The effect of salinity on the head movements of *Caenorhabditis elegans*

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ABSTRACT

The movement of N2 wild-type strain of *Caenorhabditis elegans* was studied at different salinity concentrations to see if there was a relationship between salinity and movement. We immersed *C. elegans* in 3 different NaCl concentrations (5.86 g/L, 9.93 g/L and 14.0 g/L) with 10 replicates each. We recorded the number of times the *C. elegans* changed their head directions for 60 seconds using a DinoXcope. We found that the number of head turns decreased with an increase in salinity. The maximum number of head turns was 196.3 ± 24.4 at 5.86 g/L NaCl, followed by 170.3 ± 29.6 at 9.98 g/L NaCl and 124.0 ± 22.4 at 14.0 g/L NaCl. After doing a t-test, we found that there was a significant difference between two treatments; 5.86g/L and 14.0g/L, thus leading us to reject our null hypothesis. We concluded that the number of head movements of *C. elegans* decreases as the concentration of NaCl increases.

INTRODUCTION

Caenorhabditis elegans is a small-roundworm species that reaches 1-2 mm in length during adulthood (Felix and Braendle 2010). As *C. elegans* are considered a model organism, they are often utilized in studies related to the understanding of human diseases (Kaletta and Hengartner 2006). The use of *C. elegans* in these significant studies requires scientists to have a comprehensive understanding of the organism itself. This is achieved by studying its habitat and the multitude of variable factors it is exposed to. Among the abiotic factors, optimal levels of pH, temperature, hardness, and salinity, are all instrumental in the development of the roundworm (Khanna *et al.* 1997). In this study, we focused on salinity and its effect on the head movements of the N2 --wild-type strain of *C. elegans.*

Roundworms, or nematodes, live in a variety of environments including areas where they are fully submerged in water; so salinity could very well be a limiting factor for nematode distribution (Capstick 1959). Additionally, nematodes, *C. elegans* in particular, populate various microbe-rich communities like soils or rotting fruit (Felix and Braendle 2010). Thus, we can reasonably deduce that depending on the environment; nematodes experience extensive exposure to salts. Khanna *et al.* (1997)

found that the organism could tolerate upwards of 15.5 g/L of NaCl. Using this value as a guide, we set a lower limit (the provided buffer of 5.86 g/L) and obtained three treatments for our experiment, by averaging the two extremes. This wide range of tolerance is not surprising, since nematodes are capable of forming special larvae when necessary, generally in unfavorable situations (Riddle *et al.* 1981). These larvae, known as dauer larvae, can survive in stressful environments for months before adverse effects become apparent (Felix and Braendle 2010).

Nematodes sense salinity levels using a nervous system with 302 neurons located at the head (White *et al.* 1986). A simple diagram is displayed in Figure 1 below. Out of these 302 neurons, 60 are used for chemical, mechanical and thermal stimuli (Felix and Braendle 2010). By way of a 1000 G-protein coupled receptors functioning in chemosensory preferences, *C. elegans* can acclimate to different environments (Felix and Braendle 2010). This system would also be proficient in sensing the salinity levels, as it is a form of chemical stimulus. Through observations in lab, the trademark sinusoidal movement always incorporated the turning of the head. Thus, by exclusively looking at head turns, we managed to obtain a good indicator of movement.

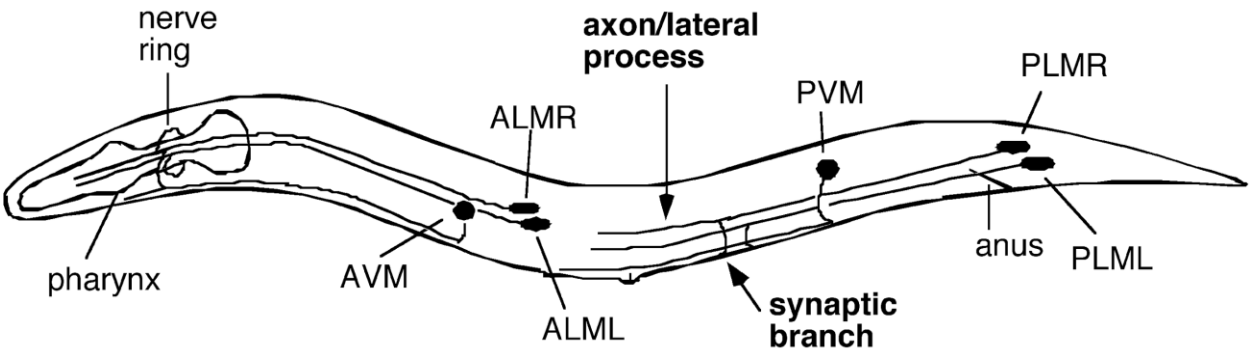

Figure 1. The sensory system of *C. elegans* including a nerve ring and some specific neurons (Nonet 2012)

In this experiment the nematodes will be subjected to higher levels of salt (NaCl) concentrations, such that it would increasingly deviate away from normal levels of 3.075 g/L (Williams and Dusenberry 1990). Ward (1973) observed that *C. elegans* makes frequent stops in higher concentrations of salt

relative to moving around in lower concentrations. It makes sense that the nematodes are active in concentrations closer to the salinity to which they are accustomed. Thus, it is justified to expect that the nematodes would have more twitches in the lower of the tested salt concentrations. These conclusions led to our hypotheses:

H_0- Environments of higher NaCl concentrations increase or will have no effect on the number of head turns performed by *Caenorhabditis elegans*.

H_a- Environments of higher NaCl concentrations decrease the number of head turns performed by *Caenorhabditis elegans*.

METHODS

In this experiment, we used two Kyowa dissecting microscopes at 7X magnification; one for picking up the nematode and the other for observing and recording its behavior using the DinoXcope. The set up is shown in Figure 2 below.

Figure 2. General set up of the experiment. Left microscope was used to aid us while picking the nematode. Right microscope was connected to DinoXcope to observe and record the nematode's head movement

We investigated the N2 wild-type hermaphrodites *C. elegans* in this experiment. We tested them in three different sodium chloride (NaCl) concentrations; 5.86 g/L, 9.98 g/L and 14.0 g/L, where we set 5.86g/L as the control solution. We chose 14.0 g/L to be the maximum concentration, because in an earlier experiment performed by Khanna *et al.* (1997), it was found that the maximum concentration tolerance for *C. elegans* is 15.5 g/L. Therefore, keeping a lower concentration than 15.5 g/L wouldn't harm the nematodes to a greater extent for our experiment. To make the 9.98g/L solution, we used 50mL of stock 5.86 g/L solution with 50mL of 14.0 g/L stock solution. In each of the treatments, there were ten replicates (n=10), and we used sterile techniques to handle the nematodes and the treatment solutions.

The main objective was to pick up an adult nematode from the 100mm *E. coli* plate, which is in its last L4 stage, and transfer it into the centre of a new 60 mm agar dish with 20 µL salt solution. Before each transfer, we sterilized the worm pick by flaming it for 5 seconds. We tried to pick the adult nematodes for a few reasons; because they are easier to see at a lower magnification and therefore easier to pick, and also because there is a higher chance of observing their activity in the salt solution since they are likely to survive longer than younger nematodes. We then left the worms for 80 seconds to acclimate to the salt solution. We counted the number of head turns for every replicate; each time the worm changed its direction was counted as one head turn. We counted for 60 seconds after the 80-second acclimation period. In our data, we only included the worms that were moving for the entirety of the 140 seconds, and discarded those that died upon contact with the solution. This is because those worms might have been damaged while we were picking them up; further explanation is mentioned in the sources of error. We recorded the behavior of each nematode for a total of about 140 seconds using the

DinoXcope. Figure 3 below shows a screenshot of one of the videos that were recorded during the experiment. These videos were useful for making qualitative observations for our experiment.

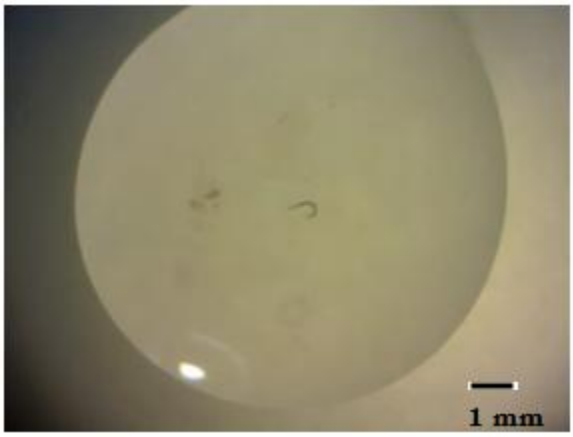

Figure 3. Video screenshot of *C. elegans* movement in salt solution at magnification of 7X.

We used 7X magnification so that the 20 µL salt solution and the worms are shown in the field of vision. This enabled us to view the nematode's movement in the entire solution and made it easier for us to observe and count the number of head turns.

Finally, we collected the data for all 30 replicates and organized it into a data table. Using this data, we calculated the means and 95% confidence intervals, and plotted them on a scatter plot. For further analysis of the results, we also performed a t-test on our data, the details of the results are outlined below.

RESULTS

For qualitative observations, we saw that nematodes turned their heads back and forth the fastest compared to the other two treatments when immersed in 5.86 g/L NaCl. The head turn is shown in Figure 4 below, where the nematode would come together in a circle as shown and then open up. Nematodes in both the 5.86 g/L and 9.93 g/L salinity concentration were found to swim around the corners of the bubble of solution instead of moving in the centre spot (Figure 5). The nematodes in the 14.0 g/L NaCl treatment were found to move slower on average than the other two treatments.

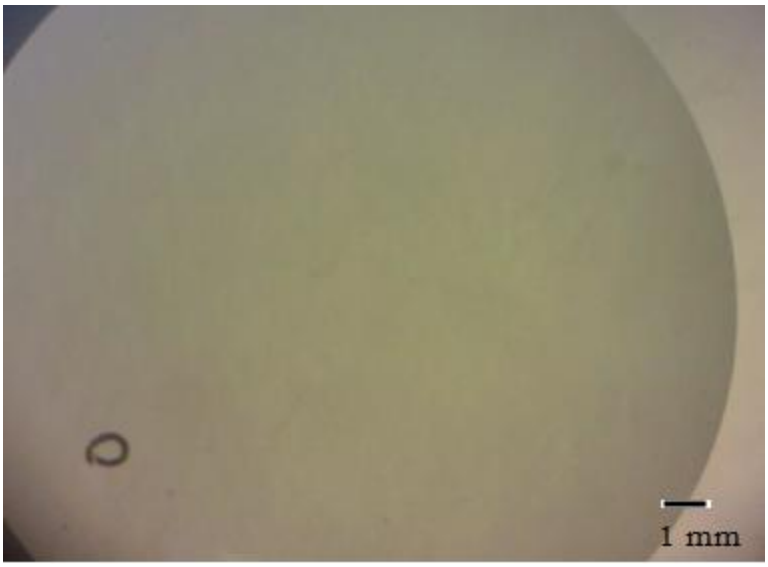

Figure 4. *C. elegans* in 5.86 g/L NaCl in the middle of a head turn.

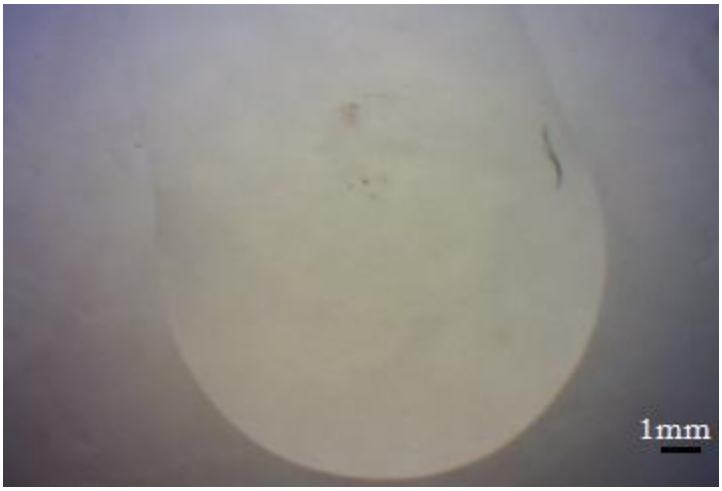

Figure 5. *C. elegans* in 9.93 g/L NaCl solution swimming around the edges of the solution.

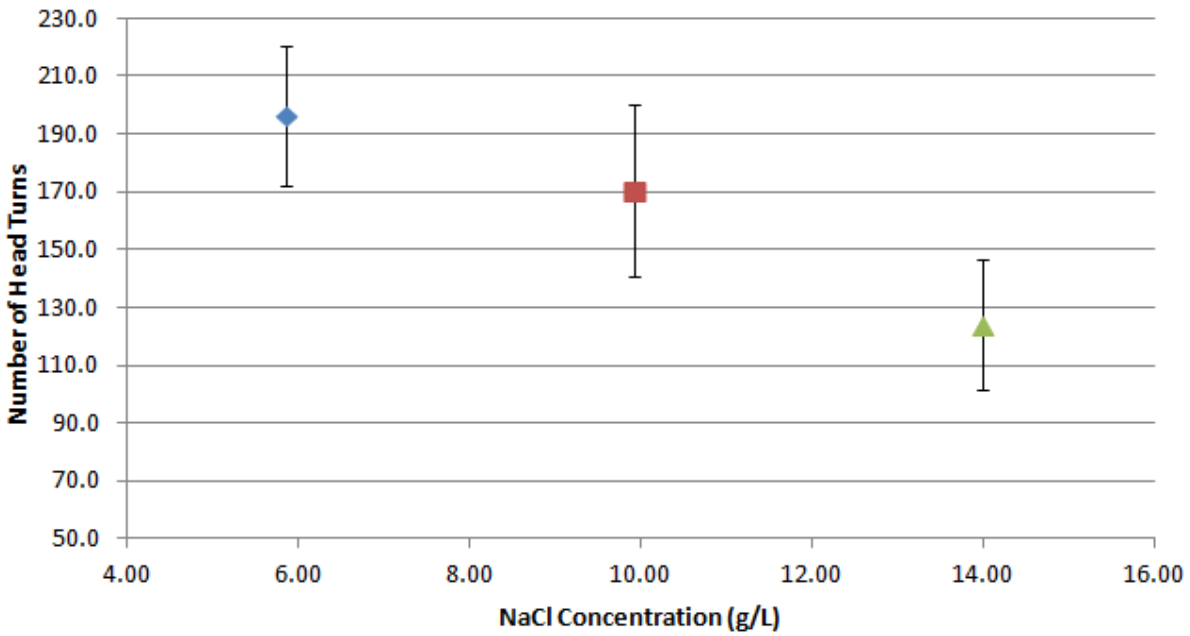

Figure 6. The number of head turns of *C. elegans* at different salinities. Treatments include 5.86 g/L NaCl, 9.93 g/L NaCl and 14 g/L NaCl, with error bars indicating 95% confidence intervals. N= 10 for each treatment.

The data were graphed by calculating the 95% confidence interval for each treatment. The 95% confidence intervals for the 5.86 g/L, 9.93 g/L and 14.00 g/L NaCl treatments were 196.3 ± 24.4 head turns, 170.3 ± 29.6 head turns and 124.0 ± 22.4 head turns, respectively. From Figure 6, a trend showing the average number of head turns decreasing as the salinity increases can be observed. The data were analyzed by comparing the 95% confidence intervals. The 95% confidence intervals for treatment 5.86 g/L NaCl and 14.00 g/L NaCl do not overlap. We further analyzed this difference by doing a t-test. We inserted the specific values of our results into the following formula to see if there was a significant difference between the two treatments.

$$t = \frac{\bar{x}_1 - \bar{x}_2}{s\sqrt{\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$$

Our calculated t value was 4.278. The two-sided 95% theoretical t value, which is 2.101, was found to be lower than our calculated t value. Thus, there is a significant difference in the number of head turns between the two treatments, 5.86 g/L NaCl and 14.00 g/L NaCl.

A sample calculation for the 5.86 g/L NaCl treatment group is shown below:

1) Mean:

$$\bar{x} = \frac{\sum x}{n}$$

= (179 + 164 + 177 + 146 + 227 + 176 + 274 + 174 + 236 + 210) / 10= 196.3 head turns

2) Variance:

$$s^2 = \frac{\sum (x - \bar{x})^2}{n - 1}$$

= [(179-193.3)² + (164-193.3)² + (177-193.3)² + (146-193.3)² + (227-193.3)² + (176-193.3)² + (274-193.3)² + (174-193.3)² + (236-193.3)² + (210-193.3)²] / 9= 1544.2 head turns

3) Standard Deviation:

$$s = \sqrt{s^2}$$

√1544.2 head turns= 39.3 head turns

4) 95% Confidence Interval:

$$C.I. = \bar{x} \pm 1.96\frac{s}{\sqrt{n}}$$

196.3 ± 1.96*(39.3/(√10))= 196.3 ± 24.4 head turns

DISCUSSION

According to the statistical analysis in our results, the alternate hypothesis that *C. elegans* have a lower number of head turns in an environment of higher NaCl concentration is supported. The null hypothesis that *C. elegans* have a higher number or same number of head turns in an environment of higher NaCl concentration is rejected. Our results show that *C. elegans* turn their heads significantly less when being in a medium of 14.0g/L NaCl compared to when being in a medium of 5.86g/L NaCl, since the confidence intervals of these two concentrations do not

overlap. Further analysis of the data shows that there is an overall negative correlation between the concentration of NaCl and the number of head turns performed by *C. elegans* (Figure 6).

C. elegans can tolerate NaCl concentrations up to 15.5 g/L (Khanna *et al.* 1997). A possible explanation of our results is that since 14.0 g/L NaCl is an environment of high NaCl concentration to *C. elegans*, they do not find the need to turn their heads to seek for even higher concentrations of NaCl and therefore, they perform fewer head turns. According to Ward (1973), *C. elegans* use chemosensory organs on their heads to detect NaCl molecules. These chemosensory neurons include ASE neurons, which play an integral role in chemotaxis, with NaCl in particular (Uchida *et al.* 2003). Through their chemosensory organs, these worms sense and subsequently compare the different concentrations and recognize a concentration gradient by traveling through them, and eventually gravitate towards areas of higher attractant concentration (Ward 1973). As 14.0 g/L NaCl is only slightly below the lethal NaCl concentration to *C. elegans*, 15.5 g/L (Khanna *et al.* 1997), *C. elegans* do not need to perform as many head turns to seek for areas of even higher NaCl concentrations.

Another possible explanation of our results is that *C. elegans* turn their heads less frequently as they are not able to choose a direction to avoid the high NaCl concentration. According to Menini (2010), if the NaCl concentration is too high, *C. elegans* will move away from that area to avoid high osmotic pressure to their body; this response is mediated by the ASH neurons (Hilliard *et al.* 2004). As mentioned in the above paragraph, 14.0 g/L of NaCl is near the highest concentration that *C. elegans* can tolerate (Khanna *et al.* 1997). However, *C. elegans* need to detect a concentration gradient to determine the direction of its movements (Ward 1973). Since the NaCl concentration is constant within the droplet that *C. elegans* is

immersed in, it will be difficult for the *C. elegans* to find a direction to avoid the high NaCl concentration; thus, leading to lower frequency of head turns.

Most obstacles we encountered during the experiments pertained to the transport and transfer of the nematodes. Since these nematodes are very small in size, we had some difficulties transferring them to the agar with the salt solution without damaging either the nematode or the agar itself. A few of the worms died on contact with the salt solution, and this may have been because of previous damage from the transport. The nematodes are quite fragile, and picking them up with a sharp platinum worm pick increases the risks of injury, hence it is likely that those of worms died upon placement. This eventually results in fewer number of head turns than theoretical number of head turns. Another human error that may affect the results is the different reaction time of each individual in counting head turns. Since the task was divided amongst the group members, it is likely that all our reaction times vary. This discrepancy in counting could have lead to an increase in variation and subsequently lead to the overlap of results.

Biological variations also exist in our experiment. *C. elegans* of different life-stages may display varied number of head turns when immersed in salt solution. In order to minimize biological variations in our results, we decided to only pick the large nematodes from our culture, ensuring they are all of similar size and at the same life stage (adults). We also increased the number of replicates for each treatment to minimize any external and biological variations. Since we had to use our judgments while picking these worms, it is possible that those we thought were adults were actually only L3 or L4, thus increasing human errors which lead to an increase variation and subsequently to the overlap of results. We also minimized the amount of error by using sterile technique. We sterilized the worm pick right before picking up the worms, and the

flasks containing the solutions were sterilized before and after the extraction of solution using a fresh micropipette tip each time.

Future studies of *C. elegans* regarding salinity should include techniques to minimize the damage to the organisms when transporting. They should also account for a wider range of NaCl concentrations by starting with a lower concentration of NaCl as one of the treatments, as a visible change may be observed between the lowest and highest concentrations. Furthermore, the exposure time for each number of replicates and the number of replicates could also be increased. Future studies should also focus on long-term effects of increasing salt concentrations and observe whether the changes are genetically linked and can or cannot be passed through the generations.

CONCLUSION

After analysing the number of head movements of *C. elegans* in three different concentrations of NaCl (5.86 g/L, 9.98 g/L and 14.00 g/L), we found that the head movement decreased as we increased the concentration of NaCl. Therefore, we reject our null hypothesis (H_0) that *C. elegans* have a higher number or same number of head turns in an environment of higher NaCl concentration, and we support our alternate hypothesis (H_a) that *C. elegans* have a lower number of head turns in an environment of higher NaCl concentration.

ACKNOWLEDGEMENTS

First and foremost, we cannot express enough gratitude for Dr. Carol Pollock. Without her continual support and guidance we could not have formulated a working experiment by making the necessary adjustments. Similarly, we would also like to thank Mindy Chow for her

invaluable role in preparation of supplies and input in experimental design. Additionally, we are very grateful to Haley Kenyon for the indispensable advice she provided prior to and during our experiment. Lastly, we would like to thank the University of British Columbia and the Combined Major in Science program for giving us the unique opportunity to create our own experiments and fully gather the Biology 342 experience.

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