

# Exercising *Caenorhabditis elegans*

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**Abstract— Muscles deteriorate with age, restricting mobility. It is thought that regular exercise can lessen the rate of muscle deterioration, but it is not known if it affects longevity. Using the nematode *Caenorhabditis elegans*, we wish to determine a relationship between exercise and the aging process. To do this, we need to find a way to induce the animals to exercise. A method of exercising *C. elegans* was identified by exposing them to a fluid flow. Without food, the *C. elegans* maintained a constant low activity level until the external flow reached a threshold velocity. Once this threshold velocity was exceeded, the *C. elegans* assumed a heightened activity level. The effect of food on the activity level was also examined. In the presence of food, the animals' activity level declined since they were not actively searching for sustenance. Lastly, using different strains with mutated sensory neurons, it was determined that an elevated activity level is caused by the animals' cilia. This opens the way to find how exercise affects mobility level as a function of age and longevity.**

## I. INTRODUCTION

*Caenorhabditis elegans* have been studied since 1974 when Sydney Brenner proposed using the nematode to study the nervous system [1]. Since then, *C. elegans* have been studied extensively. They are a model organism often used in medical research due to their small size, transparency, short life cycle, low number of neurons, and availability [2]. Genetically, there is little difference between generations because they are hermaphrodites [3]. These characteristics make *C. elegans* ideal for controlled experiments.

*C. elegans* move with an undulatory motion, but they have two distinct movements: crawling on a surface and swimming in fluid [4]. They move faster in fluid than on a surface. It is hypothesized that introducing the nematodes to a fluid flow will increase their activity level, hence exercising them. This would enable one to compare the aging process with two different populations, one that has been exercised and one that has not. It is also thought the touch sensory neurons are what cause this heightened level of activity.

## II. BACKGROUND

### i. Genetics

Much has been learned about genetics through studying *C. elegans*, including their complete genome sequence [5]. With this knowledge, scientists have been able to identify the genetic

causes of mutations and also create mutated strains that focus on a particular set of genes [6]. *C. elegans* have 302 neurons making their nervous systems much easier to study than other model organisms. There are many mutant strains of *C. elegans* that alter their nervous system [7]. This allows us to see the effects of different neurons.

### ii. N2

The N2 strain, commonly known as wild-type, is the genetic baseline for *C. elegans*. They are of the same genetic structure as the *C. elegans* that live in the soil, but they are grown on petri dishes in a lab. Mutant strains are often compared to N2 to see the effects of different genes [8].

### iii. Strains

#### *MEC-3* CB1338

*C. elegans* have six mechanosensory neurons that detect touch [9]. The *mec-3* gene causes these touch receptors not to function so the nematode can no longer detect its surroundings [10].

#### *MEC-4* TU253

The *mec-4* strain is similar to the *mec-3* strain, but rather than the mechanosensory neurons being suppressed, they degenerate over time [11].

#### *OSM-6* PR881

Like the mechanosensory neurons, *C. elegans* also have chemosensory neurons which respond to chemical stimulation. At the ends of these neurons there are cilia, small hair-like structures that are used to detect their environment [12]. Studies have shown that cilia deficient *C. elegans* cannot swim and try to crawl through the fluid [13]. The *osm-6* strain mutates the worm's sensory cilia so the worm's mechanosensory and chemosensory behaviors are altered [14].

#### *CHE-2* CB1033

The *che-2* strain is similar to the *osm-6* strain since the cilia are also altered. In the *che-2* strain, however, the cilia are shorter than they normally are. This also alters the behaviors mediated by

the mechanosensory and chemosensory neurons [15].

#### *TAX-4* PR678

The *tax-4* strain is also deficient in chemosensory behaviors, but they still respond to mechanosensory stimulation. They have no response to temperature change as the other strains of *C. elegans* do [16].

### III. MATERIALS AND METHODS

#### Set-up

A microfluidic device made of 1/8 inch acrylic was used in this experiment. The device consisted of circular holding chambers for the *C. elegans* and conduits to allow fluid to flow through. The fluid used was Nematode Growth Media (NGM) which is a common fluid environment used in studies of *C. elegans*. All worms used in this experiment were picked at the fourth larvae state the day before use to ensure they would all be at the same age. They were placed into the holding chambers, each of which had a conduit going through it. Each conduit was connected to plastic tubing which connected to a syringe. The flow was introduced to the system and kept constant by a multi-barrel syringe pump.

#### Exercising *C. elegans*

First, a method of exercising *C. elegans* was determined. Worms put in the NGM buffer move at a higher rate than those not in liquid, but this is not considered exercising. The hypothesis tested was that exposing *C. elegans* to a fluid flow would increase their activity level. Wild-type *C. elegans* were loaded into the holding chambers and allowed to acclimate for 30 minutes with no fluid flow. At the end of the 30 minutes, the flow started and a recording of the worms began. Data was recorded for the next three hours. Trials were first done with fluid flows at 0, 25, 50, and 100  $\mu\text{L/hr}$ . After analyzing this data, four more trials were added at 8, 17, 33, and 42  $\mu\text{L/hr}$ . The data was analyzed in one minute intervals at the beginning of each hour, and each trial lasted for three hours. Six worms experienced the flows for each trial, and each worm was analyzed individually for each time interval. The average number of body bends was then found for each fluid velocity

#### Food

No food was used while determining a method of exercise. This does not affect the results short term, but after a few hours, the worms will begin to starve. For a long term experiment, there will have to be food in the holding chambers so the *C. elegans* can survive. LG buffer was inoculated with *E. coli* bacteria and would then spend overnight in an incubating shaker. The next day, 15mL of the LG buffer would be spun in a centrifuge to separate the bacteria from the buffer, and then the bacteria would be washed with NGM. The bacteria would then be suspended in 6.5mL of NGM creating a food mixture concentrated approximately two-fold. Using the same conditions as without the food, trials were run at

velocities 0, 25, 50, and 100  $\mu\text{L/hr}$  for the same time intervals as above.

#### Sensory Neurons

After determining it is possible to exercise *C. elegans* with a fluid flow, the next step was to determine what caused the worms to have a heightened activity level. To do this, different mutant strains were tested against wild-type. It was hypothesized that the nematode's mechanosensory neurons are responsible for the heightened activity level. To test this, half of the holding chambers in the device contained *mec-3* and the other half contained wild-type. This increased accuracy, as the only variable was the different strains. Using the same time intervals as above, the nematodes were tested at 0 and 50  $\mu\text{L/hr}$ . The same set up was used for the *mec-4*, *osm-6*, *che-2*, and *tax-4* strains.

### IV. EXPERIMENTAL RESULTS

#### Exercising *C. elegans*

Introducing *C. elegans* to a fluid flow did increase their activity level. There was not a large increase from 0-25  $\mu\text{L/hr}$  and there was little difference from 50-100  $\mu\text{L/hr}$ , but there was a big jump from 25-50  $\mu\text{L/hr}$  (see Figure 1). This shows there exists a threshold flow rate below which *C. elegans* maintain one level of activity and above which they maintain an elevated level of activity. Since the fluid flow increased the activity level, this is a method of controlled exercising of *C. elegans*.

#### Food

The activity level was fairly consistent for each flow rate immediately after putting the *C. elegans* into the microfluidic device. The activity level change occurred after letting them settle into the new environment for a short period of time. Adding food into the system drastically slowed the movements of the *C. elegans* (see Figure 2). Within the three hour period, the activity level went from highly active to barely active at all. This is due to the worms eating the food. As seen in Figure 2, the higher flow rates caused the number of body bends to decline faster than at the slower flow rates. A possible reason for the decline in motion is because they were not starved so they did not have to maintain an elevated activity level to search for food. This could indicate the *C. elegans* were not exercising in the presence of food. Using the same set-up and the same food concentration, another experiment was done to determine if *C. elegans* exercise in the presence of food. This time, the *C. elegans* put into the holding chambers did not experience a flow for two hours and then experienced a flow of 50  $\mu\text{L/hr}$  for the next two hours. After two hours with no flow, the worms had slowed to the point where they were barely moving. When the flow began, the activity level increased, though not by as much as when there was no food present. This shows that *C. elegans* will exercise in the presence of food.

#### Sensory Neurons

##### *MEC*

The data from the tests with *mec-3* and *mec-4* did not support the hypothesis. Both the *mec* and wild-type's activity level increased in the presence of a fluid flow which shows there is little or no connection between the mechanosensory neurons and the activity level.

#### TAX-4

The activity level of the *tax-4* strain increased with the fluid flow, more so than the wild-type or *mec* strains. This shows there could be a connection between either chemosensory neurons or temperature detection and exercise, but there is no definitive evidence there is.

#### OSM-6 and CHE-2

Unlike the other strains tested, *osm-6* and *che-2* did not have an elevated activity level when exposed to the fluid flow. Their activity level decreased with time indicating that they did not exercise. This shows the cilia are a possible cause of exercise.

Figure 3 shows the percent difference of the activity levels between 0 and 50  $\mu\text{L/hr}$  for the different strains. For example, the number of body bends of N2 *C. elegans* increased by slightly over 20% when it experienced the fluid flow.

### V. DISCUSSION AND CONCLUSION

Introducing *C. elegans* to a fluid flow was determined to be an efficient method of exercise both in the presence and the absence of food. It was found there exists a threshold velocity below which the worms maintain a constant level of activity and above which they maintain an elevated level of activity. This leads the way to age related experiments. It can be seen if exercise extends the lifespan of *C. elegans* and if exercised worms maintain a higher mobility rate as they age. Though before longer term experiments can be done, the device should be re-designed so that the conduits are large enough to allow for the eggs to flow out of the holding chambers while still containing the adult worms. Otherwise, the holding chambers would become overcrowded which would not be ideal. By testing different strains of *C. elegans*, it was also determined that cilia causes *C. elegans* to exercise rather than the mechanosensory or chemosensory neurons. This can lead to further experiments with motion and cilia in areas such as propulsive power. It can be determined if the strains with mutated cilia have a weaker propulsive power which could be why they cannot exercise.

#### Appendix

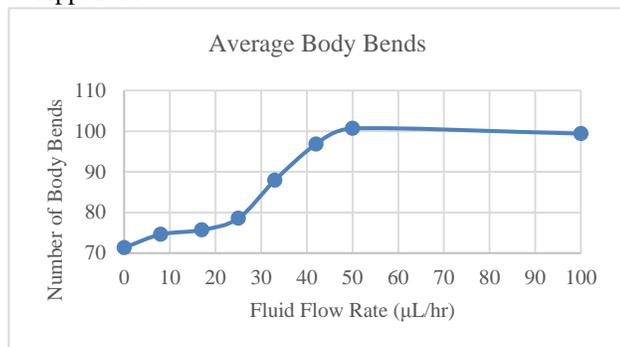


Figure 1: This graphs shows the average number of body bends per flow rate. It can be seen that the number of body bends increases with flow rate only until reaching a certain point. After this point, the number of body bends remains constant.

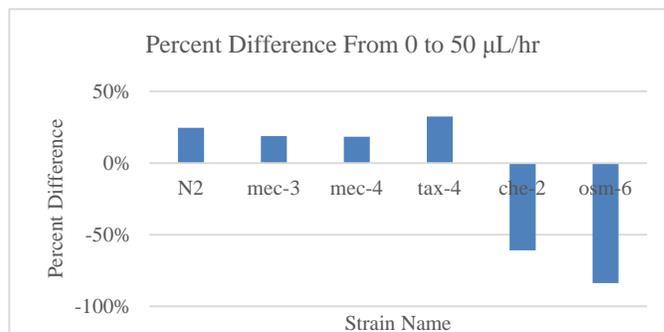
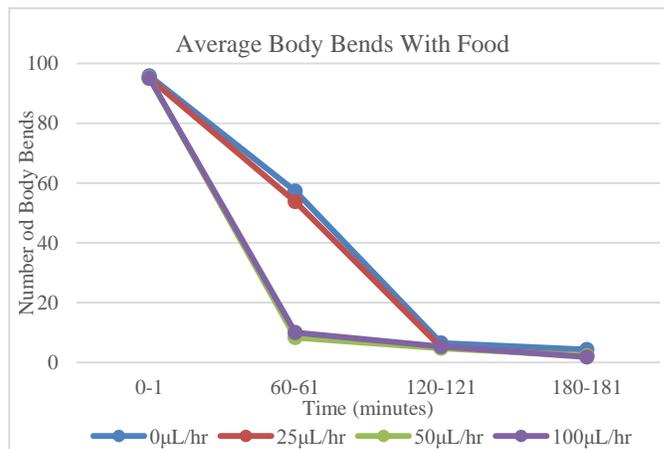


Figure 2: *C. elegans* experiencing higher flow rates had a faster decline in activity level.

Figure 1: Percent increase or decrease of the number of body bends after adding flow

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