Effects of attractant concentration on pirouetting response of *Caenorhabditis elegans*

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ABSTRACT

The nematode *Caenorhabditis elegans* (*C. elegans*) use a pirouetting motion to re-orientate themselves with respect to the concentration gradient of the attractant. The number of pirouettes performed by *C. elegans* during a three-minute span in the presence of the attractant sodium acetate (NaOAc) was counted. Two separate trials were performed. In trial one, 0.5 M, 1.0 M, and 1.5 M treatments of NaOAc and 0.0 M water controls were used. In trial two, 0.5 M, 1.5 M, and 3.0 M treatments of NaOAc and 0.0 M water controls were used. Individual worms were followed and the number of pirouettes performed was counted. In trial one, the results showed a gradual decrease in the number of pirouettes performed as NaOAc concentration increased. In trial two, there was no evident trend in the results. By combining the results of both trials, we see that as the NaOAc concentration increased from 0.5M to 1.5M, there was a gradual decrease in the average number of pirouettes, but at 3.0M, there was a spike in the number of pirouettes. Our findings suggest that an increase in the concentration of NaOAc causes a decrease in the number of pirouettes performed by *C. elegans*, but beyond a certain, optimum concentration, toxicity may cause an increase in the number of pirouettes.

INTRODUCTION

One of the most basic needs of living organisms is the requirement for high quality food and resources. This instinct can be easily observed in mammals such as rats (Young 1932) or human infants (Davis 1928); when they were offered different choices of food, they selected the diet that provides an optimal balance of nutrients for their growth and health.

A free-living nematode, *Caenorhabditis elegans (C. elegans)* also shows this behavior where they pursue higher quality food over hard-to-eat and less nutritious bacteria (Shtonda and Avery 2006). To obtain the optimal food, the worms show two different types of locomotion: a sinusoidal-swimming movement known as a “run”, and a turning motion known as a “pirouette” in which the worm’s head curls back, touching or crossing the tail, before the worm continues to move forward in the opposite direction (Shontda and Avery 2006, Pierce-Shimomura *et al.* 1999). Running is a rapid straight,

wave-like movement that propels the worm forwards whereas pirouetting is the worm's method of re-orientating its body position to change directions.

This kind of physical response to the presence of attractants, such as a chemical, is known as chemotaxis. Chemoreceptors called amphids and phasmids, which are located at the head and the tail of *C. elegans* respectively, sense the presence of the chemical in the gradient, and then induce a physical response from the worm (Hilliard *et al.* 2002). One of the common attractants used to test chemotaxis of *C. elegans* is sodium acetate (NaOAc), where a study done by Matsuura *et al.* (2007) suggests that 1.0 M of sodium acetate is the optimal concentration for adult worms.

Despite numerous studies done on chemotactic response of *C. elegans*, it is not clear how the presence of different concentrations of an attractant will affect their pirouetting behavior. Thus, this current study investigates the relationship between the numbers of pirouettes performed by *C. elegans* with an increasing concentration of NaOAc. This experiment provides a stronger understanding of the pirouetting behavior of *C. elegans* in the presence of an attractant, and will provide guidelines and data that may be useful in future studies.

We worked with the idea that a higher concentration would indicate to the organism that a higher amount of attractant was available in their immediate environment. Thus, we hypothesized that the worms would pirouette more in higher concentrations as there is a higher amount of attractant present, giving two hypotheses:

H_A: An increase in the concentration of sodium acetate will increase the number of pirouettes performed by *Caenorhabditis elegans.*

H_O: An increase in the concentration of sodium acetate will decrease or will not affect the number of pirouettes performed by *Caenorhabditis elegans.*

METHODS

Figure 1. Preparation of the filter paper circles. In this figure, the filter paper circle is being placed into a centrifuge tube to soak.

For trial one, we made dilutions of 0.5 M, 1.0 M and 1.5 M from a 3.0 M NaOAc stock solution for our different treatments. Water (0.0 M) was used as the control. Using a hole puncher, we punched out filter paper circles with a diameter of 7.5 mm. Next, we placed them in a centrifuge tube containing one of the different treatments or the control, as seen in Figure 1. Each circle was soaked for at least three minutes. To create a gradient, the filter paper circles were each set near the edge of a 60 mm agar plate. Each filter paper circle sat on the agar plate for the attractant to spread and create a gradient for at least 45 minutes before it was removed.

For trial two, we used 0.5 M, 1.5 M and 3.0 M NaOAc for our different treatments. Again, water (0.0 M) was used as the control. We followed the same procedure as trial one except we set up the agar plates approximately 19 hours before the experiment was conducted in order for the attractant to spread across the plate as much as possible. For this trial, we did not remove the filter paper circles from the agar plates.

Using the diffusion coefficient $D = 10^{-5}$ cm^2s^{-1} (Luo *et al.* 2010), we calculated the distance travelled by the attractant for trial two. The attractant moved approximately 1.6 cm away from the filter paper during the 19 hours before the experiment.

For both trials, we used N2 wild type strain *C. elegans*. Using the Kyowa dissecting microscope and a worm pick, we relocated adult worms from their original growth plate to a plain agar transfer plate using sterile technique. We left the worms on the transfer plate for at least two minutes to allow the adult worms to separate from younger worms, eggs, and any food that may be on their body. For each plate being tested, we transferred one adult worm, using sterile technique, from the transfer plate to the treatment plate. For trial one, we placed the worm near the edge of the concentration gradient. We placed the worms approximately 1 cm away from the filter paper for trial two. Once on the plate, we observed the worms for three minutes.

Figure 2. DinoXcope set up. The camera was placed in the eyepiece of the microscope and connected to a laptop.

For trial one, we used the DinoXcope apparatus to view the worms during the experiment. This allowed us to take photos of the worms while they were pirouetting. For trial two, the DinoXcope was used for some of the 0.5 M and 1.5 M replicates. For the remaining replicates, observations were made simply using the Kyowa microscope. The set up for the DinoXcope apparatus in the microscope is seen in Figure 2. The camera was placed in the eyepiece of the microscope and then connected to a laptop for viewing.

Table 1. Example of the data table used for data collection. The data here is from the water control treatment of trial one.

Treatment	Replicate Number	Time Observed	Pirouettes
0.0 M (water control)	1	3:00 mins	2
0.0 M (water control)	2	3:00 mins	2
0.0 M (water control)	3	3:00 mins	4

Qualitative Observations:
#1 – moved away from location of concentration gradient, moved back and forth
#2 – stayed in same relative area, moved back and forth
#3 – stayed in same relative area, moved back and forth

Data were obtained using a data table with headings for treatment, replicate number, time observed and number of pirouettes, as demonstrated in Table 1. We counted the number of pirouettes each worm performed in three minutes after being introduced to the plate. We counted a pirouette as when the worm's head touched its tail, to form a circular shape, as seen in Figure 3. Qualitative observations were made regarding the direction in which the worms seemed to be moving, if they were moving back and forth, or any other peculiarity that should be noted.

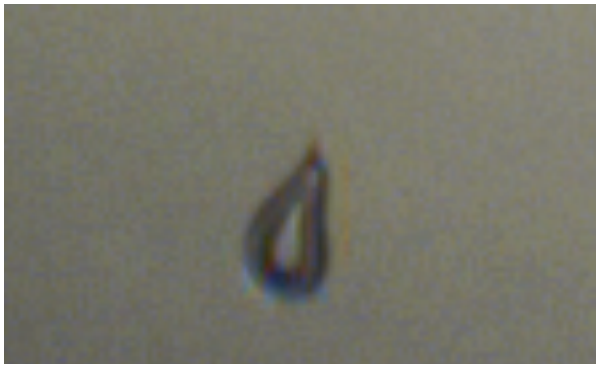

Figure 3. A *C. elegans* performing a pirouette. Pirouettes were counted as when the head of the worm touches the tail, as seen here.

We analyzed our data using 95% confidence intervals after calculating the mean and the standard deviation for the data.

RESULTS

The two separate trials were performed on two different days. The results of trial one and trial two have been graphed and labeled in Figure 5 and 6, respectively.

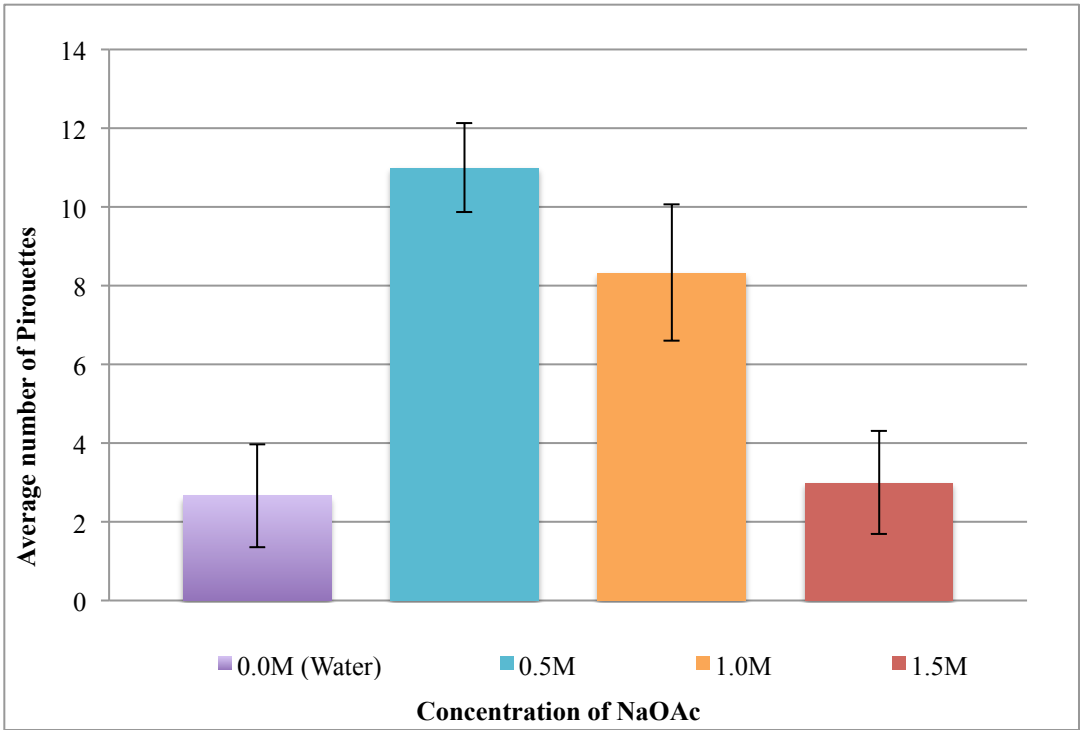

Figure 5. Average number of pirouettes performed by *C. elegans* for different concentrations in the first trial. At 0.0 M, 0.5 M, 1.0 M and 1.5 M the average number of pirouettes was 2.67, 11, 8.3 and 3, respectively. Error bars represent 95% confidence intervals.

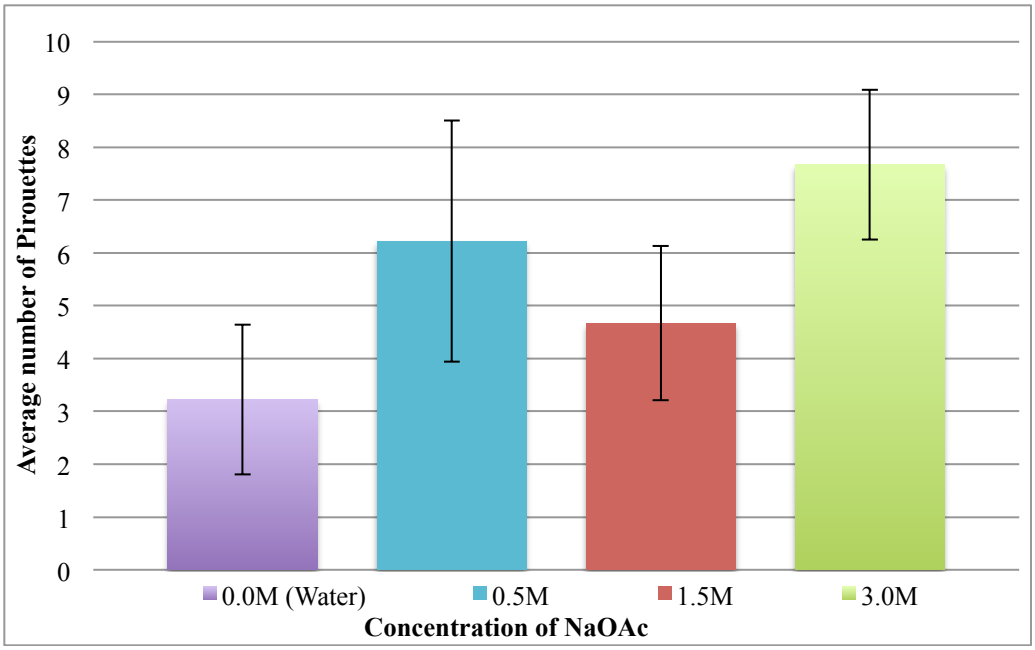

Figure 6. Average number of pirouettes performed by *C. elegans* for different concentrations in the second trial. At 0.0 M, 0.5 M, 1.5 M and 3.0 M the average number of pirouettes was 3.2, 6.2, 4.67 and 7.67, respectively. Error bars represent 95% confidence intervals.

In Figure 5, there was a gradual decrease in the average number of pirouettes performed as the concentration of sodium acetate (NaOAc) increased. The water control treatment had the lowest average number of pirouettes. There was a significant difference between the 0.5 M treatment and the water control, the 1.0 M treatment and the water control, the 0.5 M and 1.5 M treatments, and the 1.0 M and 1.5 M treatments because there is no overlap between the 95% confidence intervals. Although it appears to overlap, the lower 95% confidence interval for the 1.0 M treatment is 6.60480757 and the upper value for the 1.5 M treatment is 6.3947572. However, there is no significant difference between the 95% confidence interval of the 1.5 M treatment and the water control.

In Figure 6, there was no overall gradual trend as the concentration of NaOAc increased. However, all three NaOAc treatments had a higher average number of pirouettes than the water control. There was no overlap between the confidence intervals of the 1.5 M and 3.0 M treatments, as well as the 3.0 M treatment and the water control, signifying a significant difference between these treatments. However, there was overlap between the other confidence intervals.

To demonstrate sample calculations, we calculated the average, the standard deviation, and the 95% confidence intervals for the 0.5 M NaOAc treatment in the first trial.

Average: Average = $\bar{x} = \Sigma x_i / n = (12 + 10 + 11) / 3 = 33 / 3 = 11$

Standard Deviation: Standard Deviation = $\sigma = \sqrt{(\Sigma (x - \bar{x})^2 / (n - 1))} = \sqrt{(((12-11)+(10-11)+(11-11))^2 / (3-1))} = 1$

95% Confidence Interval: 95% CI $= \bar{x} \pm ((1.96 * \sigma) / \sqrt{n}) = 11 \pm ((1.96*1) / \sqrt{3}) = 11 \pm 1.3158573 = (9.86841427, 12.13158573)$

DISCUSSION

In trial one, our data shows a clear trend that increasing the concentration of NaOAc decreases the number of pirouettes that *C. elegans* performed. Therefore, we fail to reject our null hypothesis. In trial two, we observed a significant increase in the number of pirouettes that *C. elegans* performed from 1.5 M to 3.0 M. Therefore, based on our trial two data, we have sufficient evidence to reject our null hypothesis.

The experimental procedure changed between trial one and trial two, which caused variation and error in our data. In trial one, the three attractant concentrations were 0.5 M, 1.0 M and 1.5 M. The filter paper soaked close to the edge on each agar plate for 45 minutes making a small and strong NaOAc gradient. We removed the filter paper before the experiment placed each replicate by the edge of the NaOAc gradient.

In trial two, we changed the treatment concentrations to 0.5 M, 1.5 M and 3.0 M. We placed the filter paper in the center of each agar plate and soaked for a minimum of 19 hours, prepared the day before trial two. Therefore, the gradient should have evenly covered the majority of the 60 mm agar plate. As a result, the gradient was weaker in trial two. Also, we didn't remove the filter paper and observed some experimental worms to briefly interact with the filter paper. We had three successful replicates per treatment in trial one whereas we had nine replicates per treatment in trial two.

C. elegans behavior could differ depending on its response to the strength of the gradient (Gray *et al.* 2005, Pierce-Shimomura *et al.* 1999). Therefore, our pirouetting observations between trial one and two could be affected as a consequence from changing from a steep to shallow gradient. The change in gradient slope is important in interpreting our data. Literature states the pirouetting rate is more dependent on the gradient rather than the concentration present (Miller *et al.* 2005, Pierce-Shimomura *et al.*

1999). Specifically, *C. elegans* pirouette in response to a very recent gradient change in its environment (Pierce-Shimomura *et al.* 1999). In our trials, we successfully observed our replicates to pirouette after moving down the gradient and then have runs back up the gradient. However, the gradient doesn't fully explain the behavior we observed in our study.

Our data can be explained more clearly by taking into consideration the combined effect of the gradient strength and the attractant concentration. According to Gray *et al.* (2005), pirouettes become less frequent in preferable environments. We also predicted the worms to prefer the stronger gradients. However, in both trials the 0.5 M and 1.5 M treatments showed a similar decreasing trend in pirouettes with increasing concentration. A possible interpretation of our decreasing trend could be the replicates preferred the 1.0 M, from trial one, and the 1.5 M, from trial two, concentrations. So, we observed fewer pirouettes because the replicates were already in a preferable gradient and didn't need to re-orientate themself (Gray *et al.* 2005).

In contrast, we observed significantly more pirouettes in the 3.0 M replicates in trial two. This could be explained based on the idea that the concentration was too strong for the replicates (Miller *et al.* 2005). The attractant concentration's toxic effect on the behavior of the replicates needs to be taken into consideration. Observations from the 3.0M treatment showed that five out of the nine replicates immediately moved down the gradient. Also, all of the 3.0 M replicates remained at the agar plate's edge where the gradient would be weakest. One study found high attractant concentrations repelled *C. elegans* causing an avoidance response down the gradient (Miller *et al.* 2005). The high pirouetting could be a result of the *C. elegans* being distressed by the concentration and continuously seeking a lower gradient (Miller *et al.* 2005).

Possible variation between our replicates is presumably experimental error during the experimental procedure. Also biological variation such as age, sex, size and health could have affected the behavior between replicates. Our procedure was also much more modest and simpler than the scientific literature studies' procedures which could not be replicated in lab.

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CONCLUSION

Chemotaxis is vital for *C. elegans* to be able to assess their environment and re-orientate themselves towards attractants through pirouetting (Pierce-Shimomura *et al.* 1999). It was found that when increasing the concentration of the attractant, sodium acetate, from 0.5 M to 1.0 M and 1.5 M, *C. elegans* performed fewer pirouettes. It was found that increasing the concentration from 1.5M to 3.0M increased the number of pirouettes performed possibly because of toxicity. Understanding the relationship between optimal and toxic concentrations and *C. elegans* behavioral response is important to further understanding chemotaxis.

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LITERATURE CITED

Davis, C. M. 1928. Self-selection of diet by newly weaned infants. American Journal of Diseases of Children, **36**(4): 651-679.

Gray, J. M., Hill, J. J., and Bargmann, C. I. 2005. A circuit for navigation in *Caenorhabditis elegans*. Proceedings of the National Academy of Sciences of the United States of America, **102**(9): 3184-3191.

Hilliard, M. A., Bargmann, C. I., and Bazzicalupo, P. 2002. *C. elegans* responds to chemical repellents by integrating sensory inputs from the head and the tail. Current Biology, **12**(9): 730-734.

Iino, Y., and Yoshida, K. 2009. Parallel use of two behavioral mechanisms for chemotaxis in *Caenorhabditis elegans*. The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, **29**(17): 5370-5380.

Luo, L., Greenwood, J., Soucy, E., Kim, D., and Samuel, A. 2010. Making linear chemical gradients in agar. The Worm Breeder's Gazette, **18**(3): 10-11.

Matsuura, T., Endo, S., Iwamoto, R., Takahashi, H., and Ichinose, M. 2007. Developmental changes in chemotactic response and choice of two attractants, sodium acetate and diacetyl, in the nematode *Caenorhabditis elegans*. Comparative Biochemistry and Physiology - Part A, **147**(4): 920-927.

Miller, A. C., Thiele, T. R., Faumont, S., Moravec, M. L., and Lockery, S. R. 2005. Step-response analysis of chemotaxis in *Caenorhabditis elegans*. Journal of Neuroscience, **25**(13): 3369-3378.

Shtonda, B. B., and Avery, L. 2006. Dietary choice behavior in *Caenorhabditis elegans.* The Journal of Experimental Biology, **209**, 89-102.

Pierce-Shimomura, J. T., Morse, T. M., and Lockery, S. R. 1999. The fundamental role of pirouettes in *Caenorhabditis elegans* chemotaxis. Journal of Neuroscience, **19**(21): 9557-9569.

Young, P. T. 1932. Relative food preferences of the white rat. Journal of Comparative Psychology, **14**(3): 297-319.