24th COBEM - 2017

24th ABCM International Congress of Mechanical Engineering
December 3-8, 2017, Curitiba, PR, Brazil

COBEM-2017-1613
ON THE CRAWLING WAVE MOTION OF NEMATODES UNDER THE PRESENCE OF A MAGNETIC FIELD

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Abstract. *The current study experimentally investigated the possible effects of static magnetic fields on the kinematics-wave motion of suspended active particles in a biological fluid. The fluid is an active suspension of Caenorhabditis elegans immersed in a gel like biological structure, moving at low Reynolds number. A permanent magnetic field is applied so the magnetosensory abilities of C. elegans can be analyzed from the dynamical perspective. The relevant physical parameters are analyzed for three different samples including starving and well-fed nematodes. Dynamical system tools, such as FFT allowed us to physically interpret the kinematics and wave motion of nematodes when under the presence of a magnetic field. In addition, kinematic quantities such as slip velocity and frequency spectrum helped us to draw conclusions that are different from those drawn in previous studies, where different magnetic fields were applied.*

Keywords: *active fluids, creeping flow, C. elegans, magnetosensory*

1. INTRODUCTION

The evolution of several microorganisms is related to complex fluids rheology, whose characteristics can substantially affect the microorganism swimming behaviour. An interesting aspect on the mechanics of swimming is that the biomechanics of a living being must be adapted to its surroundings. Several works have focused on studying a specific nematode called *Caenorhabditis elegans* regarding its physical properties and aspects of low Reynolds number locomotion. Nematodes inhabit almost every environment and are one of the most commonly found organisms on Earth. The reasons for using this nematode as a benchmark go from the easiness to manipulate them to the simplicity of its motion. Higher deformation speeds associated with lower internal viscous resistance are related to the shear thinning feature of an individual worm. The shape of an undulating crawler is defined by a dynamic balance between elastic, hydrodynamic, and muscular forces (Malvar *et al.*, 2017). As a result, the shear thinning property of the worm may influence the dynamics of motility, and shear thinning should be integrated into a full locomotory model (Backholm *et al.*, 2013).

Nematodes normally respond to stimuli by swimming in particular directions. Such responses are called taxes. Taxes of importance are gravitaxis (or geotaxis), a response to gravity or acceleration; phototaxis, a response to light; and chemotaxis, a response to chemical gradients. According to Vidal-Gadea et al. (Vidal-Gadea *et al.*, 2015), some bacteria contain magnetic particles (i.e., magnetosomes), which cause them to swim along the magnetic field lines (magnetotaxis). As a matter of fact, many organisms are also affected by the Earth's magnetic field. For a few animals, central neurons responsive to earth-strength magnetic fields have been identified. However, birds and higher vertebrates have a large and complex nervous system that can be difficult to interrogate during experiments. Thus, there is an increasing interest to study magnetoreception in model organisms, such as *Caenorhabiditis elegans*. This soil-dwelling nematode worm have shown different feeding behaviour when under the presence of a magnetic field (Vidal-Gadea *et al.*, 2015). The short lifespan of *C. elegans* makes it attractive for whole-organism compound screening (Hertweck *et al.*, 2003). The neuron sporting a magnetic field sensor, called AFD neuron, was already known to sense carbon dioxide levels and temperature. The researchers discovered the worms' magnetosensory abilities by altering the magnetic field around them with a special magnetic coil system and then observing changes in behavior. In turn, increased dynamical analyses allow for the quantification of behavioral change that may be represented by speed of locomotion. This could then help in furthering the connection in *C. elegans* between genetics, behavior and dynamics (Angstman *et al.*, 2016).

In this work, the typical crawling motion of *C. elegans* was studied considering the geometry and variables defined in Fig. 1, where a is the worm's diameter, λ represents the nematode's motion wavelength, d is the *C. elegans* motion amplitude, θ represents the bending angle, and v is the centroid velocity. The nematode's length is given by L.

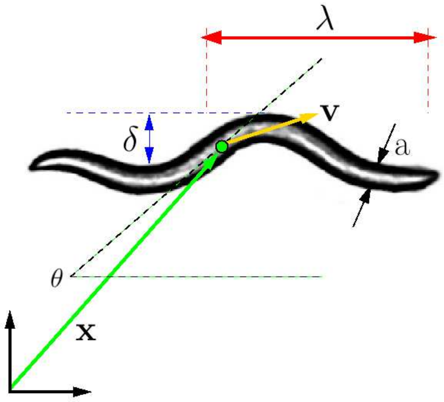

Figure 1. Geometrical sketch of variables a, δ, λ, θ, and v.

2. EXPERIMENTAL PROCEDURE

A high viscosity gel-like medium was used in order to study the nematode (N2 wild type) crawling motion. The gel is a mixture of water and agar molecules and during the worms movement, fluid is released from the medium due to the breaking of agarose molecules bonds. This maintains a lubrication regime. Sealed acrylic chambers with $2cm$ in diameter and $1mm$ in depth containing NGM-agar filled with *Escherichia coli* (OP50) are used in order to study the nematode's dynamic behaviour. Incubation temperature was 22^{o}C. The travelling waves are generated by organisms with approximately $400\mu m$ in length and $80\mu m$ in diameter. The main part of the experiment was the nematode tracking, which is used for obtaining kinematic data such as swimming speed, beating frequency, and amplitude. The individuals were observed through a Olympus UC30 CCD camera coupled to a Olympus BX51 microscope. Images were recorded with the focal plane at the centre of the chamber to avoid movies with nematode-wall interactions; out-of-plane recordings were discarded. Kinematics data were an average of 10 individuals of each sample analyzed in 10 different records. In these experiments, three samples were used: well-fed nematodes, starving nematodes and well-fed nematodes under the presence of a magnetic field. The images were manually analyzed using the WormLab software (Roussel *et al.*, 2014).

The magnetic strength was provided by a neodymium permanent magnet as shown in figure Fig. 2. This magnet made from a combination of neodymium, iron and boron is extremely strong, but fragile at temperatures above 120^oC and has typical values of remanence and coercitivity $0.38T$ and $600kA/m$, respectively. In order to compute the decay of the field produced by the magnet, two different gaussmeters were used: a LakeShore 410 Gaussmeter and F.W.Bell 5070 Gaussmeter. Both equipments measure the strength of the magnetic field at a point in space based in the Hall Effect. The nematode was put under the presence of a $1mT$ magnetic field, which is approximately 20 times the Earth's.

3. RESULTS AND DISCUSSIONS

When the magnetic field was applied at the side of the plate, no noticeable change in trajectory was seen. However, the slip velocity changed considerably. As a matter of fact, when a magnetic field was applied, nematodes tended to move faster, as seen in Fig. 3. This result is somehow distinct. (Hung *et al.*, 2010) reported reduced crawling speeds when worms were exposed to static magnetic fields. Another study showed that when *C. elegans* were submited to a 1.7 Tesla magnetic field at 60 hertz for 84 hours the worms were not able to locomote or reproduct (Njus *et al.*, 2015). However, on both cases, the magnetic strength was way stronger than the one provided by the Earth's magnetic field. Apparently, the application of a small magnetic field favorably modifies the locomotion. As a matter of fact, Njus (Njus *et al.*, 2015) concludes that the investigations regarding the directional ability of worms in magnetic fields have been less conclusive. On the other hand, even though the authors conclude that there was no considerable change in the nematodes velocity or turning percent, the average velocities without field are lower than the ones obtained when the magnetic field was applied.

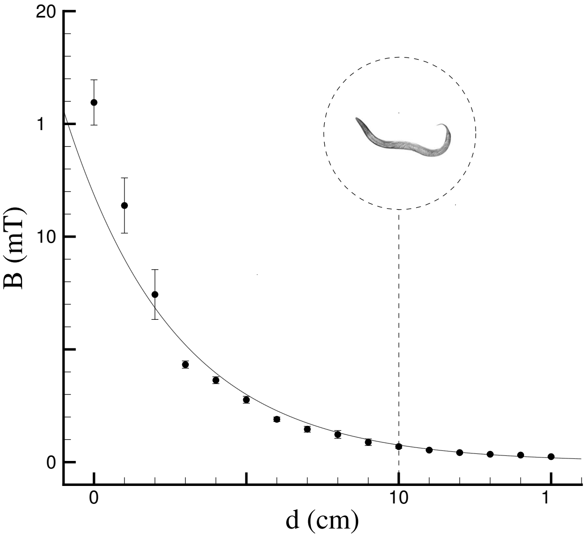

Figure 2. Magnetic field $\mathbf{B}$ in militesla as a function of the distance from the Petri dish center in centimeters. The position of each nematode is shown on the insert.

This result is consistent with our experiments.

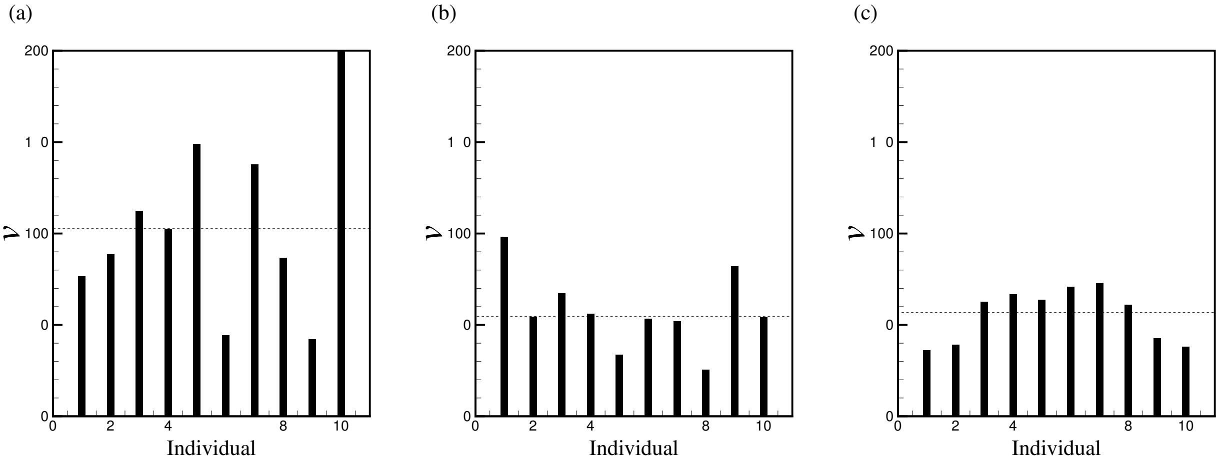

Figure 3. Individuals velocities (in $\mu m/s$) considering (a) the magnetized sample; (b) the well-fed sample and (c) the starving sample. The dashed lines represent the average velocity from the sample.

Figure 4 shows the fast Fourier transforms of both centroid and head for the three analyzed samples. Based on that, we may understand how numerous modes of vibration are present in the complex nematode motion. The FFT were performed in *Scilab* using a script developed by the authors. The head presents a much more complex motion composed of several vibrational modes (degrees of freedom). Thus, there is a non-negligible energy in slightly higher frequencies. This is a direct consequence of the fact that the nematode's head must command the rest of the body, and so it responds at higher frequencies and develops a complex bending motion in the absence of food. The centroid follows an almost harmonic pattern showing only one mode corresponding to the fundamental frequency, i.e., having a minimum bending effort. However, this kind of behavior can be changed due to the environment characteristics.

The first column shows the magnetized sample, the second is the well-fed sample and the last one is the starving sample. In this particular case, the first harmonic was not considered since it actually represents the principal oscillatory mode, which is very similar for all samples. However, it is possible to observe that the first frequency of the magnetized sample is higher. The frequencies and amplitudes are then related to the nematode's size. It's interesting to notice that when there was food available but no magnetic field, the worm's head exhibited several moving frequencies. Besides, the velocity of the head's turning became larger, since the nematode was feeding. However, when considering the starving sample, there was no significant change on centroid and head's dynamic behaviour. Thus the worm is searching for food while moving and not feeding. On the other hand, the motion becomes extremely nonlinear due to the constant search for food and the necessity to move faster. Thus, in the absence of food, the individuals tend to move faster and in different directions tracking the non-harmonic trajectories. Moreover, the worms increase their velocities and tend to crawl through higher distances. However, when a colony of bacteria is present, all individuals tend to crawl with similar velocities, i.e., proportional to the nematode's size. Under this condition, they also present fewer non-harmonic oscillations and do not explore the NGM (nematode growth medium) plate, reducing its crawling path. This result was previouslly explained by Malvar et. al (Malvar *et al.*, 2017).

The most intriguing fact was observed in sample three, when well-fed nematodes are subjected to a magnetic field. Not only there were less harmonics, but also their energies were smaller. Again, there were no observable differences between head and centroid. The nematodes movements become harmonic and stable.

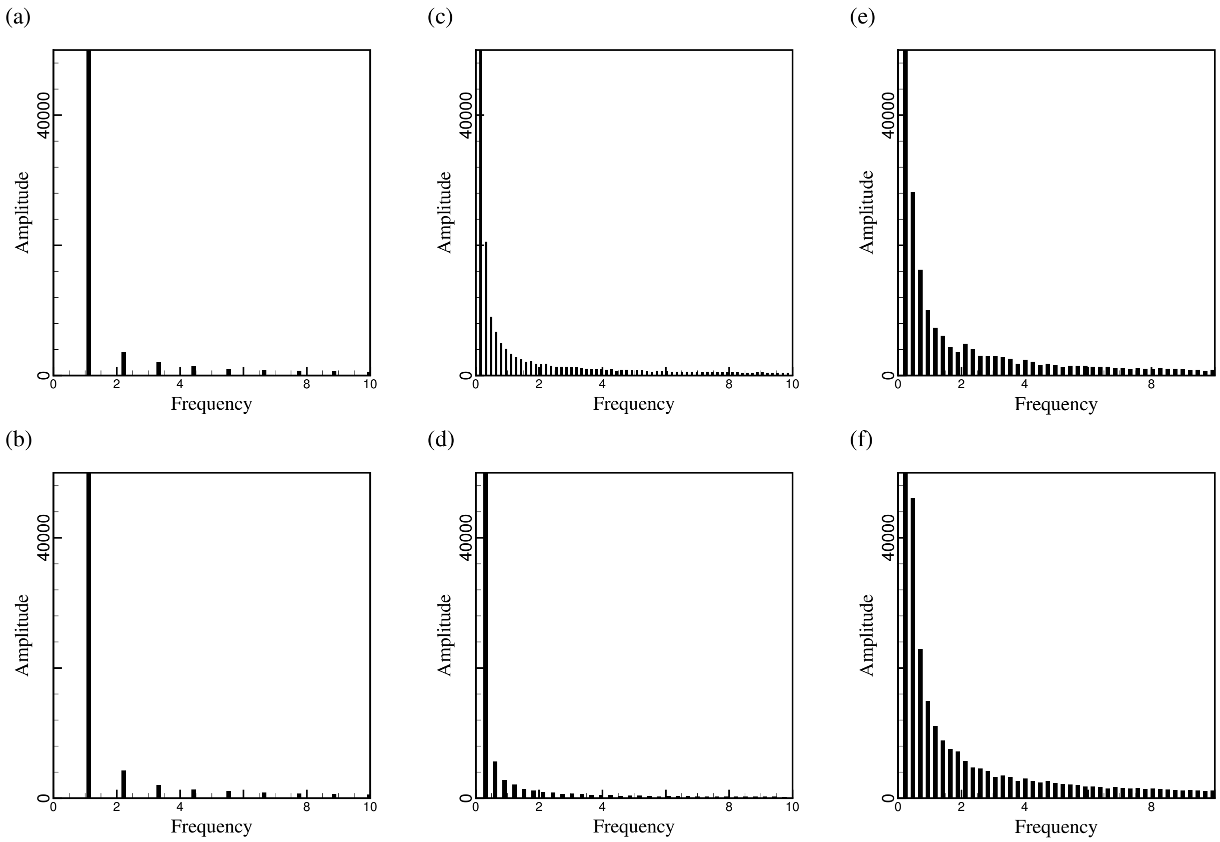

Figure 4. Fast Fourier Transform of the samples head and centroid positions (in $\mu m/s$). (a) Represents the head of the magnetized sample; (b) is the centroid of the same sample; (c) shows the head of the well-fed sample; (d) is the centroid of the same sample; (e) the head of the starving sample and (f) presents the centroid of the starving sample.

As explained before, the magnetized sample first frequency was higher than the others. Considering that, we observed that the average amplitude of the starving sample is greater than both magnetized and well-fed ones. The interesting fact here relies on the fact that when there is no food available, the nematodes crawling motion becomes more nonlinear. Moreover, the magnetic field does not appear to influence the motion amplitude. On the other hand, the food availability is a determining factor.

It is importat to highlight that the nematodes search for food is directly related to *E. coli* availability. Previous studies suggest that the rate growth of bacteria tend to decrease as a magnetic field is applied. Though the effects of magnetic fields are not bacteriostatic, the number of bacteria increased during exposure of the growing culture to the magnetic fields but was less than in the control culture. Magnetic fields have no effect on the metabolism of the bacteria. From all the results, it was assumed that the magnetic fields kill a part of bacteria exposed (**?**).

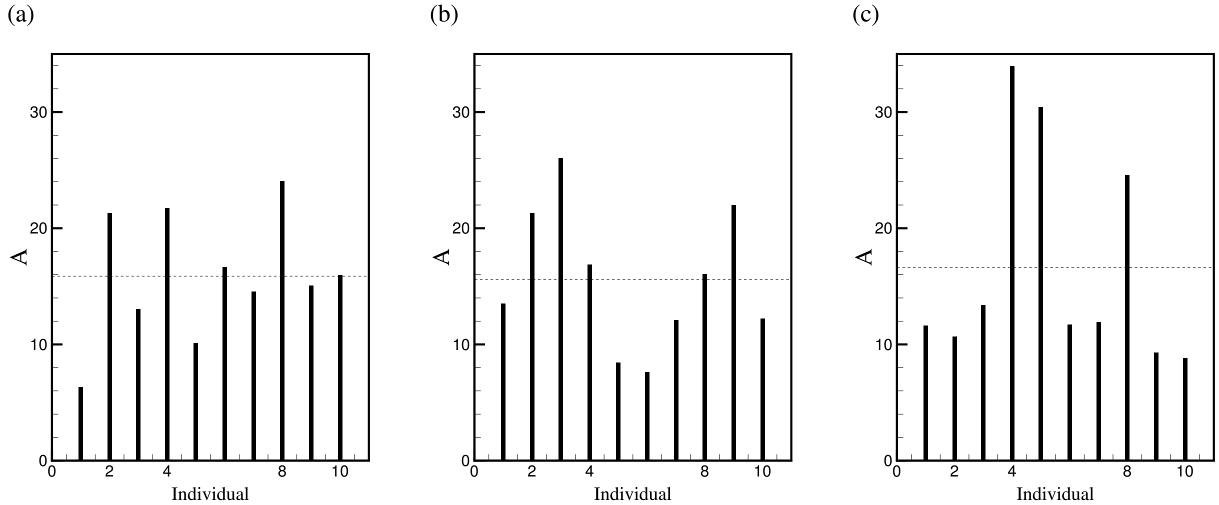

Figure 5. Individuals amplitude (in $\mu m/s$) considering (a) the magnetized sample; (b) the well-fed sample and (c) the starving sample. The dashed lines represent the average velocity from the sample.

4. CONCLUSIONS

Here, we have shown that exposing nematodes to low strengh permanent magnetic fields significantly increased the slip velocity of *C. elegans*. Previous works observed that this application accelerated the nematodes development and reduced their life cycle Lee *et al.* (2010). The results are consistent with those obtained in previous studies. However, the effects of long-term exposure have not been studied. Neither lethal nor mutagenic effects were noticed during the present experiments: all the individuals survived the exposure. In addition, we have observed that not only the slip velocity increased when the sample is magnetized, but the head's movements tended to decrease and the motion became more harmonic. This kind of behaviour is expected since the nematode tend to use all stored energy to propulsion instead of food searching. The starving sample, for example, have shown a nonlinear oscillatoy behavior and a greater amplitude of motion. Experiments using magnetic fields and nematodes are very promising. However, many biological aspects must be considered before taking any conclusions about *C. elegans* kinematics. In addition, different combinations of magnetic fields can be used changing frequency, intensity and experiment time.

5. ACKNOWLEDGEMENTS

The work was supported in part by the Brazilian funding agencies FAPESP - São Paulo State Research Support Foundation (process 2016/14337-5) and CNPq (process 552221/2009-0/142303/2015-1). We wish to aknowledge the support of Professor Carlos Winter from the Nematode's Molecular Biology Laboratory (ICB-USP) and Professor Vicente de Paulo Martins from Pathogens Molecular Analysis Laboratory (IB-UnB) in obtainment and cultivation of *C. elegans*.

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7. RESPONSIBILITY NOTICE

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