The effect of temperature on the speed of locomotion in *Caenorhabditis elegans*

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ABSTRACT

Changes in environmental temperature are known to have strong impacts on the biochemistry and behaviour of ectothermic organisms such as *Caenorhabditis elegans*. In this experiment, the effect of temperature on the speed of locomotion of N2 wild-type *C. elegans* at 12.0°C, 15.0°C and 20.8°C (control) was studied. The total distance travelled along an agar-filled petri dish per unit time was calculated for each replicate, and subsequently converted into speed. A positive correlation between temperature and the average speed of *C. elegans'* locomotion was found. Significant differences between the mean locomotion speeds were found to exist between select temperatures (ANOVA, p=0.002). Specifically, the mean speeds of *C. elegans'* locomotion were significantly slower at 12.0°C (3.8 ± 1.6 mm/min) and 15.0°C (5.2 ± 1.5 mm/min) than that at 20.8°C (8.6 ± 1.7 mm/min) (Tukey Kramer HSD, 12.0°C vs. 20.8°C p=0.004 and 15.0°C vs. 20.8°C p=0.03). However, the differences in mean locomotion speed at 12.0°C and 15.0°C were not statistically significant (Tukey Kramer HSD, p=0.48). Treatment temperatures below the control temperature could induce a cold shock response in *C. elegans* where TRPA plasma membrane channels prevent Ca^{2+} from exiting the cell. In motor neuron cells, the increased intracellular [Ca^{2+}] reduces the cell's excitability; whereas in muscle cells, high intracellular [Ca^{2+}] results in decreased ATP availability. Both scenarios reduce *C. elegans'* muscle contractions and consequently, their locomotion speed at temperatures below their optima. Overall, results from this study give insight into how the competitive abilities of these and other nematodes may be impacted by global temperature changes.

INTRODUCTION

Caenorhabditis elegans are small, soil-dwelling nematodes that have a short, predictable lifespan and a completely mapped genome, making them useful model organisms for research studies. *C. elegans,* like other ectotherms, are highly sensitive to changes in the surrounding abiotic factors, such as temperature (Huey and Kingsolver 1989). Previous studies have found that temperature influences many aspects of nematode life including hatching, life cycle, and motility (Davide and Triantaphyllou 1968, Bird 1972). This experiment was performed in order to further investigate the potential relationship between temperature and *C. elegans'* locomotor behaviour; specifically whether temperature affects the speed of locomotion of N2 wild-type *C. elegans*.

Our null hypothesis was that temperature does not affect *Caenorhabditis elegans'* speed of locomotion, while our alternate hypothesis was that temperature does affect *Caenorhabditis elegans'* speed of locomotion. We predicted that *C. elegans'* speed of locomotion would be slower in any temperatures below its 20°C optimum. Previous studies have determined that these nematodes exhibit a maximal reproductive and growth rate at 20°C, thus locomotor speed is also expected to be greatest at this optimal temperature (Byerly *et al*. 1976; Vassilieva *et al.* 2000). A number of physiological and chemical changes occur within the cells of ectothermic organisms upon their exposure to suboptimal environmental temperature. At temperatures at and above 26.5°C, for example, *C. elegans* have been found to cease reproduction and display very limited motion (Fatt and Dougherty 1963, Prahlad *et al.* 2008, Lee and Kenyon 2009). Moreover, enzymatic reaction rates are known to decrease significantly in temperatures below an organism's optimal range (Somero 1978). Additionally, cooler temperatures are associated with decreased kinetic energy and membrane fluidity, both important components of passive and facilitated cross-membrane molecule transport (Mackey 1975). Furthermore, the tertiary structure—and thus the function—of certain transmembrane channels are also compromised by drops in environmental temperature, resulting in unfavourable chemical gradients across the plasma membrane. Ca^{2+} efflux, for example, is also affected by temperature in *C. elegans* (Gomez *et al.* 2001). As seen in Figure 1A, Ca^{2+} can properly enter and exit the cell via the TRPA channel at *C. elegans*' optimal temperature of 20°C. Conversely, in Figure 1B, it is evident that at temperatures below 20°C, the TRPA channels inhibit the exit of Ca^{2+}, leading to two consequences in different cells (Gomez *et al.* 2001). The outcome is that ATP becomes inaccessible within muscle cells and excitability potential decreases dramatically within motor nerve cells, resulting in reduced muscle contraction (Gao and Zhen 2011).

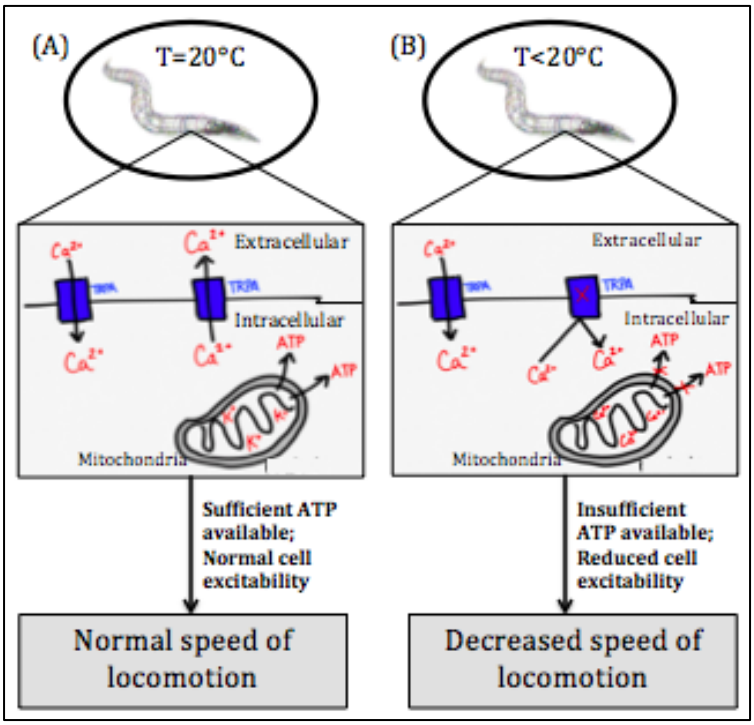

Figure 1. Model showing calcium entry and exit and its effects at the cellular and organismal level for *C. elegans* at (A) 20°C and (B) temperatures below 20°C.

Gaining a better understanding of how suboptimal temperatures affect *C. elegans'* locomotion speed can help us predict how other nematodes may be affected by climate change. If the motility of nematodes is reduced by changes in temperature, their competitive abilities may be compromised thus leading to habitat loss or even species extinction. This is of grave concern because many nematode species are decomposers; they convert decomposing organic matter into essential inorganic products, usable by primary producers (Wang and McSorely 2005). Without nematodes, primary producer abundance will be reduced and thus entire ecosystems would be negatively impacted (Wang and McSorely 2005).

METHODS

In this experiment, there were five replicates (N=5) in each of the three treatment temperatures, 12.0°C, 15.0°C, 20.8°C. We chose the latter temperature as our control treatment

because *C. elegans* have been found to grow and reproduce maximally at 20°C (Prahlad *et al.* 2008). Unfortunately, we did not have access to a temperature controlled room at 20°C, therefore, our control treatment room was 20.8°C (Byerly *et al.* 1976). We obtained three 60-mm Petri dishes each containing N2 wild-type *C. elegans* colonies grown at 20°C. Each Petri dish also contained *Escherichia coli,* which is a food source for the nematodes (Brenner 1974). To ensure both the nematodes and the agar in the prepared Petri dishes were at the appropriate treatment temperature, each dish containing the *C. elegans* was transferred into its respective treatment room four hours before data collection began. Five 60-mm Petri dishes were filled with agar, which would later be used as movement-surface for each replicate, were also placed into each temperature-controlled room at this time. *E. coli* was not added into the replicate dishes because we did not want the food source to affect the directionality of locomotion. Figure 2A and Figure 2B illustrate the experimental setup prepared in each room.

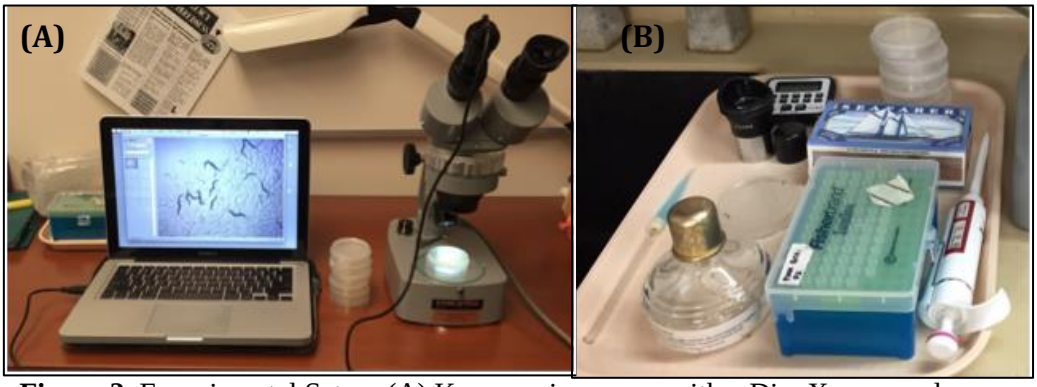

Figure 2. Experimental Setup: (A) Kyowa microscope with a DinoXcope ocular camera attached to a computer for video analysis (B) Materials used including: worm pick, flame for sterile technique, and micropipette.

Because age has been found to affect *C. elegans'* movement behaviour (Hart 2006), we chose to use only L4 stage individuals in our experiment in order to minimize inter-replicate age

variation. L4 stage *C. elegans* are identifiable by a white crescent-shaped mark in the vulval region, seen in Figure 3 (Koelle and Horvitz 1996; Hart 2006).

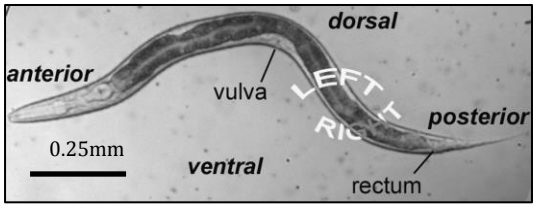

Figure 3. Image of an L4 larval stage *Caenorhabditis elegans*. (Source: Wormbook)

Within each treatment room, we used a Kyowa dissecting microscope and a sterile, platinum worm-pick to transfer one *C. elegans* worm at L4 larval stage into one of five prepared Petri dishes. While on the microscope stage (lighting setting 'IT'; magnification 15x), we allowed the replicate to acclimatize for five minutes in its new dish. Next, after ensuring that the replicate was in the field of view (FOV), we began the video recording of its movements using a DinoXcope ocular camera. In order to coax the worm to move across the FOV, 1 µL of 10% isoamyl alcohol, a chemoattractant for *C. elegans*, was added onto the opposite end of the dish using a micropipette (Bargmann *et al.* 1993). The video recording continued until the worm left the FOV. Qualitative observations, such as behaviour, movement, white crescent visibility, and notes on any procedural changes, were recorded for all replicates. The same procedure was followed for all replicates at all treatment temperatures. Finally, using the DinoXcope ocular camera, a photo was taken of a ruler under each microscope in order to calibrate the FOV.

After conducting the experiment in the lab, we analyzed all of the replicate video recordings to determine the speed for each replicate. First, we trimmed each video so that only continuous movement by *C. elegans* was observed. We recorded the length of each cropped video as the time of travel. Next, a global calibration was set on ImageJ using the picture of the ruler by determining how many pixels equalled 1 mm on the ruler. We tracked the distance travelled by each worm using both ImageJ and MTrackJ programs; the tail of each worm was

tracked frame by frame, as shown in Figure 4. The speed of each replicate was subsequently calculated by dividing the total distance travelled by the time of the trimmed video. The speeds of the five replicates in each treatment were then averaged to yield one mean speed of locomotion per treatment. These calculations, along with their respective 95% confidence intervals, were calculated using Excel. Further data analysis was performed using a one-way analysis of variance (ANOVA) to determine whether there was a significant difference between any of the three mean speeds. A post-hoc Tukey Kramer HSD test was subsequently performed to determine which mean values differed significantly.

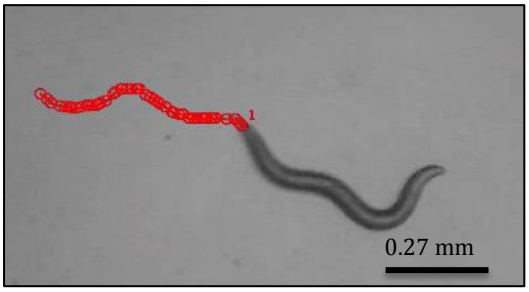

Figure 4. Image captured from ImageJ using the MTrackJ software to track the movement of *C. elegans* at 15x magnification.

RESULTS

Throughout the experiment, *C. elegans* replicates moved with their normal, sinusoidal locomotor patterns in all three of the treatment conditions. In addition, no anomalies were observed in regards to directional changes or stopping frequency in any of the 15 *C. elegans* individuals used. Overall, locomotive behaviour patterns appeared similar across replicates and treatments. However, upon analyzing the data, the average speed of locomotion was found to differ significantly between certain temperature treatments (ANOVA, p=0.002). As Figure 5 illustrates, there was a positive correlation between average speed of locomotion and temperature. The average locomotion speed of *C. elegans* at 12.0°C (3.8 ± 1.6 mm/min) was found to be significantly slower than that of *C. elegans* at 20.8°C (8.6 ± 1.7 mm/min), as seen in Figure 5 (Tukey Kramer HSD, p=0.004). Additionally, average *C. elegans'* speed in the 15.0°C treatment (5.2 ± 1.5 mm/min) was also found to be significantly slower than that in the 20.8°C

treatment (Tukey Kramer HSD, p=0.03). However, no significant difference was found between the average locomotion speeds of *C. elegans* at 12.0°C and 15.0°C (Tukey Kramer HSD, p=0.48).

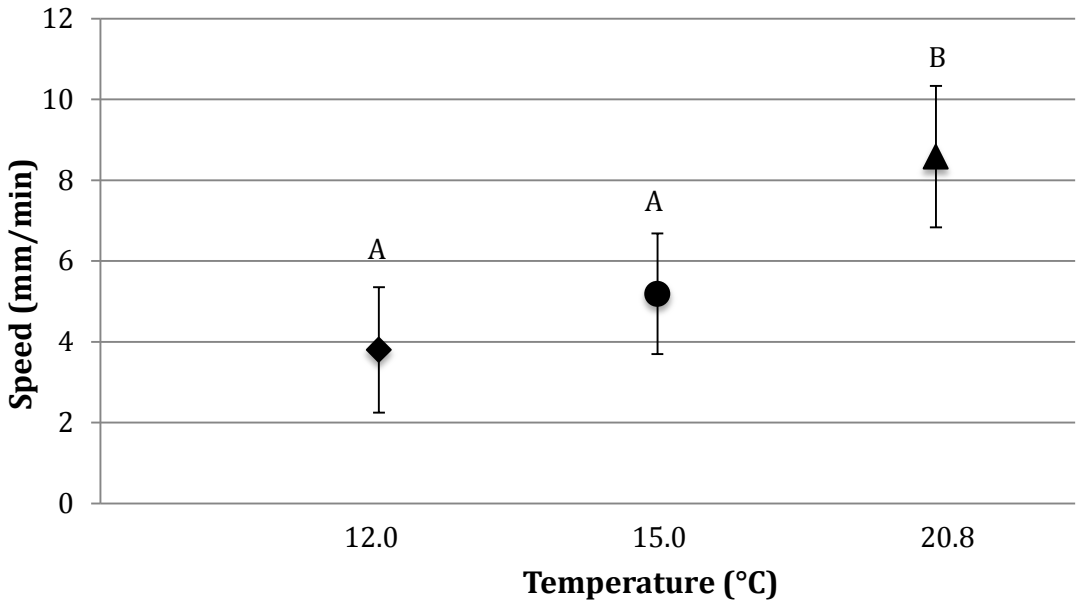

Figure 5. Average speed (mm/min) of *Caenorhabditis elegans* locomotion at 12.0°C, 15.0°C, and 20.8°C. Error bars represent 95% confidence intervals. N=5 for each treatment. Means with different letters are significantly different (Tukey Kramer HSD, p<0.05).

As is evident by the three similarly sized 95% confidence intervals seen in Figure 5, variation in the speed of replicates within each treatment of 12.0°C, 15.0°C, and 20.8°C was similar. Specifically, speed data from the 20.8°C treatment contained the greatest variation, represented by the largest 95% confidence interval of ± 1.7 mm/min, while speed data from the 15.0°C treatment contained the least variation, illustrated by the smallest 95% confidence interval of ± 1.5 mm/min.

DISCUSSION

The data collected from this experiment indicate that temperature does have an effect on *Caenorhabditis elegans* speed of locomotion, allowing us to reject the null hypothesis and

provide support for the alternate hypothesis (ANOVA, p=0.002). Results from this study show that *C. elegans* in colder temperatures (12.0°C and 15.0°C) move significantly slower than those at the optimal temperature (20.8°C) (Tukey Kramer HSD, 12.0°C vs. 20.8°C p=0.004 and 15.0°C vs. 20.8°C p=0.03). However, the difference between the mean speeds of locomotion of *C. elegans* at 12.0°C and 15.0°C was not statistically significant (Tukey Kramer HSD, p=0.48).

There are 959 somatic cells found within *C. elegans*, 75 of which are motor neurons that run longitudinally along the body and are responsible for locomotion (Gjorgjieva *et al.* 2014). Motor neurons respond to stimuli received through the thermosensory system; movement is induced by an attraction or repulsion to different stimuli such as temperature (Gjorgjieva *et al.* 2014). Once the sensory information is processed, efferent signals are sent through motor neurons as action potentials (Luo *et al*. 2014). Action potentials involve a change in the electrical potential across a neural cell membrane where Na^+ and Ca^{2+} enter the cell and K^+ leaves causing a depolarization and repolarization event (Gao and Zhen 2011). When an action potential is generated, it stimulates muscle fibers at a target location causing muscle contraction, leading to movement (Gao and Zhen 2011). In cold temperatures, a cold shock response is observed where the TRPA channel of cell membranes allows Ca^{2+} to enter but prevents its exit, as seen in Figure 1B. This inhibits cellular repolarization, decreasing the cell's excitability and consequently reduces action potential propagation (Xiao *et al.* 2013). The reduced excitability of efferent motor neurons leads to a reduction in muscle contraction and thus decreased movement at below-optimal temperatures. This pathway's effect on *C. elegans'* movement behaviour is consistent with the results obtained in the present study. The mean locomotor speeds of *C. elegans* in the two cold treatments (12.0°C and 15.0°C) may have been slower than that of the control (20.8°C) due to the negative cascading effects of TRPA malfunction on the excitability of motor neurons and consequently motor muscle contraction. Because TRPA channels are fully functional at

20°C, the motor neuron excitability of *C. elegans* in the control was not reduced and thus their muscles contracted at a frequency that lead to a greater mean locomotion speed.

Increasing levels of Ca^{2+} within the cell due to the aforementioned cold shock response also has a negative effect on mitochondria (Wojtovich *et al.* 2008). As seen in Figure 1B, when the TRPA channel prevents Ca^{2+} from exiting the cell, the intracellular concentration of Ca^{2+} increases. As a result, Ca^{2+} takes the position of K^{+} in the mK_{ATP} channel of the mitochondrial membrane (Wojtovich *et al.* 2008). This, in turn, prevents ATP from leaving the mitochondria and entering the cytoplasm for use by the cell (Xiao *et al.* 2013). ATP is a source of energy that is required for the active process of muscle contraction; therefore, low levels of ATP lead to muscle fatigue (Korzeniewski 1998). This relationship between temperature and its effect on ATP availability may also explain our results. Due to TRPA channel malfunction and increased intracellular [Ca2+] at low temperatures, low intracellular ATP available in *C. elegans* muscle cells at 12.0°C and 15.0°C may have led to reduced contractile force, and thus the slow locomotor speeds observed. Conversely, at 20.8°C, where TRPA channels are functional, *C. elegans* would have had sufficient cytoplasmic ATP to generate regular muscle contractions resulting in faster locomotion.

It should be noted that this experiment did not aim to identify which temperature-dependent mechanism led to the differential locomotor speeds at the various treatment temperatures. Further studies should be conducted to examine how the physiology of *C. elegans* nerve and muscle cells is affected by decreases in temperature as well as how other factors, such as enzyme kinetics, may play a role in the observed temperature-variable locomotor behaviour.

The difference in mean speed of *C. elegans'* locomotion at 12°C and 15°C was found not to be statistically significant (Tukey Kramer HSD, p=0.48). This could be attributed to the fact that these temperatures are only 3°C apart and at least 5.8°C below the control temperature.

Perhaps there is a threshold at which the cold shock response occurs and speeds below this temperature are all similarly affected. If this threshold is between 15.0°C and 20.8°C, it is expected that the speed of locomotion at 12.0°C and 15.0°C would not differ, as seen in our results. Another reason for the lack of difference in the speeds of *C. elegans* locomotion at the two colder temperatures could be attributed to a lack of replicates performed in this study. With N=5, variation is relatively high and the sample may not accurately represent the population at their respective temperatures. It is recommended that further studies be conducted with a greater number of replicates to determine whether the difference between the speeds at these two temperatures remains statistically insignificant.

The relationship between temperature and locomotor speed found in this study closely parallels those found in previously published studies. First, a study by MacMillan *et al.* (2011), which analyzed *C. elegans'* rate of movement at 11°C, 17°C, and 25°C, found that there was a positive correlation between temperature and speed of *C. elegans* movement. Specifically, they found the average speed of *C. elegans'* locomotion to be significantly slower at 11°C than at 25°C (MacMillan *et al.* 2011). This trend is consistent with the results obtained in our study. Secondly, a study examining the effects of temperature on locomotion of the freshwater snail, *Lymnaea stagnalis*, also found similar associations between these two variables (Sidorov 2003). In temperatures below the snail's optimal range, speed of locomotion was found to be significantly slower than that at their optimal temperature (Sidorov 2003). Because nematodes and gastropods are both ectothermic, their behavioural responses may be similarly affected by changes to environmental temperatures. Overall, in terms of the relationship between temperature and locomotion speed, the findings in the literature are consistent with those of the present study.

However, it is important to consider factors that may have affected the precision and accuracy of our data, and thus results. Sources of error related to our experiment fall into four main groups: the volatility of the chemoattractant (isoamyl alcohol) used, inaccuracy of L4 stage nematode identification, imprecision of measuring tools, and software (MTrackJ) tracking errors. Firstly, the volatile nature of isoamyl alcohol may have made it difficult for the nematodes to determine the precise location of the chemoattractant. This may have resulted in increased lateral head movements (location of olfactory organs) in order to locate the dispersed chemoattractant, but not necessarily forward movement. This would affect our results because *C. elegans'* speed of locomotion was calculated using the distance traveled by the tail of the organism, not the head. Thus replicates that had uninhibited muscle movement but did not move forward very much would have yielded similar speeds of locomotion as those who had generally poor muscle movement, potentially misrepresenting the actual locomotor abilities of certain replicates. To minimize the effects of volatility, further studies may choose to use benzyaldheyde, a similar but more stable chemoattractant than isoamyl alcohol (Parida *et al.* 2014). Another source of error may have stemmed from misidentification of L4 nematodes. L4 individuals were identified based on the presence of a white crescent structure found near the middle of their bodies (Schindler *et al.* 2014). High resemblance of the L4's to adults and the difficulty experienced during identifying the unique white crescent made it challenging to locate them accurately under the microscope. As previously mentioned, behaviour varies with age; therefore, selecting incorrect replicate individuals may have affected the accuracy and precision of our results. Another source of error may be attributed to the ruler used for calibration of each microscope's FOV. It was not a scientific-grade ruler, therefore, its lack of precise calibration may have led to incorrect calculations of distance traveled by each replicate. This, in turn, would affect the calculated speed. Another source of error may have occurred whilst using MTrackJ, which requires that the

user manually track the worm by placing markers at every frame segment. This is problematic because imprecise marker placement would greatly affect the overall tracking, resulting in inaccurate values for distance traveled by the replicates. Again, this would result in incorrect speed values. While these sources of error may have affected our overall results, they likely played a minimal role in affecting the statistical differences in average speed values as our *p*-value is considerably less than 0.05. Furthermore, the variation among the three temperature treatments was similar; therefore, possible errors likely affected all treatments relatively evenly.

CONCLUSION

The data collected in this study reveals that temperature does have an effect on the speed of *C. elegans'* locomotion, allowing us to reject the H_0 and provide support for H_A (ANOVA, p=0.002). As predicted, the average speed of locomotion was found to be significantly slower in the 12.0°C and 15.0°C treatments than in the control, 20.8°C, treatment. Our study provides us with insight that predicts how the competitive abilities of nematodes may be affected by climate change.

ACKNOWLEDGEMENTS

We would like to thank Dr. Carol Pollock for her suggestions on how to measure the speed of *C. elegans'* locomotion. In addition we are grateful to Nicole Gladish for her suggestions on our model design. We would also like to thank Mindy Chow for preparing the organisms, equipment, and chemicals required to successfully execute this experiment. Finally, we are grateful that UBC has given us the opportunity to take Biology 342 as a part of our undergraduate degrees by providing the space and financial necessities.

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