Effect of Salinity on the Locomotion of *Caenorhabditis elegans*

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Abstract

Wild-type *Caenorhabditis elegans* have previously been observed to avoid high concentrations of NaCl and move away from highly concentrated sources (Khanna *et al.* 1996). In this experiment, the locomotion and change in rate of movement of the N2 wild-type nematode *Caenorhabditis elegans* was studied at different salinities. Three different concentrations of NaCl buffer solution, 0.0M, 0.5M, and 1.0M, were applied to *C. elegans* with 16 replicates for each concentration. Each replicate was observed as their speed and movement was recorded for 30 seconds, with the DinoXcope. They then were exposed to a specified concentration treatment and were left to acclimatize for 30 minutes before being recorded for another 30 seconds to track any changes. Using the software WormLab, measurements of the overall change in movement and changes in speed were obtained. When exposed to 0.0M NaCl buffer solution the average change in speed was -1.81 µm/s, when exposed to 0.5M NaCl buffer solution the average change in speed was -10.55 µm/s, and when exposed to 1.0M NaCl buffer solution the average change in speed was -84.65 µm/s. Although the average speed and movement decreased as NaCl buffer solution concentration increased, statistical analysis showed that there was no significant difference in the means. Therefore, we concluded that increasing salinity does not significantly decrease the rate of movement of *C. elegans*.

Introduction

The nematode *Caenorhabditis elegans* are free-living terrestrial/aquatic roundworms, their average lifespan is 2-3 weeks and they can grow up to 1 mm in length (Wixon *et al.* 2000). They feed primarily on microbes in the environment they inhabit (Wixen *et al.* 2000). These organisms have simple body structures but they have a more complex nervous system; they are able to sense a variety of chemicals using chemosensory neurons through exposure to their sensory cilia, for example salts (Bargmann 2006). As seen in Figure 1, the chemosensory neurons located in the amphid and inner labial of the head region, and the phasmid of the tail region are exposed to its environment by pore openings (Bargmann 2006). *C. elegans* have the ability to rapidly withdraw when they encounter high osmolarity or bitter alkaloids such as chlorine

(Culotti and Russell 1978). Their ability to sense and respond with rapid avoidance is due to the presence of ASH chemosensory neurons (Bargmann 2006). The *tmc-1* gene encodes a sodium sensor that functions in salt taste chemosensation; it is also expressed in the ASH avoidance neuron (Chatzigeorgiou *et al.* 2013). This accounts for the natural behavior of avoiding harmful exposure to high salt concentrations. Knowing the function and response of these sensory neurons, we wanted to test how the *C. elegans* speed and overall movement changes when exposed to increasing salinity.

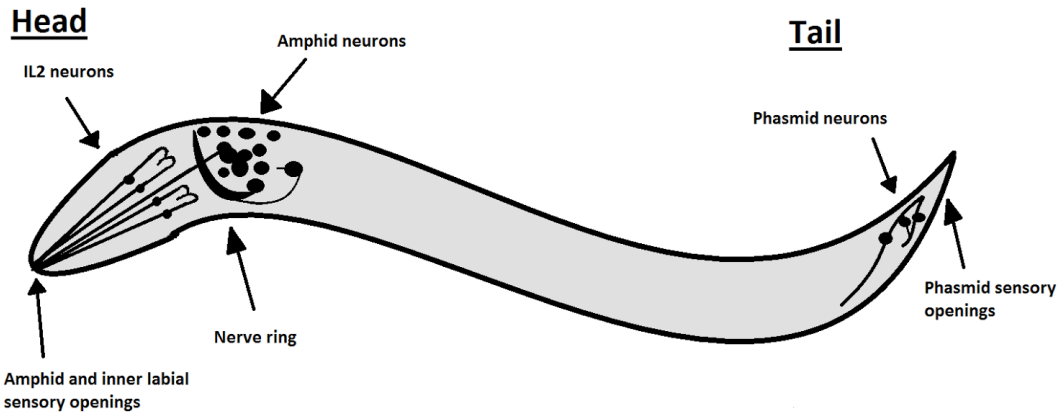

Figure 1. The sensory system of *Caenorhabditis elegans* based on Figure 1 from Bargmann 2006.

This experiment showed how increasing salinity can have an effect on the overall movement and speed of *Caenorhabditis elegans*. Our experiment focuses on the motility and speed of the organism when being exposed to multiple concentrations of NaCl buffer solution: 0.0M, 0.5M, and 1.0M. We chose a NaCl solution based primarily on the knowledge that these organisms can detect and react to both sodium and chlorine ions in an aqueous environment by using their chemosensory neurons (Culotti and Russell 1978). We wanted to record their motility after a 30 minute acclimation period when exposed to varying NaCl buffer concentrations. Studies have shown that *C. elegans* have a threshold

for high salinity before death rates increase, and can tolerate up to 0.3M NaCl for 24 hours before the environment induces significant lethality (Khanna *et al.* 1996). Having limited observation and experimental time, we used this as a guideline and increased the concentration to observe the effects on the movement of *C. elegans*.

We used this information to formulate our null and alternate hypotheses as follow:

H_0: Increasing salinity has no effect or increases the movement of N2 wild type *Caenorhabditis elegans*.

H_A: Increasing salinity decreases the movement of N2 wild type *Caenorhabditis elegans*.

Methods

Adult, N2 wild type *Caenorhabditis elegans* were used for our experiment. Worms that had observable movement and length equal to or longer than 1.0mm were selected from the 100mm *Escherichia coli* plate. Larger worms were selected because it was easier to see them and selecting worms that were already moving helped us avoid picking dead or potentially injured ones. Kyowa dissecting microscopes, magnified at 7X, along with worm picks were used to pick up and transfer worms individually onto the center of a new 60mm agar plate. Sodium chloride (NaCl) was prepared in nematode storage buffer. We tested the worms in three different NaCl buffer concentrations: 0.0M, 0.5M, and 1.0M. The 0.0M solution, which was the stock storage buffer, was used as our negative control. For each of the three treatments, there were 16 replicates (n=16).

The speeds of the worms before (t = 0min) and after (t = 30min) treatment were recorded and compared. Once a worm was safely transferred onto the new agar dish, we recorded qualitative observations of each worm such as, type of movement, apparent

speed and presence of head movement. After qualitative observations were recorded, we used the DinoXcope attachment on the Kyowa dissecting microscopes to record a 30 second video of the worm moving on the agar plate at t = 0min.

After the initial recording, we proceeded by adding 200 μL of NaCl buffer solution on top of each worm using a micropipette and a clean pipette tip using sterile technique. This was done under the microscope to ensure the worm was fully submerged in the solution. We allowed the worms to acclimate for 30 minutes on the lab bench, covered with a lid. When the 30-minute acclimation period was reached, we recorded the "after" video (t=30min) for 30 seconds using the DinoXcope (Figure 2).

Figure 2. Video screenshot at 7X magnification of *C. elegans* completely submerged in NaCl solution on the agar plate.

We used WormLab, software used to track and analyze videos of *C. elegans* developed by MBF Bioscience, to obtain quantitative data. For each video, the contrast between the worm and the agar plate was manually selected. WormLab would then use to detect the worm and apply a worm motion model on it (Figure 3). We then ran the tracking software, which processed any overlaps in the worm's track frame by frame and produced the track length in μm and average speed in μm/sec. Following this we exported the data into Excel format.

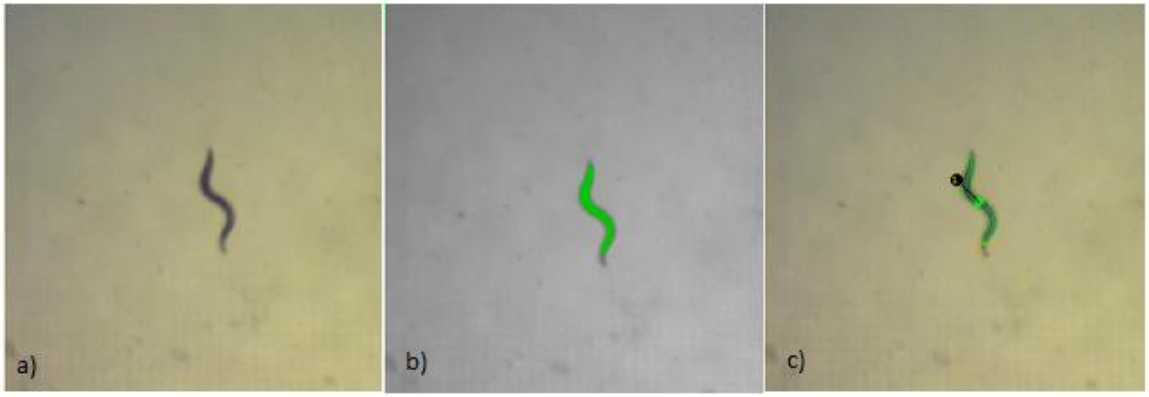

Figure 3. a) *C. elegans* before analysis b) Manual selection of contrast level for detection of *C. elegans* c) Detected *C. elegans* ready to be tracked by WormLab.

We tabulated the speeds from all 48 replicates into one single table and calculated the difference in speed. We calculated the means of the differences and obtained a 95% confidence interval for each NaCl treatment. Finally, we performed a one-way analysis of variance (ANOVA) on the results to see if there was a significant difference between the mean speeds of *C. elegans* in different treatments.

Results

Table 1. Mean decrease in speed of *C. Elegans* after 30 minutes of being exposed to treatment levels of 0.0M, 0.5M, and 1.0M of NaCl buffer solution. Error is the 95% confidence interval.

NaCl buffer concentration (M)	Mean decrease in speed (µm/sec)
0.0	-1.81±54.05
0.5	-10.55±52.21
1.0	-84.65±62.66

We observed that before treatment *C. elegans* traveled across the plate in a smooth fashion, similar to a sine wave (Figure 4). After treatment with 0.0M NaCl buffer solution for 30 minutes, the type of movement in some *C. elegans* became less smooth and more rigid, while other worms appeared to be unaffected. Overall we found that the mean difference in speed of the control was small, -1.81±54.05µm/s (Table 1). Replicate 00A was excluded as the before treatment video was unusable.

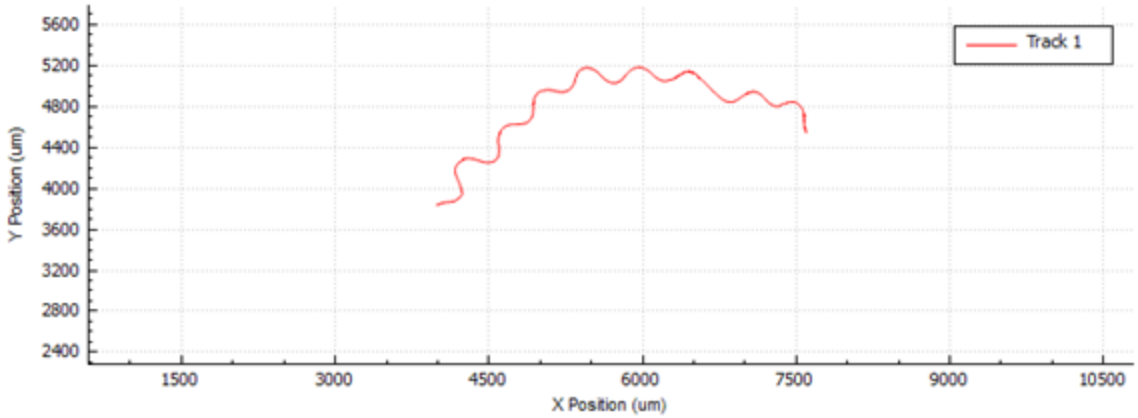

Figure 4. Track of mid-point position in µm of *C. elegans* replicate 05I before treatment with 0.5M NaCl buffer solution. This is a typical track found among pre-treatment *C. elegans*. Measured using WormLab.

We found that after 30 minutes, the 0.5M treatment group had worms that were either thrashing at varying intensities (Figure 5) or, in some cases worms were either "paralyzed" or twitching slightly (Figure 6). We found the mean decrease in speed to be -10.55±52.21 µm/s (Table 1).

After treatment of 1.0M for 30 minutes, we observed similar responses to the 0.5M NaCl buffer solution however, a greater proportion of the individuals were immobilized rather than thrashing. We calculated the mean decrease in speed to be -84.65±62.66 µm/s (Table 1).

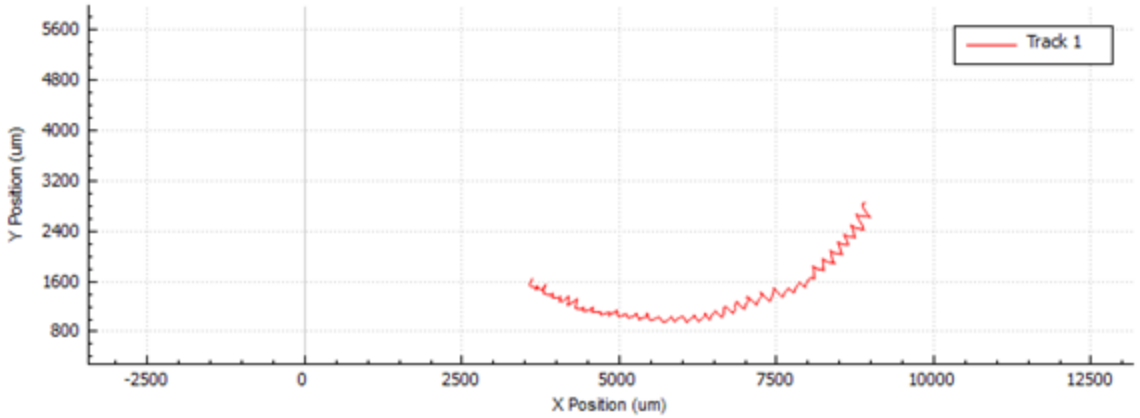

Figure 5. Track of mid-point position in µm of *C. elegans* replicate 10I after treatment with 1.0M NaCl buffer solution for 30 minutes. This is a typical track for *C. elegans* that are observed as thrashing after treatment. Measured using WormLab.

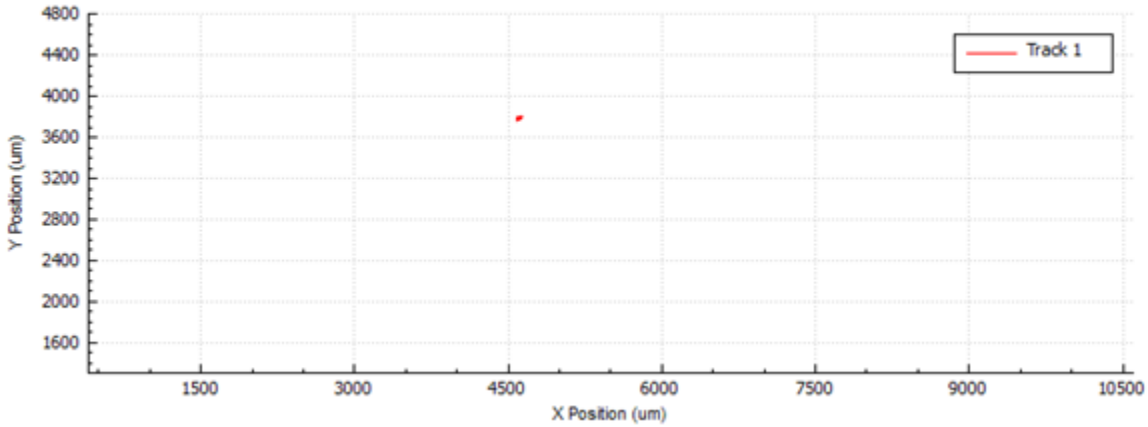

Figure 6. Track of mid-point position in µm of *C. elegans* replicate 2.1-1.0 after treatment with 1.0M NaCl buffer solution for 30 minutes. This is a typical track for *C. elegans* that are observed as immobile after treatment. Measured using WormLab.

We used one-way ANOVA to determine if the response variable, mean decrease in speed, between treatments was statistically significant. The resulting values were F_{calc}=2.498812 and p=0.084214. We concluded from the p-value being greater than 0.05 that there was no statistically significant difference in variation between the three treatments.

We also reached this conclusion by analyzing Figure 7; the 95% confidence intervals of the mean difference in speed for each treatment all overlapped as the variance within each treatment group was very large. The large variance is due to the different types of movement observed after treatment. Thrashing worms had a much smaller decrease in speed or were observed to be increase in speed, compared to worms that just decreased speed or were paralyzed. However, we still observed a general trend; that as the concentration of NaCl buffer solution increased, the mean difference in speed decreased. At all three treatment levels, we also observed that the worm would most often be found right at the edge of the droplet of NaCl buffer solution, rather than the central location where we placed them.

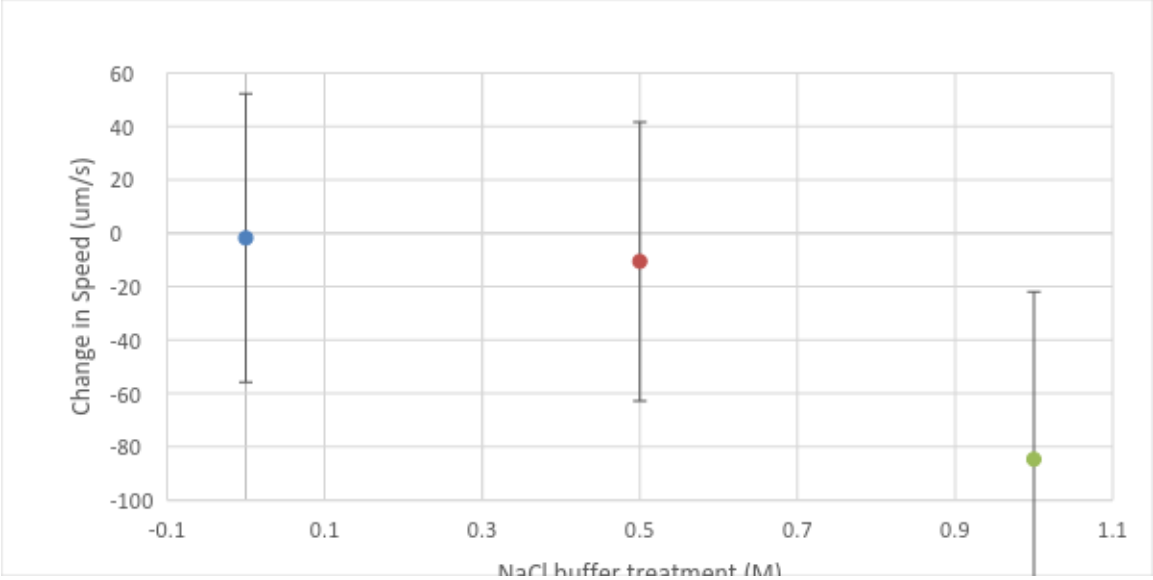

Figure 7. Mean difference in speed of *C. elegans* after 30 minutes of exposure to 0.0M, 0.5M, and 1.0M NaCl buffer solution. Error Bars show 95% Confidence Intervals. Speed was measured using WormLab software. n=15 for 0.0M treatment and n=16 for 0.5M and 1.0M treatments.

Discussion

The results tell us that there is no significant difference between the three treatment levels, therefore we fail to reject our null hypothesis and fail to provide support for our alternative hypothesis that an increase in salinity will have decrease the speed of *Caenorhabditis elegans*. This result was unexpected due to some of the qualitative observations recorded during the experiment appearing to provide support to our alternative hypothesis, but further research on past studies has shown that this result is not uncommon. However, in our search for past studies, we did not find one strictly devoted to the effect of salinity on the differences in speeds as the majority were studying how changes in salinity affected *C. elegans* behaviour.

One behaviour that was observed in our experiment that is well documented in the literature is how *C. elegans* tries to escape areas of high salinity. In the NaCl buffer solutions, it was noted that even if the worms were originally placed in the centre, after 30 minutes the majority would be found on the barrier between the solution and the agar trying to swim out. Studies done by Culotti and Russell (1978), Choe (2013), and Campbell and Gabriel (2003) found that the wild-type strain of *C. elegans* is shown to avoid high concentrations of salts and sugars. Choe (2013) has even termed the behaviour of the worms to reverse directions when encountering a rise in environmental osmolarity as, "osmotic avoidance." This avoidance is due to ASH neurons which are important for the avoidance and withdrawal actions as noted by Chatzigeorgious *et al.* (2013). However, the majority of studies differed in how the NaCl buffer solution was administered. Instead of placing individual worms in a NaCl buffer solution, the

behaviour was noticed by having the NaCl buffer solution act as a barrier around a worm although similar results were recorded.

Studies, by Campbell and Gabriel (2003) and Khanna *et al.* (1997) had *C. elegans* placed directly into a NaCl solution. Khanna *et al* (1997) discovered that worms are able to tolerate 0.35M of NaCl in water with no adverse effects shown. Campbell and Gabriel (2003) found the same results and also stated that there were no significant differences in the percentage of motile worms in dishes with and without the addition of salt (0.5M NaCl). Referring back to Figure 7 and our findings, it can be seen that our results coincide with these sources. From Table 1, we can see that the mean difference in speed in 0.0M and 0.5M solutions are not that different, being -1.81µm/s and -10.55µm/s. Campbell and Gabriel (2003) thought that an osmotic mechanism may be helping the worms prevent osmotic shock and avoid salt-enhanced deleterious effects. They also believe that any increase in salt would not affect the salt concentration at the synapses for the ion channels of *C. elegans* due to the fact salt impacted ion channel modulation agents differently depending on the agent used (Campbell and Gabriel, 2003).

Choe (2013) determined how *C. elegans* is able to determine the change in salinity. When salts are concentrated enough to generate high osmotic pressures, a pair of amphids found in the tip (head) of the worm will detect the high osmotic pressure and cause the "osmotic avoidance." Amphids are sensory structures that have pores filled with the ciliated endings of sensory neuron dendrites on both sides of the mouth of the worm (Choe 2013). Not only do these amphids cause the worm to not cross an area of high osmotic pressure, but also notify when it is safe to cross that area when all the salts are dissolved into the agar gel (Choe 2013).

Choe (2013) also looked at the behaviour of *C. elegans* when being transferred to NaCl solutions and found that there was a 90% survival rate in solutions between 51 – 400 mM NaCl. They noted that at the lower end of the range, movement is not affected after 20 – 30 minutes, with the worms moving as well as before. However, when the worms are transferred to extreme hypertonicity at the higher end of his range, the worms are seen to undergo dramatic shrinkage losing half of their total body volume, loss of turgor pressure, a more rigid posture and movement and even complete loss of motility after 20-30 minutes (Choe 2013). This result mirrors what we discovered in our experiment with not observing much change in speed differences seen between 0.0M and 0.5M salt solutions. A rather large difference in speed was seen however in the highest salinity solution (1.0M), were most worms moved very rigidly or not moving at all, vastly different from how they moved before. This rigidness can be a result of the worm not having synthesized enough of the organic osmolyte glycerol. As Choe (2013) explained, this compound is needed to balance high environmental osmolarity and stabilizes protein structures as a chemical chaperone. Synthesis of organic osmolytes like glycerol take time and could explain why at 1.0M NaCl solutions we see such a vast difference in speed as the nematode needed time to acclimated with the new environment.

With regards to uncertainty in our experimental design, we can attribute it to a few factors. The statistical tests we used to determine significance have the assumption that the data is sampled randomly, however since we only selected worms that were moving and of a certain size we can no longer call it a random sample. Also observed was that a few of the worms were found to be right up against the edge of the Petri dish, we may assume that they might have managed to come out of the solution and thus we

cannot with certainty say that the acclimation period for all worms was 30 minutes. We also encountered difficulties with WormLab detecting and tracking worms in solution, particularly the ones observed to be thrashing, many times the software lost track of the worm due to the contrast of the worm in solution being noticeably less than the worm on the agar plate due to the different refractive index of water. This resulted in several tracks being recorded for one worm and in order to get the average speed of the worm we joined them together. As a result of WormLab estimating parts of the track, we can conclude that there was uncertainty within the accuracy of the average speed of some worms.

From the information mentioned above, it would be wise for further studies to have a much longer period of observation as it would appear 30 minutes was not enough time to see the full effects that an increase in salt solutions may have on *C. elegans* movement speeds. Sznitman *et al.* (2010) found that *C. elegans* speed is affected by the surface viscosity, so to remove this unnecessary variable the worms should begin in solution and increase salinity there rather than recording the initial speed on the agar plate and then the post treatment speed in solution which have markedly different viscosities. Lastly, it may also be worth investigating how increased salinity in the liquid phase of soils where free-living *C. elegans* are found affects its speed and behaviour.

Conclusion

Our statistical analysis showed that there was no significant difference in the means of speed-difference amongst the *C. elegans* in the three different NaCl treatments ($F=2.498812$, $p=0.084214$). Thus, we were unable to reject the null hypothesis, as there

was no significant statistical evidence to support the alternate hypothesis that increasing salinity decreases the movement speed of *C. elegans*.

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