Effect of NaCl concentration on the mid-body movement of *Caenorhabditis elegans*

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Abstract:

Caenorhabditis elegans has a nervous system that enables it to explore and respond to its environment, in a process called chemosensation. *C. elegans* have been observed to be attracted to high NaCl concentrations. In our study, we used treatment levels of 0.10 M, 0.15 M, 0.20 M, and 0.25 M NaCl. Nematodes were tracked in NaCl using a DinoXcope to record their movements. Nematodes were allowed a 10 second acclimation period, and then movement was recorded for 30 seconds. The recordings were then analyzed using the software, WormLab, to calculate the average distance travelled by their mid-body at each of the four treatment levels. We performed a one-way ANOVA on our data and calculated a p-value of 0.122. For the lowest concentration, 0.10 M, the mean track length value was found to be 9.1 mm, while at the highest concentration mean track length was 22.6 mm. Although the average track length increased as NaCl concentration increased, statistical analysis showed that there was no significant difference in the means. Therefore, we concluded that increasing salinity does not have a significant effect on the mid-body movement of *C. elegans*.

Introduction

Caenorhabditis elegans, in the phylum Nematoda, is a free-living roundworm found in the soil in temperate environments in all regions of the world. They are transparent, have a cylindrical shape with bilateral symmetry and their bodies typically grow to approximately 1 mm in length (Bargmann, 2006). A cuticle surrounds *C. elegans*; the cuticle is a tough outer layer whose main function is to protect the organism from harm. *C. elegans* lacks a respiratory and a circulatory system; however, it has a nervous system which uses sensory neurons to interact with its surrounding environment and interpret various external conditions (Hilliard et al., 2002). As it travels through the environment, *C. elegans'* neurons detect sensory cues, evaluate them, and convert them into information that can lead to movement. Cues can stem from potential food sources, danger in the surroundings, or the presence of other animals. *C. elegans'* nervous system

contains more than 300 neurons and it has sensory cilia on the body surface that are key in sensing the chemical cues (Gray et al., 2005). The head, or the leftmost region of Figure 1, is where specialized amphid neurons reside. Amphid neurons are able to interact with the environment thanks to small openings in the tough cuticle (Hilliard et al., 2002).

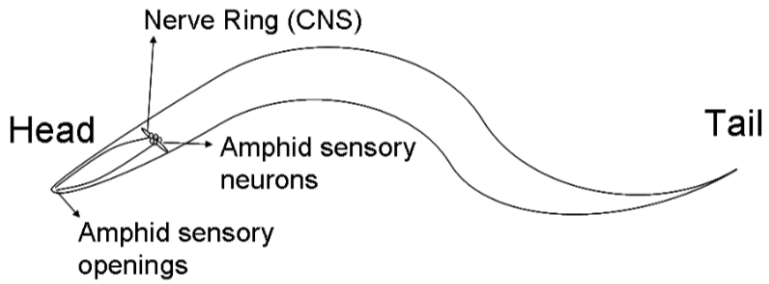


Figure 1: *C. elegans,* with neurons highlighted (image from Goldstein 2009)

Motor output to muscle cells is the beginning of the pathway that allows the organism's response to take place. At a side called the neuromuscular junction, the exchange of information between nerve and muscle takes place as the muscle receives electrical signals from neurons. This sequence of events ends in muscle contraction and allows for movement of the organism in a sinusoidal path (McIntire et al., 1993). This snakelike motion is achieved thanks to innervation on opposing sides of the organism's body, the ventral and dorsal sides, which helps muscle contraction to be synchronized and assists in propagation of the body (Ward, 1973). Studies have seen *C. elegans* make its way along a gradient of changing stimulus concentration, as it has the ability to sense the changes in attractant concentration and responds by maneuvering its body and aligning itself to facilitate chemotaxis towards high salt concentration (McIntire et al., 1993).

Khanna et al. (1997) showed that *C. elegans* could tolerate up to 15.46 g/L or 0.26 M NaCl (over 24 hours) in K-medium, (an enriched seawater medium). We used the above

information to select treatment levels that were within the limits of NaCl tolerance for *C. elegans*.

There is currently a lack of information as to why *C. elegans* prefer higher NaCl concentrations; however, we know that *C. elegans* can detect and react to both sodium and chlorine ions in aqueous environments (Culotti and Russell, 1978). Their preference for higher NaCl may stem from the fact that they are led by a concentration gradient or that their biological functions are carried out more efficiently under these conditions (Pierce-Shimomura et al., 1999; Ward, 1973). Through our experiment we hope to discover useful information about the relationship between NaCl concentrations and mid-body movement levels of *C. elegans*, which will help to further understanding of the effect of chemical signalling on movement.

H_o: Increasing salinity has no effect on the mid-body movement of *Caenorhabditis elegans*.

H_a: Increasing salinity has an effect on the mid-body movement of *Caenorhabditis elegans*.

We predicted that in environments of higher NaCl concentrations, *C. elegans* will display a higher degree of mid-body movement. This prediction is supported by a previous study conducted which found *C. elegans* to be more attracted to higher concentrations of NaCl when placed in a concentration gradient (Ward, 1973).

Methods

We placed wild-type strain N2 *C. elegans* in a buffer with 0.10 M, 0.15 M, 0.20 M, and 0.25 M of NaCl. These levels were chosen because we found through previous studies that *C. elegans'* ideal salinity levels were 0.20 M and that they were able to tolerate up to 0.30 M

without any adverse effects (Hu et al., 2015). We used two treatment levels below the ideal concentration (0.10 M and 0.15 M) and one above (0.25M) because we did not want to place them in an environment out of their tolerance range.

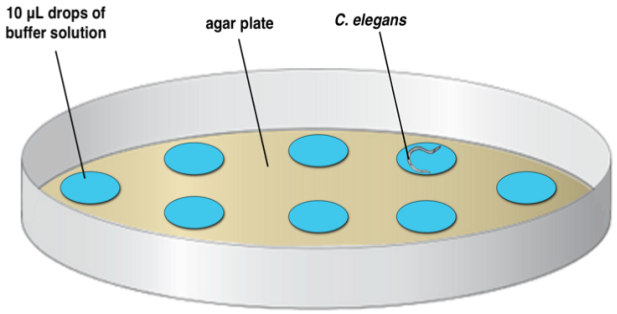


Figure 2: Setup of petri dish; 8 droplets of storage buffer on a single petri dish that contains agar. *C. elegans* placed inside of each droplet.

Experimental Setup

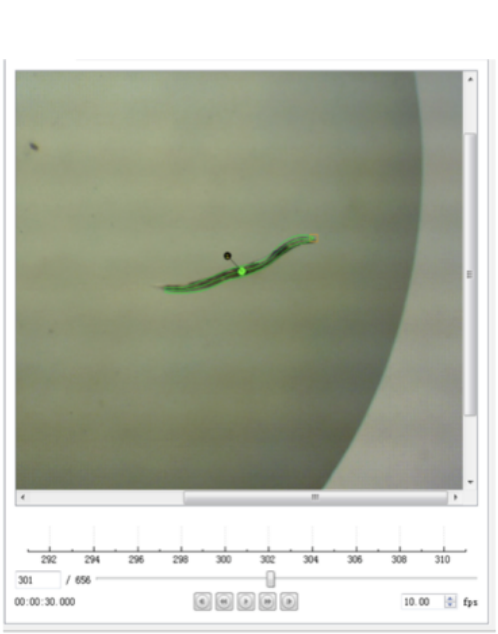
We placed 10 µL of the desired buffer onto a 60-mm diameter petri dish that contained agar with no *E. coli*. Removing *E. coli* eliminated a factor (food) that could affect our results. Before placing an organism onto a petri dish, we focused the microscope and the attached DinoXcope (ocular camera) onto the buffer solution. With a sterilized worm pick, we transferred a single *C. elegans* into the buffer under the microscope (Figure 2). When selecting each nematode to be transfer, we took the largest nematodes we could find from the petri dish containing the culture of *C. elegans*. In order to minimize the amount of variation between all treatment levels and replicates, the same person transferred each nematode and we collected all data on the same day.

Data Collection

Once we placed the wild-type the *C. elegans* into the buffer, we allowed an acclimation period of 10 seconds. After the 10-second acclimation, we recorded the movements of *C. elegans*

for 30 seconds. During these 30 seconds, we moved the petri dish around so the organism would stay within the field of view as seen in the recording. This was done to help ensure the WormLab program would be able to detect and accurately track the nematodes.

We repeated this seven more times (for a total of eight replicates) for this treatment level. The videos for each of these trials were labeled and saved to prevent confusion between video files of different treatment levels. This process was repeated for each treatment level (0.10 M, 0.15 M, 0.20 M, and 0.25 M).



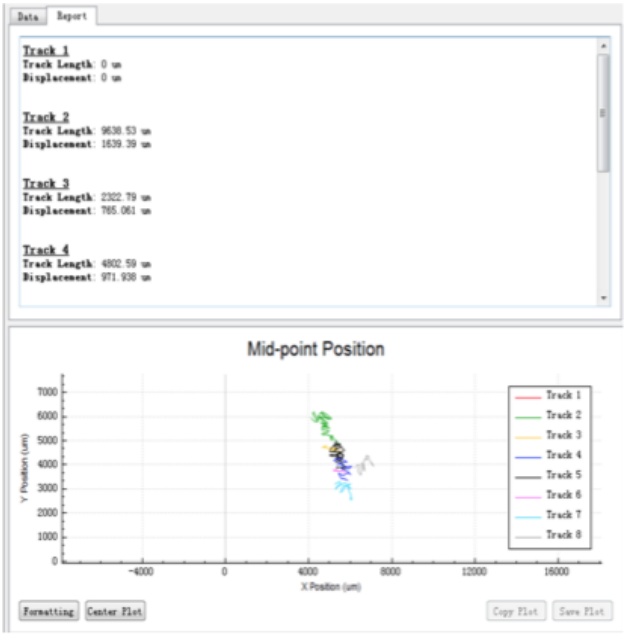


Figure 3 (left): Detection of *C. elegans* using WormLab; Figure 4 (right): Graph of mid-body position and track length data of *C. elegans* produced from WormLab.

Data Analysis

After recording and archiving all the videos from each treatment level, we processed them using WormLab. By using WormLab, we were able to upload the video files into the program, which would then detect the nematode in the field of view (Figure 3). After using the tracking portion of the software, we extrapolated the data by having WormLab graph the

tracking data of the mid-body position of each nematode. In addition to plotting the position (X- and Y-direction) over the recorded time, WormLab also gave us the length of that graph labeled as "Track Length" (Figure 4). There were some videos that produced multiple tracks for the same video, but this was due to the software losing track of the nematode and re-targeting it over the 30 second recording time. Thus the total amount the midpoint of the nematode moved was the sum of all the "Track Length" values.

Once we found the total track length for every video, we found the average for every treatment level and performed a one-way ANOVA in order to determine the statistical significance of our results.

Results

When exposed to various levels of NaCl, *C. elegans* displayed different levels of mid-body movement. It was found that in a concentration of 0.10 M of NaCl, the average mid-body movement was 9.008 mm with a standard deviation of 3.484 mm. This was fairly similar to the mid-body movement of *C. elegans* in 0.15 M of NaCl, where the average was 7.775 mm with a standard deviation of 2.784 mm. The average mid-body movements at higher concentrations of NaCl displayed a high average amount of movement: shown by 0.20 M of NaCl with an average of 22.263 mm with standard deviation of 6.244 mm and 0.25 M of NaCl with an average of 22.596 mm with standard deviation of 6.265 mm. These were found by adding up the track lengths and averaging them at each treatment level, and these results can been seen in Figure 5. It can also be see that there is a significant difference in movement between the higher two treatment concentrations and the lower two concentrations.

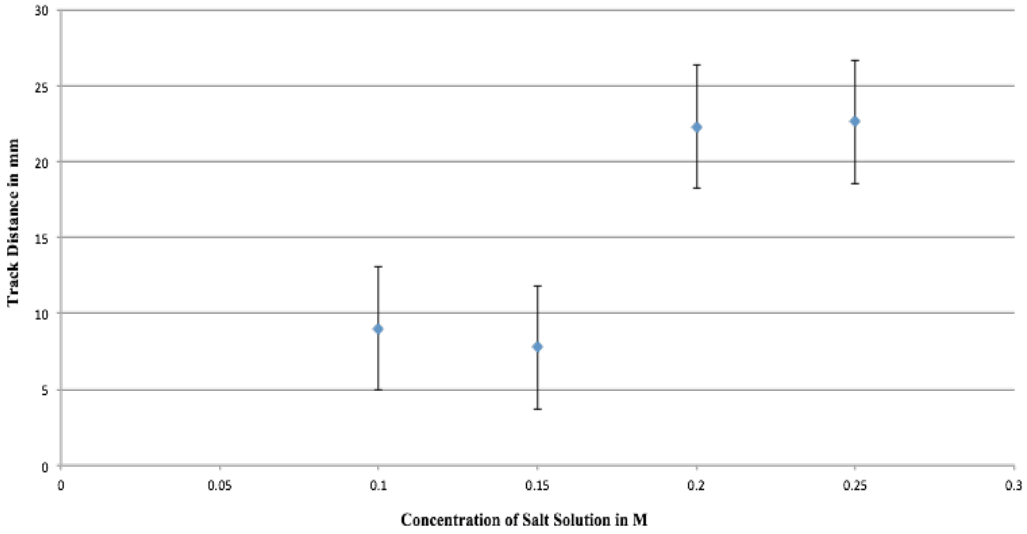


Figure 5: Graph of mean track lengths with corresponding errors bars what are these error bars? for each treatment level (0.10 M, 0.15 M, 0.20 M, 0.25 M).

We used a one-way ANOVA statistical test in order to determine the statistical significance of the data. The p-value was 0.122, which is greater than 0.05 which indicated that there was no statistically significant effect of NaCl concentrations on the mid-body movements in *C. elegans*.

Discussion

Based on the results from a one-way ANOVA test where $p > 0.05$, we failed to reject our null hypothesis that there is a statistically significant difference between the mid-body movement of *C. elegans* at various NaCl concentrations. Although there is a trend where there is increased mid-body movement with increased salinity (Figure 5), the differences in the means were not significant. Research has shown that *C. elegans* do not respond to absolute levels of concentration but do respond to a concentration gradient (Pierce-Shimomura et al., 1999). *C. elegans* navigate with a chemosensory system that uses short-term memory to determine their

optimal direction of motion. The short-term memory chemotaxis operates by comparing recent levels of concentration to current levels of concentration, ultimately guiding the organism to areas of higher concentration (Pierce-Shimomura et al., 1999). But in our experiment where there was no variation in concentration within each treatment, the *C. elegans* had no directionality to follow and therefore no response changes in their chemosensory system. This resulted in findings that were not statistically significant.

Further testing could be performed to obtain data that would allow us to confidently reject our null hypothesis and support the trend that is depicted in our graph (Figure 5). Possible improvements could be performing more replicates at each of the treatment levels, to reduce the large variation in the data. By gaining more data entries we could reduce the impact of variation on the mean: this may allow us to achieve a more precise mean, which may more accurately represent the differences among the average movements at each treatment level. Another improvement could be increasing the number of treatment levels to include higher concentrations in order to obtain data of a greater range. This can allow us to better observe and calculate, at a wider range, if there is a trend with increasing mid-body movement with increasing concentration.

In addition to increasing the range of treatment levels, exploring NaCl concentrations between our measured treatment levels would help to explain the abrupt jump in mid-body movement between 0.15 M and 0.20 M concentrations. We hypothesized that this sudden change in mid-body movement could be linked to a threshold of chemical sensing by *C. elegans* when interacting with its environment. But further testing would need to be conducted in order to more accurately explain this sudden change in mid-body movement.

Although our data does not allow us to reject our null hypothesis, other studies do not support this outcome. We predicted that when the *C. elegans* are exposed to higher salinity concentrations they will become more active and thus have more mid-body movement. The chemosensory system measures and detects salinity concentrations outside of the body by extending its sensory cilia into the environment (Ward, 1973). Most of these sensory cilia directly affect the intra-flagellar transport (IFT) system which transports proteins and influences the structure of cilia and flagella (Blacque et al., 2005). Since the sensory cilia would be exposed to the higher NaCl concentration that *C. elegans* are attracted to, it is possible that higher NaCl concentrations allow for better internal functioning of the IFT and therefore improved movement. This is supported by the research conducted by Ward in 1973 when he experimented on the behaviour of *C. elegans* when exposed to a salinity concentration gradient, and found that *C. elegans* moved towards areas of higher concentrations.

We would need to perform further tests to gain a stronger quantitative database that would allow us to confidently determine the effect of salinity on the mid-body movements of *C. elegans* and then determine if our results are consistent with the literature.

Conclusion

When *C. elegans* is placed in buffers with different concentrations of NaCl, the concentration of NaCl had no effect on the mid-body movement of *C. elegans*, thus we failed to reject our null hypothesis. These findings are consistent with previous research where scientists found *C. elegans* responded to concentration gradients, but did not respond to changes in absolute concentration (Pierce-Shimomura et al., 1999). While we fail to reject our null hypothesis, the results from the experiment, although not statistically significant, still helped to support our prediction that higher NaCl concentrations would increase the mid-body movement

of *C. elegans*. This study helps demonstrate methods of studying the influence of chemicals on mid-body movement in *C. elegans*, and how chemosensory systems of *C. elegans* may impact overall physical activity in the organism.

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Erratum

There was an error in the calculation of the one-way ANOVA. The true p value is less than 0.05. Therefore the null hypothesis is rejected and the results are as predicted with support from the literature as discussed.