. coli* into the replicate plates because we used only five transfer plates in total (one transfer plate per treatment). A minimum of 15 worms were placed in each transfer plate, thus some of the nematodes placed on the transfer plate may have picked up the bacteria of the nematodes that were transferred earlier, thus defeating the purpose of transfer plates. The accidental *E. coli* transfer added variability within replicates of same treatment level. As an example, we observed food in 0µL treatment, indicating that food was added as a consequence of accidental *E. coli* transfer.

Furthermore, inappropriate use of worm-picks was also a possible source of error. During transfer, we accidentally jabbed the agar with the worm-pick which created holes in the medium where the worms hid and later died. Also, after sterilization, the platinum of the worm-pick may still have had heat on it, thereby injuring the nematodes. As a consequence, the initial number of adult *C. elegans* on the replicate plates was unequal.

Despite using sterile technique during transfer, we had plates containing contamination with fungus. Since fungi are pathogenic to *C. elegans*, the worms on a contaminated plate would get infected and would only survive between 1.5 to 7 days (Austebel and Powell 2008). This might have affected the clumping behaviour of *C. elegans* as the plates would have external

factors that would decrease the population size. Therefore, the only course of action was to discard the plates. This also affected our data since the number of replicates decreased.

Some measurement errors include subjectivity in measuring clump size using a $1cm^2$ grid and difficulty in measuring the clumps as the worms did not remain in one location for longer than a few minutes. Thus, all our measurements of clump size should only be considered as estimates.

That being said, the largest source of error in our experiment was the effect of overcrowding as an additional stress factor. Therefore, if this experiment was to be repeated, to avoid overpopulating the replicate plates, we could take a simple random sample of each replicate by transferring a small number of adult worms (~10) to a new, empty replicate plate after the first week of starvation. This will allow us to observe the starved adults outside of a crowded environment; a relationship between food limitation and social feeding behavior could then be made. In addition, instead of measuring cluster size, it would be more representative of our objective if we calculate the proportion of the population that is socially feeding. This would be a more accurate and simpler as a method.

CONCLUSION

In conclusion, we failed to reject the null hypothesis, since there is no significant difference between clump size of *C. elegans* exposed to different amounts of *E.coli*. Thus, according to our results, limiting food source has no effect on the social feeding behaviour of *C. elegans*. However, we noticed that overpopulation is another critical stressor that activates the nociceptive neuron which induces social feeding behaviour in *C. elegans*.

ACKNOWLEDGEMENTS

We thank Dr. Carol Pollock and Katelyn Tovey for their insightful and extensive guidance on the design of the experiment. Also, we thank Mindy Chow, the course lab technician, for preparing the *C. elegans* and the agar plates used in this experiment and for providing important feedback throughout the experiment. The equipment of this experiment was generously provided by the Biology faculty of the University of British Columbia.

LITERATURE CITED

Bargmann, C. I. 1998. Neurobiology of the *Caenorhabditis elegans* genome. Science, **282**(5396): 2028-2033.

Boender, A. J., Roubos, E. W., and Van der Velde, G. 2011. Together or alone? Foraging strategies in *Caenorhabditis elegans*. Biological Reviews, **86**(4): 853-862.

Cheung, B., De Bono, M., Persson, A., and Rogers, C. 2006. Behavioral motifs and neural pathways coordinating O_2 responses and aggregation in *C. elegans*. Current Biology, **16**(7): 649-659.

Coates, J. C., and De Bono, M. 2002. Antagonistic pathways in neurons exposed to body fluid regulate social feeding in *Caenorhabditis elegans.* Nature, **419**(6910): 925-929.

De Bono, M., and Bargmann, C. I. 1998. Natural variation in a neuropeptide Y receptor homolog modifies social behavior and food response in *C. elegans*. Cell, **94**(5): 679-689.

De Bono, M., Tobin, D. M., Davis, M. W., Avery, L., and Bargmann, C. I. 2002. Social feeding in *Caenorhabditis elegans* is induced by neurons that detect aversive stimuli. Nature, **419**(6910): 899-903.

Du, W., Feng, X., Hu, L., Liu, B.F., Tang, L., Wang, X., and Xia, Y. 2013. Stress response of *Caenorhabditis elegans* induced by space crowding in a micro-column array chip. Integrative Biology, **5**: 728-737.

Meléndez, A., Talloczy, Z., Seaman, M., Eskelinen, E., Hall, D.H., and Levine, B. 2003. Autophagy genes are essential for dauer development and life-span extension in *C. elegans*. Science, **301**(5638): 1387-1391.

Odum, E. P. 1985. Trends expected in stressed ecosystems. BioScience, **35**(7): 419-422.

Powell, J. and Ausubel, F. 2008. Models of *Caenorhabditis elegans* Infection by Bacterial and Fungal Pathogens. **415**: 403-427.

So, S., Tokumaru, T., Miyahara, K., and Ohshima, Y. 2011. Control of lifespan by food bacteria, nutrient limitation and pathogenicity of food in *C. elegans*. Mechanisms of Ageing and Development, **132**(4): 210–212.

Stiernagle, T. 2006. Maintenance of *C. elegans*. WormBook, ed. doi/10.1895/wormbook.1.101.1, http://www.wormbook.org.

Wood, W. B. 1988. Nematode *Caenorhabditis elegans*. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory.