



universität
wien

DIPLOMARBEIT

Titel der Diplomarbeit

„A Screen for Magnetosensitive Nematodes “

Verfasser

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angestrebter akademischer Grad

Magister der Naturwissenschaften (Mag.rer.nat.)

Wien, Oktober 2011

Studienkennzahl lt. Studienblatt: A 490

Studienrichtung lt. Studienblatt: Diplomstudium Molekulare Biologie

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Abstract:

Magnetosensation is a sensory phenomenon that has been described in a multitude of organisms including bacteria, protists, insects and birds. The neuronal, cellular and molecular mechanisms that underlie this sense remain mysterious. Advances in the field have primarily been limited by the absence of model systems that can be maintained in a laboratory and genetically manipulated. Here, we present the preliminary results of a behavioural screen to identify magnetosensitive nematodes. It is based on the rationale that a nematode will have a selective advantage if it is able to employ the inclination of the earth's magnetic field to guide geotactic behaviour in a three dimensional environment. This hypothesis has been formed specifically with aquatic and troglobite (i.e. cave dwelling) nematode species in mind. The assay we have developed records the direction, speed, reversals and omega turns of 50 individual nematodes simultaneously, while exposing them to three different magnetic environments. To date, this screen has been applied to just a small percentage (~0.0038%) of the nematode species that reside on the planet earth. Should a magnetosensitive nematode exist, the assay described in this thesis will allow its identification.

Zusammenfassung:

Magnetorezeption ist ein Sinnesphänomen, das in einer Vielzahl an Organismen, darunter Bakterien, Protisten, Insekten und Vögeln, beschrieben wurde. Die neuronalen, zellulären und molekularen Mechanismen, welche diesem Sinn zugrunde liegen, sind aber immer noch unbekannt. Fortschritte in diesem Gebiet sind vor allem durch den Mangel an Modellsystemen, die im Labor gehalten und genetisch manipuliert werden können, begrenzt. In dieser Arbeit werden die vorläufigen Ergebnisse eines Verhaltensexperiments zur Identifikation von magnetosensitiven Nematoden vorgestellt. Dieses basiert auf der Annahme, dass Nematoden einen selektiven Vorteil haben, wenn sie die Inklination des Magnetfeldes der Erde für gravitaktisches Verhalten in einer dreidimensionalen Umgebung verwenden können. Diese Annahme wurde im Besonderen mit Blick auf aquatische Nematoden und Stygobionta (d.h. höhlenbewohnend) gebildet. Die hier vorgestellte Methode misst die Richtung, Geschwindigkeit, Reversals und Omega-Turns von 50 verschiedenen Nematoden gleichzeitig während diese drei verschiedenen magnetischen Umgebungen ausgesetzt werden. Bis dato wurde dieses Experiment nur bei einem kleinen Prozentsatz ($\sim 0.0038\%$) der auf der Erde vorhandenen Nematodenarten angewandt. Sollte eine magnetosensitive Nematode existieren, würde die im Zuge dieser Diplomarbeit etabliert Methode ihre Identifizierung ermöglichen.

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1. Introduction

1.1 Magnetoreception – A Sense Without a Receptor

Primary sensory cells turn physical dimensions into perceivable sensory modalities. They are, so to say, our windows to the world and it is through them that any living system can interact with its environment (Kandel et al., 2000). Almost all senses can be described mechanistically except for one: the magnetic sense, which enables an organism to perceive the earth's magnetic field and to extract navigational information from it. In spite of the quantity of behavioural findings supporting its existence and the large body of theoretical models, the cellular and molecular mechanism remains elusive. Hence the magnetoreceptor is still to be discovered.

1.1.1 The Stimulus – Magnetic Fields

A sensory system is defined by the physical properties that it detects; in the case of magnetoreception these are the different components of a magnetic field. Magnetic fields can be generated in two ways: either by a permanently magnetic material, possessing an intrinsic dipole (e.g. magnetite), or by moving electrical charges (Meschede, 2005). The resulting magnetic field can be described as a vector field, applying a force of certain magnitude and direction onto a ferromagnetic material within it. Magnetic field strength (or magnetic flux density) is measured in Tesla (defined as $1\text{T} = 1\text{V}\cdot\text{s} / \text{m}^2$). Alternatively Gauss is used, whereas one Gauss equals 10^{-4} Tesla. Graphically, magnetic fields are often depicted as vector fields where magnitude and direction at any given point are represented by an arrow with corresponding length and direction (always pointing towards the south pole). Magnetic fields can also be depicted as field lines, in which the vectors are connected to lines and the density of the lines, rather than the vector's length represent the magnitude of the magnetic field (Meschede, 2005).

1.1.2 The Earth's Magnetic Field

1.1.2.1 Origin

Although humans have used the earth's magnetic field since the discovery of the compass, its origin has long been a matter of debate and the problem is not entirely resolved to this day. The most widely accepted theory claims that the earth's magnetic field is a dynamic field, whose origin lies in the convective motion of the liquid outer core of the earth, which mostly consists of iron alloys and is marked by a constant drift of the axis of the field (Buffett, 2000). Indeed, the magnetic south pole has moved more than 600km since 1900 and complete reversals of the field occur sporadically (Meschede, 2005). It can be compared to a giant bar magnet, constituting a uniform magnetic field, meaning that all field lines run parallel (Figure 1.1). Counter intuitively the magnetic north pole lies close to geographic south pole and vice versa. The north pole of a compass needle points towards the geomagnetic south pole and geographic north pole, respectively. The different parameters that can be extracted from the field are explained in more detail below.

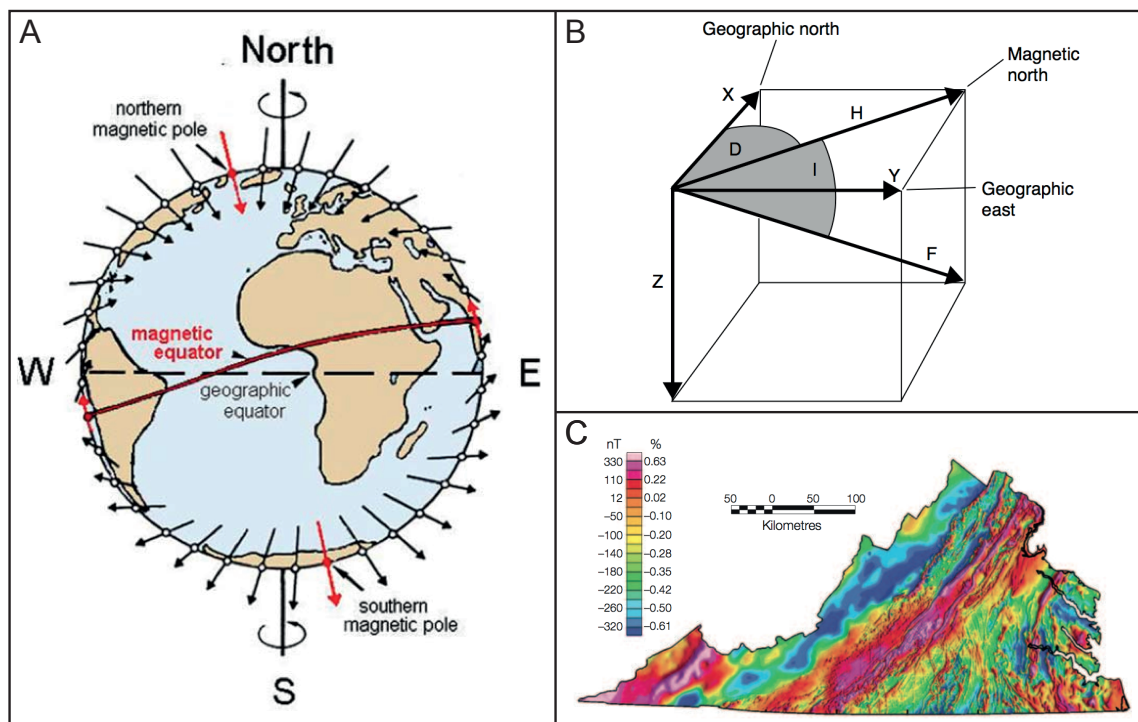


Figure 1.1: The geomagnetic field: (A) taken from Wiltshko and Wiltshko, 2005; showing the inclination angle and size (length) of the magnetic field vectors at the surface of the earth, being weakest at the equator (Inclination= 0°) and strongest at the poles (inclination= 90°). Note that the magnetic and geographic axes are not aligned. (B) shows a 3D reconstruction of a magnetic field vector F and its horizontal component H . The angles D and I are the Declination and Inclination,

respectively. The former is defined as the angle between magnetic and geographic north and the latter as the angle between F and the horizontal plane. (from Walker et al., 2002.) **(C)** shows a map of Virginia colour-coding the intensity deviations from the uniform dipole field (520mG), due to the heterogeneous composition of the earth crust. (Figure taken from Johnsen and Lohmann, 2005)

1.1.2.2 Direction

The most prominent parameter encoded in the geomagnetic field is its horizontal direction. Every compass needle aligns with the horizontal component of the magnetic field and therefore points towards one pole (usually the geographic north pole). Declination is the angle that the magnetic south pole deviates from the geographic north pole. The detection of the horizontal direction relies on the polarity of the magnetic field vector.

1.1.2.3 Inclination

The inclination, or dip angle, is the angle with which the magnetic field lines penetrate the surface of the earth at a given point (Figure 1a). At the magnetic poles the field lines run perpendicular to the surface, and consequently the inclination angle is 90° . When moving from the pole they steadily tilt until, at the magnetic equator, they run parallel to the surface yielding a 0° declination angle. When continuing towards the other pole the dip angle increases again. Hence the inclination provides two types of information: first, positional information of the geographical latitude within one hemisphere, and, second, directional information, either towards increasing or decreasing inclination towards the poles or the equator, respectively. It is important to mention that the inclination does not provide direct information about the polarity of the magnetic field (Johnsen and Lohmann, 2005; Wiltchko and Wiltchko, 2006).

1.1.2.4 Intensity

Although always uniform, the total intensity of the local magnetic field vectors around the earth underlie two types of variations. The first type is the systematic intensity change that corresponds to the inclination angle, being weakest at the magnetic equator (~ 0.3 Gauss) and strongest (~ 0.6 Gauss) at the poles (Wiltchko and Wiltchko, 2011). The second type of variation, which is biologically more interesting,

is a result of the heterogeneous distribution of ferromagnetic materials in the earth's crust, which causes magnetic anomalies. These intensity variations usually contribute only a few percent of the total intensity, but nonetheless might be used by animals to generate a type of magnetic representation that could resemble a topographic map, see Figure 1.1(C). (Johnsen and Lohmann, 2005).

1.1.2 The Sense

Behavioural experiments in the last 50 years have generated an extensive body of data, reporting the sensitivity of a variety of animals to various changes in magnetic fields (reviewed in: Johnsen and Lohmann, 2005; Lohmann and Johnsen, 2000; Ritz et al., 2002; Rodgers and Hore, 2009; Wiltschko and Wiltschko, 2006, 2011; Wiltschko and Wiltschko, 2005).

1.1.2.1 Magnetoreceptive Species

The simplest organisms to exploit information provided by the geomagnetic field are “magnetotactic” bacteria. They form a morphologically diverse and polyphyletic group of aquatic bacteria that possess membrane enclosed “organelles” containing the iron oxide magnetite, Fe_3O_4 (Frankel et al., 1979). These organelles, called magnetosomes, align the whole microorganism with the magnetic field lines constraining its movement along this axis and thereby facilitating their search for microoxic environments, such as Oxic/Anoxic Transition Zones (OATZ) (Blakemore, 1982; Faivre and Schüller, 2008). However, this form of magnetotaxis is not restricted to prokaryotes; protists, unicellular eukaryotes, have been reported to employ the very same mechanisms following the geomagnetic field's inclination in order to reach microoxic layers of salt ponds (Bazylinski et al., 2000).

Within the animal kingdom a multitude of species has been reported to be able to perceive magnetic fields in various ways. Mud snails (Brown Jr et al., 1964) and spiny lobsters (Lohmann et al., 1995), for example, both invertebrates, were reported to be sensitive to the magnetic field direction. Magnetotactic behaviour has also been reported in various insect species, such as the fruit fly *Drosophila melanogaster* (Gegear et al., 2008), the cockroach *Periplaneta americana* (Vacha et al., 2010), the

honeybee *Apis mellifica* (Walker and Bitterman, 1985, 1989), ants and termites (reviewed in: Wajnberg et al., 2010). Furthermore, vertebrate species such as the newt, *Notophthalmus viridescens* (Phillips, 1986), the sea turtle *Caretta caretta* (Lohmann, 1991), the trout, *Oncorhynchus mykiss* (Walker et al., 1997), the tuna, *Thunnus albacares* (Walker et al., 1984a), the salmon, *Oncorhynchus nerka* (Walker et al., 1988), zebrafish, *Danio rerio* (Shcherbakov et al., 2005) and even mammals like the naked mole rat, *Heterocephalus glaber* (Marhold and Wiltschko, 1997), the mouse *Mus musculus* (Muheim et al., 2006), the bat, *Eptesicus fuscus*, (Holland et al., 2008) and ruminants (Begall et al., 2008) have displayed magnetosensitive behaviour of some sort. Most evidence, however, comes from migratory songbirds, in particular European robins, like *Erithacus rubecula* (Wiltschko, 1972), and the homing pigeon *Columbia livia* (Walcott and Green, 1974; Ritz et al., 2000; Wiltschko and Wiltschko, 2006; Wiltschko, 1996; Wiltschko and Wiltschko, 2005).

1.1.2.2 Proposed Mechanisms

Despite the large amount of behavioural data the molecular mechanism underlying magnetoreception is unknown. Two fundamentally different models have been proposed and each can account only for certain behavioural findings. The models and behavioural evidence are described in more detail below

1.1.2.2.1 Chemical Magnetoreception

The first model is based on the idea that the earth's magnetic field can influence the outcome of a chemical reaction. Since the energy necessary to alter chemical bonds is by orders of magnitude larger than the energy provided by the earth's magnetic field this kind of interaction is only feasible in certain chemical species. One of those species, which was proposed by Klaus Schulten (Schulten, 1982), is a radical pair, usually formed by photoexcitation. The hypothesis holds that an electron is transferred from a donor to an acceptor group (or molecule), leaving each with an unpaired electron. This radical pair can exist in two states, either singlet (the two electrons have anti-parallel spin states) or triplet (the two electrons have parallel spin states). The interconversion rate between these states depends on the surrounding magnetic field's orientation and intensity, but not on its polarity. Each state potentially

has different chemical characteristics and could thus lead to a detectable downstream response (Lohmann, 2008; Rodgers and Hore, 2009). As this mechanism relies on light, Ritz and colleagues (Ritz et al., 2000) proposed that the sensory cells may reside in the retina. They further argued that Cryptochrome, a blue-light sensitive flavoprotein, could be the radical pair receptor. Two types of Cryptochromes exist (only one is light sensitive) and in many organisms they are involved in the regulation of circadian rhythms (Chaves et al., 2011; Liedvogel and Mouritsen, 2010). Animals employing this mechanism would be expected to behave in a light-dependent manner, which was confirmed in a variety of species. Robins for example lost their navigational abilities if exposed to only red light (Wiltschko et al., 1993), (Wiltschko, 1999), (Wiltschko et al., 2010), as did flies (Phillips and Sayeed, 1993) and newts (Phillips and Borland, 1992; reviewed in Deutschlander and Phillips, 1999). Furthermore, the pineal gland was reported to be sensitive to magnetic alterations in a light-dependent manner (Semm et al., 1980; Deutschlander et al., 1999).

1.1.2.2.2 Magnetite Based Magnetoreception

The second mechanism was inspired by the discovery of the aforementioned magnetotactic bacteria (Frankel et al., 1979). Depending on its size and axial ratio, magnetite can occur in three states with distinct properties. The first state consists of single-domain (SD) magnetite crystals (about 50nm in diameter). Their interaction with the geomagnetic field is the strongest because they possess a permanent magnetic dipole, which physically aligns with an external magnetic field. According to the model, these crystals can form chains and could thus provide directional information to a sensory system by either direct or indirect attachment to ion channels. (Johnsen and Lohmann, 2005; Kirschvink and Gould, 1981)

The second state consists of superparamagnetic (SPM) crystals. They are smaller than SD-magnetite, do not possess their own magnetic moment and hence do not physically align with an external field. Instead, their magnetic moment depends on the external field and is always induced in the opposite direction. Through secondary structures, clusters of superparamagnetic crystals could also induce forces, large enough to be integrated into cellular signalling pathways (Davila et al., 2003). Theoretical analysis has provided the necessary background to suggest different

cellular mechanisms that could explain the transduction of the magnetic information to nervous stimuli (Solov'yov and Greiner, 2007; Walker et al., 2002; Winklhofer and Kirschvink, 2010). The third state are multidomain (MD) magnetite structures, which are even larger than SD-magnetite and consist of several magnetic domains with a very low overall magnetic moment, leading thereby to weak interactions with the geomagnetic field and are thus unlikely to play a role in magnetoreception (Walker and Diebel, 2006).

Unlike the light-dependent mechanism, the magnetite-dependent mechanism could account for the perception of polarity and intensity of the geomagnetic field (Kirschvink and Gould, 1981; Kirschvink et al., 2001).

Single domain crystals have been detected in the heads of magnetosensitive fish such as trout (Diebel et al., 2000; Walker et al., 1997), tuna and salmon (Walker et al., 1984b; Walker et al., 1988). In trout, the roV nerve (a branch of the trigeminal nerve) that innervates the olfactory rosette (which was found to contain magnetite), has been shown to be sensitive to alterations in magnetic intensity by electrophysiological experiments (Walker et al., 1997). Magnetite structures have also been detected in the beak of Bobolinks (a migratory bird) and electrophysiological recordings from this species implicated the trigeminal nerve in the detection of magnetic intensity variations (Semm and Beason, 1990). The role of the trigeminal nerve in magnetoreception is further supported by lesion studies in pigeons which have shown that it is required for discriminating between the absence and presence of a magnetic field in a conditioning assay (Mora et al., 2004).

Taken together, the current evidence suggests the existence of both mechanisms, sometimes even within the same organism (Wiltschko, 1995). Although this might appear counter intuitive, given the complexity of each mechanism, it is possible that they are responsible for the detection of different components of the magnetic field. The chemical mechanism is likely to mediate the directional information of the geomagnetic field, whereas the magnetite-dependent mechanism could perceive magnetic field intensities and be used to generate magnetic “maps” containing positional information (Mouritsen and Ritz, 2005; Wiltschko and Wiltschko, 2011).

1.1.3 Disciplines in Magnetoreception

The nature of the magnetic sense has resulted in a scientific field that is, in many respects, very heterogeneous. It is dominated, however, by theoretical physicists, who generate models of the potential mechanisms, and behavioural biologists, who empirically observe very complex behaviours. The gap between those fields has only recently starting to fill with the involvement of new disciplines such as cellular and molecular biology.

A second source of heterogeneity is the multitude of different species, ranging from bacteria to higher mammals, all of which might extract different information for different purposes from the geomagnetic field. As a result, there is no accepted model system in the field, which results in a dearth of genetic and molecular tools available to investigators who wish to take a reductionist approach to the problem.

Although the discovery of magnetosensitive behaviours in the genetic model organisms fruit fly (*Drosophila melanogaster*) and zebrafish (*Danio rerio*) is promising, the biological relevance of magnetoreception in these species is unclear to this date (Lohmann, 2008; Lohmann and Johnsen, 2000; Mouritsen and Ritz, 2005).

1.2 Nematodes – *Caenorhabditis elegans*

Although *C. elegans* was first discovered by Emily Maupas in 1900 (Maupas, 1900), it was 70 years later that Sydney Brenner brought it to world fame (Brenner, 1974).

C. elegans has proved to be a perfect model system for a number of reasons.

First, it is easy to handle in the lab because it is of small size (approximately 1mm), feeds off *E. Coli* and can be maintained on agar plates at room temperature. Second, it is transparent throughout its life cycle, which is very short (about three days, depending on the culturing temperature) and follows distinct developmental stages. Third, because of its hermaphroditic reproduction it is very easy to propagate, although males can be generated and used for crossing (Blaxter, 2011). Fourth, it has an invariant cell lineage, which has been completely mapped, (Sulston, 1983;

Sulston and Horvitz, 1977; Sulston et al., 1983). Fifth, the nervous system is relatively small consisting of 302 neurons, connected by 7600 synapses, but still complex enough to study neurobiology on a behavioural level. Sixth, its genome has been sequenced, and there is a multitude of genetic tools available

1.2.1 The Nematode Phylum

Although *C. elegans* is the most prominent nematode it is not the only one.

Nematodes comprise an entire phylum within the animal kingdom. They are unsegmented roundworms, usually smaller than 1cm and belong to the pseudocoelomates (Abebe et al., 2008). Despite their small size and simple basic body plan, they constitute one of the largest and most diverse animal phyla on earth. Current estimations suggest that there are more than one million different species, 97% of which are still unknown (Abebe et al., 2008). Taxonomic classifications divide the phylum into 2 classes, 19 orders and 221 families. The various nematode species differ not only in morphology and size but also in the reproductive cycle (hermaphroditic, parthenogenetic or gonochoristic), ecology (plant and animal parasites or free living) and feeding type (Blaxter, 2011; De Ley, 2006a).

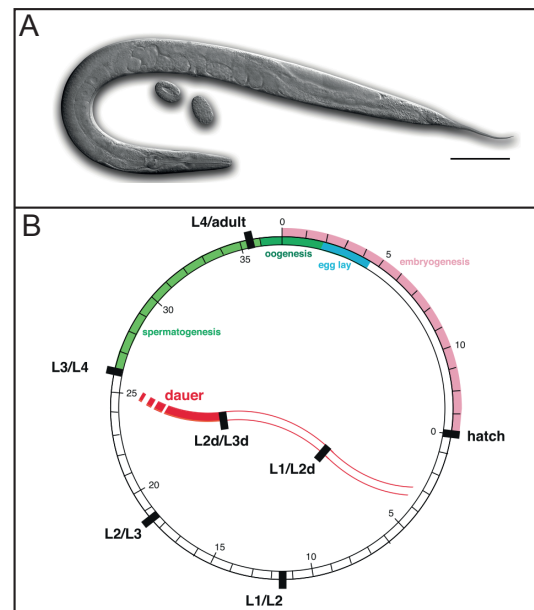


Figure 1.2: *C. elegans*: (A) Micrograph of an adult hermaphrodite (scale bar=0.1mm) (taken from <http://www.wormatlas.org/>) (B) Schematic illustration of the life cycle of *C. elegans*. After hatching, the animals undergo four larval stages (L1-L4) before maturing to an adult. After L2, they can enter a “dauer”-stage, which can be induced by starvation, crowding and high temperature and can last for several months. Upon encountering more favourable conditions, “dauer” larvae mature to L4. (Figure taken from Blaxter, 2011)

1.2.2 Habitats

Nathan Cobb, one of the most influential nematode taxonomists, most figuratively described the abundance of Nematodes on our planet:

"In short, if all the matter in the universe except the nematodes were swept away, our world would still be dimly recognizable, and if, as disembodied spirits, we could then investigate it, we should find its mountains, hills, vales, rivers, lakes, and oceans represented by a film of nematodes. The location of towns would be decipherable, since for every massing of human beings there would be a corresponding massing of certain nematodes. Trees would still stand in ghostly rows representing our streets and highways. The location of the various plants and animals would still be decipherable, and, had we sufficient knowledge, in many cases even their species could be determined by an examination of their erstwhile nematode parasites." (Cobb, 1914)

Indeed, nematodes are found in every imaginable niche, ranging from arctic ice to sulfur-rich marine sediments (De Ley, 2006a) and the deep subsurface of South Africa (Borgonie et al., 2011). The majority of nematode species are free living and only a small part (7%) derive from freshwater environments (Abebe et al., 2008).

Of particular interest, in respect to this study, are freshwater nematodes residing in caves. This environmental niche has several special characteristics, such as a constant temperature throughout the year and the absence of light. Several nematode species were found in caves around the world (Pearse and Banks, 1938; Sarbu et al., 1996) although just a few are believed to be truly cavernicolous (e.g. *Stenonchulus troglobites*; Eder, 1975) .

1.2.3 Sensory Systems and Behaviour

Most of the research on nematode sensory systems and behaviour has been conducted in *C. elegans*, where remarkable findings have been made. *C. elegans* exhibits a variety of behaviours that depend on the integration of various information, such as sensory inputs and previous experience, age and feeding status (Hart, 2006). Chemosensation is one example of a well-studied sensory system. *C. elegans* possesses the ability to detect a variety of volatile and soluble chemicals, and following integration of this information results in an array of different behaviours (Bargmann, 2006). Another sensory modality that *C. elegans* can perceive with remarkable accuracy is temperature (Hedgecock and Russell, 1975). Similarly to chemosensation, this ability can be investigated by examining the associated behaviours like thermotaxis, food conditioning and heat avoidance. It is also possible to use hallmark behaviours such as locomotion speed and omega turn frequency (i.e. an avoidance behaviour that consists of the worm making a sharp turn resembling the greek letter Ω ; Croll, 1975) to assess how a worm responds to changes in

atmospheric oxygen concentrations (Zimmer et al., 2009). In addition, *C. elegans* (and other nematodes) also display phototaxis behaviour (Croll et al., 1972), mediated by light sensitive neurons (Ward et al., 2008), and electrosensory behaviour (galvanotaxis) (Gabel et al., 2007; Sukul et al., 1975). The sensory diversity for such a simple organism is remarkable.

1.3 Hypothesis – A Magnetoreceptive Nematode?

The hypothesis of this study is that a magnetoreceptive nematode exists. This idea is conceivable for a number of reasons.

For one thing, the sheer diversity and size of the nematode phylum; Nematodes inhabit every thinkable niche of the planet and some of them have undergone extreme adaptations to make their survival possible (De Ley, 2006a). One of those niches is caves, which are sensory-deprived environments, characterized by a lack of light. This might lead to the evolution of other, non-visual sensory systems such as a magnetic sense, as suggested in the case of urodeles (Schlegel, 2008). If such a magnetic sense was present in cavernicolous animals, it could naturally not be based on the light, but rather on an alternative mechanism, such as magnetite. Evidence supporting this possibility comes from the finding of biogenic magnetite in *C. elegans* (Cranfield et al., 2004). But what would a cavernicolous nematode use the magnetic sense for, since it displays neither migrating nor homing behaviour?

Although models have suggested that a magnetic sense could also be useful in short distance navigation (Wyeth, 2010), the more plausible option is to use the magnetic field information as an indirect mechanism to sense gravity. In nematodes, there is no evidence for direct geotaxis (Eo and Otobe, 2008), therefore, one could question the necessity for geotaxis in nematodes in the first place. Yet, aquatic sediments, at the bottom of waters constitute some of the nutrient richest microenvironments and freshwater nematode are upon the most abundant metazoa found there (Abebe et al., 2008). It has been hypothesized that negative phototaxis (photoavoidance) found in numerous terrestrial nematode species (Croll et al., 1972) has evolved to constrain them in the soil. An analogous mechanism not dependent on light, might have

evolved in aquatic environments, guiding nematodes along magnetic field lines towards nutrients-rich sediments.

1.4 Project Aims

Given the explicit need for a genetically tractable model organism in the field of magnetoreception and the plausibility of the existence of a magnetoreceptive nematode, the aims of my project are:

1. To establish an assay to screen nematodes for magnetoreceptive behaviour;
and
2. To employ this assay to screen a diverse group of nematodes for magnetoreceptive behaviour with a special emphasis on troglobite species.

2. Materials and Methods

2.1 Materials:

2.1.1 Nematode Plates

All plates and solutions were prepared by the IMP-IMBA media kitchen according to the following recipes.

NGM Plates:

For 1 litre NGM medium, 3g NaCl, 2.5g Peptone and 17g Serva Ager were added to 972ml ddH_2O and autoclaved. Thereafter 1ml cholesterol (1mg/ml in Ethanol), 1ml 1M CaCl_2 , 1ml 1M MgSO_4 and 25ml 1M KH_2PO_4 (pH6) were added aseptically, poured into 6cm Petri dishes and left to dry.

Assay Plates:

For 1 litre, 16g Bacto Agar was added to 993ml ddH_2O and autoclaved. Thereafter 1ml 1M MgCl_2 , 1ml 1M MgSO_4 and 5ml 1M KH_2PO_4 (pH6) were added aseptically, poured into 9cm Petri dishes and left to dry.

“1%-Bacto-Agar” Plates:

For 1 litre, 10g Bacto Agar was added to 1000ml ddH_2O and autoclaved. Thereafter they were aseptically poured into 9cm Petri dishes and left to dry.

M9 Solution:

For 1 litre, 3 g MgCl_2 , 6g Na_2HPO_4 , 5 g NaCl and 1ml 1M MgSO_4 were added to ddH_2O and autoclaved.

2.1.2 Other Materials

OP50:

OP50 strain is a uracil auxotroph *E. Coli* strain that is used as a food source for *C. elegans*, because its growth is restricted to NGM plates (Stiernagle, 2006).

Bleach Solution:

Bleaching solution consisted of 250µl household bleach (stored at 4°C) and 50µl 5M NaOH. It was always freshly prepared before use.

2.2 Methods

2.2.1 Nematode Maintenance

2.2.1.1 Strain Sources

All strains except for SB408 and *H. Mephisto* were received from the Caenorhabditis Genetics Centre (CGC) of the University of Minnesota (<http://www.cbs.umn.edu/CGC/>). SB408 was kindly supplied by Dr. David Fitch from the New York University Rhabditis Centre (NYURC) and *H. Mephisto* from Prof. Esta van Heerden (University of the Free State, South Africa).

2.2.1.2 Culturing Worms

All CGC strains were cultured according to standard procedures (Stiernagle, 2006). In short, they were maintained on 6cm NGM plates, seeded with OP50 as a food source. Worms were transferred to a new plate either by picking individual animals (usually 4-7 hermaphrodites or around 5 females and at least 10 males for gonochoristic strains) or by cutting out an entire piece of agar. The latter was necessary if plates were overgrown. The transferring frequency depended on the temperature and the type of strain, but ranged between 3 days up to several weeks. Strains were either maintained at 20°C or 16°C to reduce growth rate. All maintenance work was conducted using a Leica M125 stereomicroscope.

H.Mephisto and SB408, in contrast were cultured on “1%-Bacto-Agar” Plates. They were transferred via chunks of agar and fed off bacterial cultures that were co-transferred with the worms from the original plates.

2.2.1.3 Seeding Plates

500ml of LB medium were inoculated with *E.Coli* OP50 bacteria and incubated at 37°C over night. The bacterial culture was then directly used or aliquoted and stored at 4°C. For seeding 6cm NGM plates, 200µl pre warmed OP50 culture was placed at their centre under aseptic conditions. The plates were then dried for 2 days at room temperature before further use or storage at 4°C (Stiernagle, 2006).

2.2.1.4 Bleaching Worms

Worm colonies were bleached when contaminated with either bacteria or fungi. Ten to 20 gravid hermaphrodites or females were transferred to an intermediate NGM plate and covered with approximately 20µl bleaching solution and left there until they stopped moving. Subsequently the worms were transferred to a new NGM plate. Depending on the strain, the eggs (which survived the bleaching) hatched within the next days (Stiernagle, 2006).

2.2.2 Isolation:

2.2.2.1 Sample Collection

Sediment samples were collected by taking a volume of approximately 1 litre of water including sediments, mud or both from the bottom of water holes and puddles. The sample was then transported to the laboratory as fast as possible for further processing.

2.2.2.2 Nematode Extraction

The nematodes from the sediment samples were extracted using a Baermann funnel (Barrière and Félix, 2006). The sample was carefully placed into the cone of a funnel (either glass or plastic; approximately 15 cm in diameter), which's inside was coated with two layers of regular cleaning tissues. The stem of the funnel was tightly attached to a plastic tubing, which was sealed at its bottom (Figure 2.1). The funnel was then filled with tap water until the entire sample was covered and incubated at room temperature over night. The following day the water was collected and examined for the presence of nematodes under the stereomicroscope, which were then transferred, either by picking or pipetting, to a seeded NGM plate covered with M9 solution.

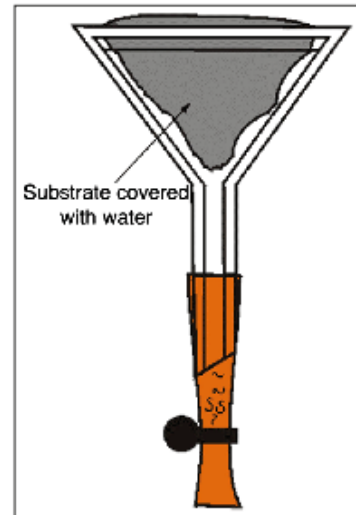


Figure 2.1: A Baermann Funnel was used to extract nematodes from the collected sediments. (Figure taken from: Barrière and Félix, 2006)

2.2.3 Experimental Procedures

2.2.3.1 Tracking experiment

A Plexiglas box was custom-made to hold the Merritt four-coil system (Merritt et al., 1983) with the dimensions 65x60x60 (WxHxD). The inner coils, consisting of 11 turns, were 7.686 cm, and the outer coils, consisting of 26 turns, were 30.33 cm from the centre of the box (in both directions). The copper wire, 0.8 mm in diameter, was stabilised by epoxy resin. The custom-made digital relay, programmed in LOGO! Soft Comfort Software (Siemens) was based on a double-pole, double-throw (DPDS) configuration as suggested by Kirschvink (Kirschvink, 1992) and controlled the stimulus application. It was connected to three independent power supplies (EA PSI 8032-20T and ISOTECH IPS 2303; possesses two independent outlets) and hence allowed the application of three different stimulus intensities of variable length.

Prior to the experiment, approximately 50 worms were transferred (by picking) from the NGM culture plate to an intermediate, “starvation” plate (9cm assay-plate) for

1 hour. A 0.5 cm thick “arena” of Whatman paper with the internal measurements of 3.1x2.4 cm was soaked in 20 mM CuCl₂ and placed at the centre of a new assay plate. After starvation, the worms were transferred into the arena and the plate was placed at the centre of the Plexiglas box. Directly underneath the worms an LED light source provided homogenous illumination. Approximately 0.8 m above the assay plate, held by an aluminium-plastic stand, a Sony XCD-SX900 CCD camera, attached to an objective (Nikon Micro NIKKOR 105mm), was focused on the assay plate. The camera recorded 7.5 frames per second with a resolution of 1280x960 pixels, which approximately corresponded to the 3.1x2.4 cm large arena. Movies were captured using VirtualDub (<http://www.virtualdub.org/>) and saved as uncompressed avi-files. The trial was initiated by simultaneously starting the stimulus application and capture program and lasted for 25 minutes, however, the first five minutes were discarded. Each experiment consisted of two trials, as described above.

2.2.3.2 Application of CO₂

The CO₂ was applied by putting dry ice pellets into a bottle of warm water. The opening of the bottle was equipped with a valve and connected to a fine plastic tubing which was fixed right next to the assay plate and pointing directly onto the worms inside the arena. The stimulus was switched on by opening the valve.

2.2.4 Data Analysis

2.2.4.1 Worm Tracking

The movies were analyzed with worm tracker software (The Parallel Worm Tracker (Ramot et al., 2008) run in Matlab (Mathworks, Version 2010b, including the Image Acquisition and Processing Toolboxes). The tracker converts each frame to a binary image and defines objects as clusters of pixels. Single worms are defined by object-size, depending on the thresholds minimum and maximum worm-length. Another script then extracted speed, direction, change in X and Y-position and omega turn frequency of the worm paths. The data from the different paths (worms) was first averaged to produce one “population” value for each parameter and frame, which

were subsequently binned and plotted (further described by Chalasani et al., 2007) and then saved as a matrix with the dimensions frames and tracks, which was further used for statistical analysis. Data from both trials were further consolidated to generate one data set deriving from approximately 100 worms at a time.

2.2.4.2 Statistical Analysis

Prism5 (Graphpad Software) was used for statistical analysis. The data was imported and two two-tailed paired t-tests were performed comparing (1) the ST-pre (15 seconds before stimulus onset) with ST-post (15 seconds after stimulus onset) and (2) the LT-pre (60 seconds before ST-pre) with LT-post (60 seconds after ST-post).

2.2.5. Magnetic field measurements:

Magnetic field changes were measured using two fluxgate magnetometers (AlphaLab Inc.) An AlphaLab DC Milligaussmeter ModelMGM has a range of +/- 2 Gauss and a resolution of 0.01 milligauss, and an AlphaLab DC Gaussmeter Model VGM with a range of +/-799 Gauss and an accuracy of 1% reading. The latter model allows the measurement of total intensity as well as the vector magnitudes along the three cardinal axes.

3. Results

3.1 Establishment of the Screen

Technical advancements in the field of *C. elegans* neurobiology have made it possible to detect subtle behavioural changes by tracking a multitude of worms in parallel to automatically extract hallmark behaviours such as speed, direction, omega-turn frequency, as well as small and large reversals (Ramot et al., 2008). Based on this technique, I set out to establish a setup which enables automatic simultaneous tracking of approximately 50 worms, while manipulating three components of the earth's magnetic field: the intensity; the inclination; and the declination. My goal was to alter these parameters without producing any other sensory inputs for the worms. The latter point is of particular importance because any behavioural response to magnetic fields might be very subtle. Thorough precautions were therefore taken to control all other stimuli such as sound, airflow, temperature and vibrations. The entire screen was designed in accordance with the suggestions given by Kirschvink (Kirschvink, 1992; Kirschvink et al., 2010) for biomagnetic experiments, further described below.

3.1.1 The Apparatus

The setup I designed had to fulfil two major objectives: (1) automatically recording of moving nematodes for further behavioural analysis, while (2) generating magnetic stimuli. This was achieved in the following way. The assay plate containing the animals was positioned in the exact centre of a Plexiglas box (constituting the electrical coil system). An underlying LED light source made the worms appear as dark objects on a bright background on the CCD camera recording from exactly above the centre of the box (approximately 0.8 m above the plate). The captured movies were then saved as .avi (Audio Video Interleave). The magnetic stimuli were generated using a Merritt four-square coil design (Merritt et al., 1983) which was chosen because it is easy to construct, while yielding a large volume of uniform field space in its centre (Kirschvink, 1992).

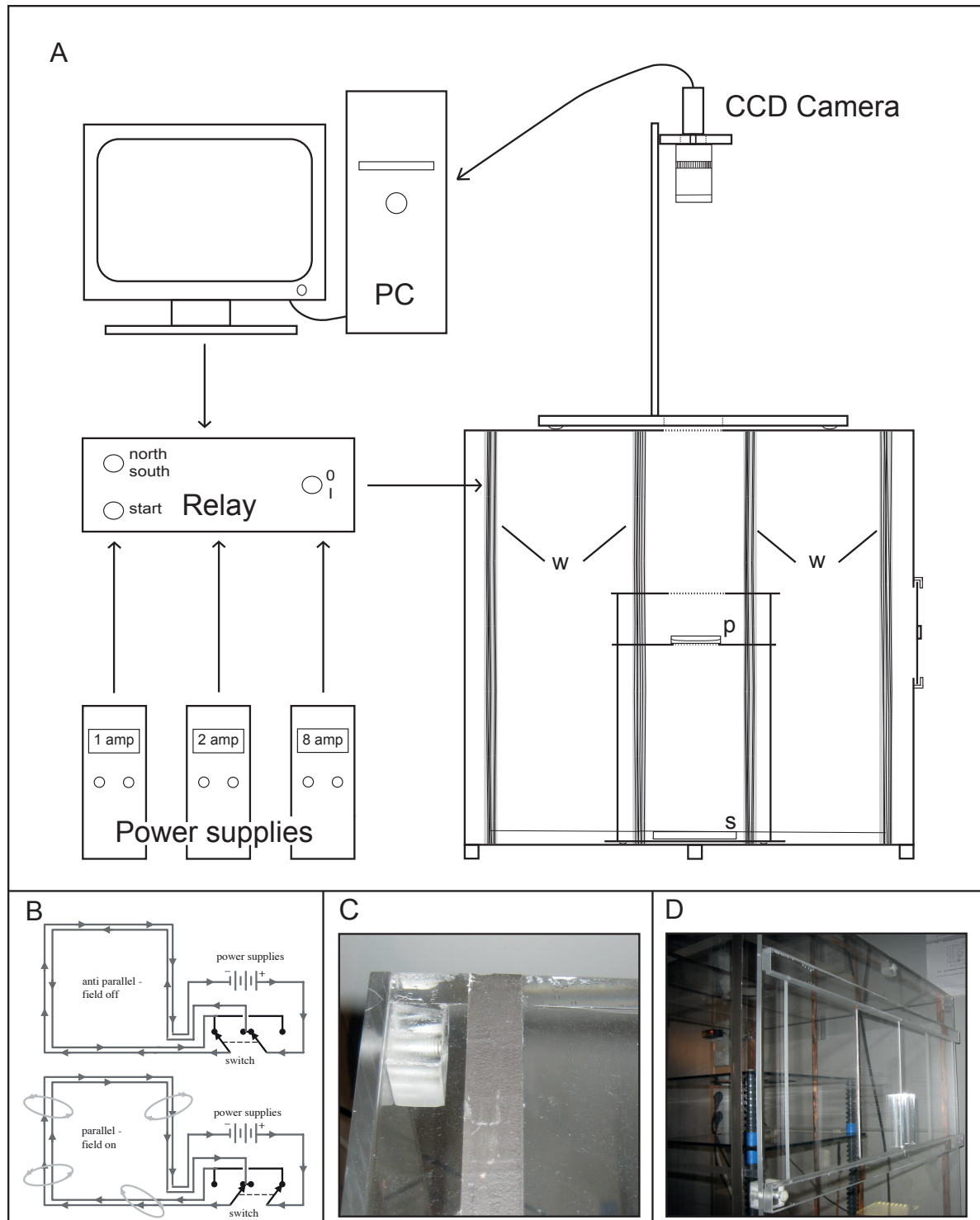


Figure 3.1: The Behavioural Setup for the nematode magnetosensitivity screen. The entire system is schematically depicted in **(A)**. The digital relay, which receives electricity from three power supplies, controls the flow of current through the Merritt four square coil system (w) that constitutes the magnetic stimuli. It allows regulating direction and duration of the 3 stimuli. The worms are on an assay plate (p), which resides in the centre of the apparatus. A LED light source (s) illuminates the plate from the bottom and makes the worms appear as dark objects on a light background. During an experiment the CCD camera above the apparatus records the worms. **(B)** The principle of a double-wrapped coil design: the coil consists of two wires wrapped in parallel. The relay (switch) controls the direction of current running through the two wires and can take either an anti-parallel or parallel state, yielding a net zero or additive field, respectively. (Taken and adapted from Kirschvink et al., 2010.) **(C)** shows the epoxy-covered wires. The epoxy resin enclosed the coils and solidified, thereby fixing them

and preventing vibrations. The inside of the box was sealed with Plexiglas, preventing airflow above the assay plate. The worms were placed in the box via a custom made sliding door (**D**).

The intensity and duration of the magnetic stimuli were controlled by a relay, which was powered by three power supplies, generating different currents (1amp, 2amp and 8amp). Figure 3.1 shows the principle design of the experimental apparatus pointing out its most important components.

The flow of electrons through a wire results not only in a magnetic field but also in the production of heat and vibrations. As suggested by Kirschvink (Kirschvink, 1992), the adoption of a double-wrapped coil system can help in controlling these factors.

3.1.1.1 Heat

The principle of a double-wrapped coil system is to uncouple heat development from the generation of the magnetic field. This was accomplished by wrapping two identical wires in parallel around the coil, yielding two independent coils, connected in series. A schematic wiring diagram is depicted in Figure 3.1 B.

A relay was constructed that regulates the direction of current flowing through the two coils in respect to each other. The two possible configurations of the system are either parallel or anti-parallel. If currents of equal magnitude flow through the coils in the anti-parallel configuration, the resulting magnetic fields perfectly cancel each other out, yielding a net zero magnetic field. In contrast, if the two coils are set in parallel, the flow of electrons through the coils generates an additive magnetic field focused in the centre of the system. Hence, the advantage of a double-wrapped design lies in the fact that it is not the current that is being switched on or off in order to generate the magnetic field but the orientation of two coils in respect to each other. Likewise, the size of the system (the minimum distance from the coils to the worms was 30 cm) was chosen to minimize a direct influence of heat development.

3.1.1.2 Vibrations

Vibrations originating from magnetomechanical interactions between the wires in the coil could coincide with the stimuli onset. To eliminate this possibility the wires were

coated with epoxy resin and fixed in position, thereby making any vibration impossible. (Figure 3.1 C)

3.1.1.3 Airflow

Another possible influence on the nematodes' behaviour that could lead to a directional bias is airflow. In order to minimize its influence on the results, the behavioural apparatus was completely shielded with Plexiglas, excepting a sliding door, which was closed during the experiments. (See Figure 3.1 D)

3.1.1.4 Noise

Yet another sensory modality that could coincide with the onset of the magnetic stimulus is the noise produced by the mechanical switches in the control relay and power supplies. This was avoided by using only digital switches which did not produce any sound when changing the direction or onset of a current.

3.1.2 The Assay

Having constructed the aforementioned experimental apparatus, which permits the controlled presentation of the stimuli, I proceeded with my behavioural screen.

In designing a behavioural assay employing magnetic stimuli I drew on existing paradigms published in the field of *C. elegans* neurobiology (Ramot et al., 2008). The assay, as schematically depicted in Figure 3.2, consisted of distinct steps described in more detail below. Two assays, conducted consecutively, with worms from the same plate made up one experiment. This approach increased the number of animals analysed and generated two sets of data.

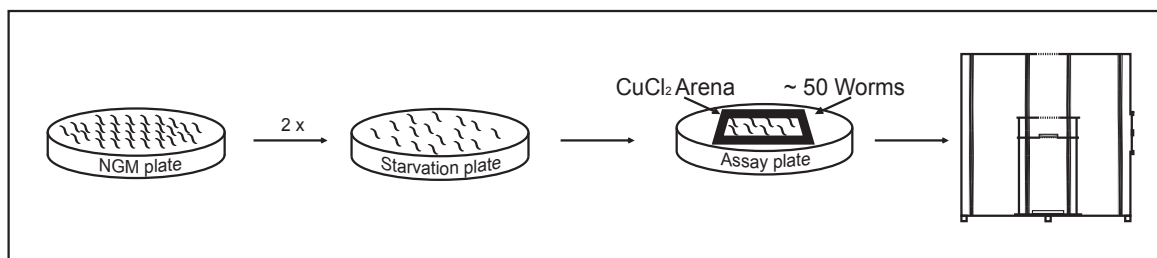


Figure 3.2: Experimental procedure: Nematode strains were cultivated on NGM plates. In preparation for the experiment approximately 50 L4 female or hermaphrodite worms were transferred

onto a starvation plate for one hour. Following starvation, worms were transferred to an assay plate and surrounded by a CuCl_2 soaked Whatman paper arena to contain the worms. This plate was placed in the centre of the testing box and subsequently tested.

3.1.2.1 The Worms

As in all nematode behavioural experiments, it is very important to set strict criteria for selecting the worms analysed. Parameters such as sex, stage in life cycle and age can influence the innate behaviour of the animal. I decided to test only hermaphroditic (or female) animals in the L4 (young adult) stage of development. This stage is visually easy to distinguish from other stages and the animals' nervous system is well developed. In addition to the animals' sex and life cycle stage, I also made sure not to use animals from plates that were contaminated with either fungi or bacteria. Both factors could potentially alter the worms' behaviour (Hart, 2006 and references therein).

3.1.2.2 Starvation

Prior to behavioural testing approximately 50 worms were transferred onto an intermediate starvation plate for one hour. The rationale behind this additional step was to make the animals more accessible for potential sensory cues, thereby increasing the probability of eliciting a behavioural response.

3.1.2.3 Testing

Following starvation, the worms were transferred onto the assay plate. The centre of the plate was surrounded by a rectangular "arena" consisting of CuCl_2 -soaked Whatmann paper. CuCl_2 , which nematodes find aversive, serves as a chemical barrier preventing them from leaving the arena. (Zimmer et al., 2009). The assay plate was then positioned in the centre of the electrical coil system in a way that the camera's field of view was aligned with the arena. The assay was initiated by simultaneously switching on the capture and coil system. Thereafter all other light sources were switched off and the lab was vacated.

3.1.2.4 The Stimuli

I employed a magnetic stimulus that altered in intensity, inclination and declination simultaneously to maximise the diversity of potential cues encoded in a magnetic field.

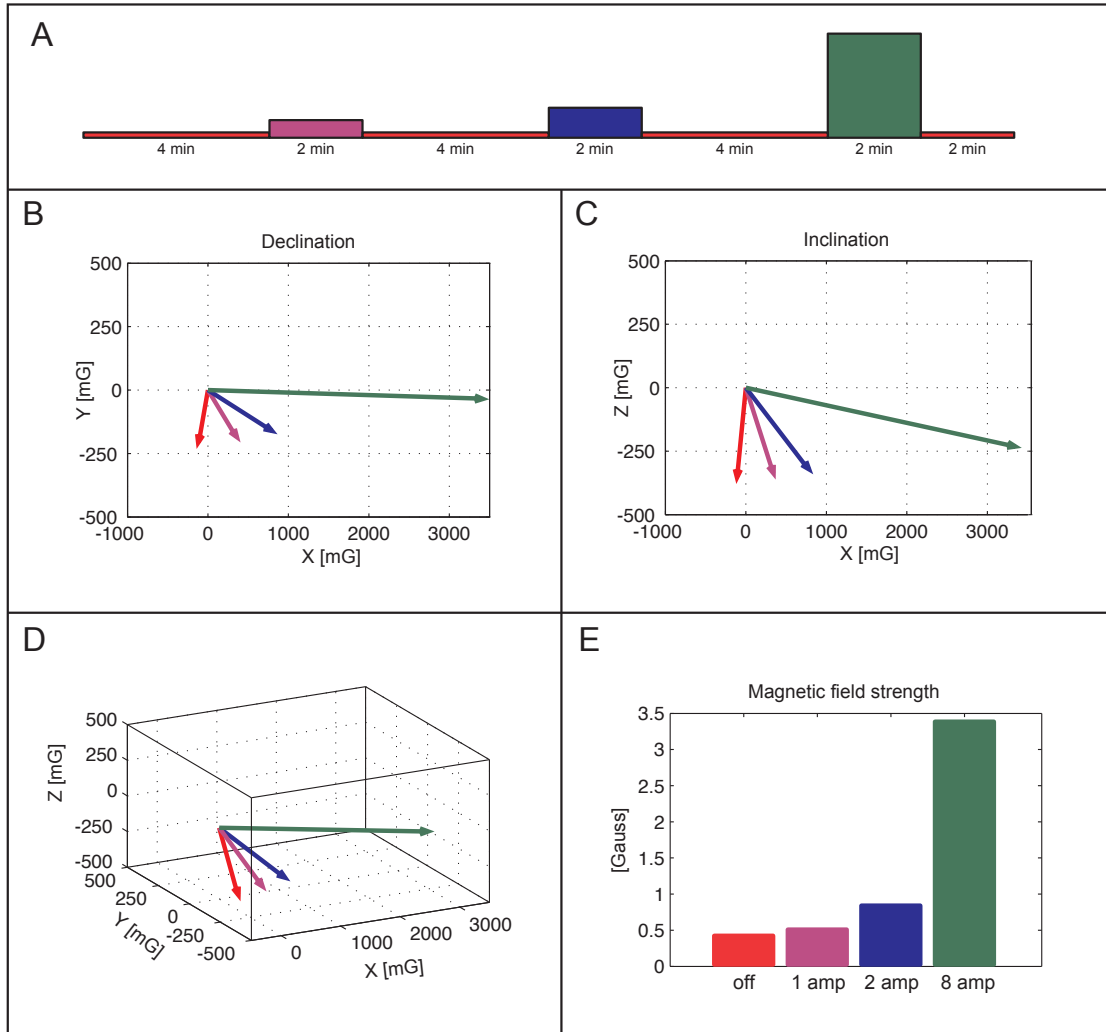


Figure 3.3: Magnetic stimuli: (A) Time course of the experiment. One 20-minute trial consisted of the application of three different two-minute stimuli, separated by four-minute pauses. (B-E) Representations of the different magnetic environments showing the “off” state in red and the three stimuli defined by 1 ampere (amp), 2 ampere and 8 ampere applied to the coil in purple, blue and green, respectively. (B-C) represents the alterations to the horizontal (declination) and vertical (inclination) components of the magnetic field during the assay. (D) A three-dimensional model of the different magnetic field conditions. (E) The total intensities of the magnetic field, defined by the application of the three different currents.

3.1.2.4.1 Time Course

One experimental trial lasted for a total of 25 minutes, the first five minutes, however, were included only to let the worms adapt to the environment and were discarded

directly after capturing, leaving 20 minutes of real experimental trial. During this time three 2-minutes stimuli of increasing strength were applied. The stimuli were separated by four-minutes lag phases. (See Figure 3.3 (A)) A two-minute time interval for stimuli presentation was adopted, so that the animals would have sufficient time to react to the altered magnetic environment.

3.1.2.4.2 Magnetic Stimuli

The red arrows in Figure 3.3 (B-C) represent the external geomagnetic field in the centre of the box without alteration (i.e. when the coil system was either switched off or in anti-parallel mode). The application of the additive magnetic field resulted in a change in the intensity, declination and inclination of the field in the centre of the box. The extent to which the direction of the field is altered is a function of the magnitude of the induced field produced by the current. Figure 3.3 (B-E) shows the relationship between the direction of the magnetic field and the current flowing through the coil.

The 1 amp condition rotated the field with a only slight increase in magnitude. The 2amp condition doubled the magnitude of the magnetic field, from approximately 0.4 to 0.9 Gauss, rotating the field further. The 8amp condition, in contrast, increased the magnitude of the magnetic field by a factor of 10 to 3.4 Gauss, and rotated the field almost parallel to the electrical coil system. Magnetic fields of this magnitude do not naturally exist at the surface of the earth, but may be employed to elicit a more robust behavioural response (Gegear et al., 2008; Yoshii et al., 2009).

3.1.2.5 The Readout

The captured movies were analysed with Matlab-implemented software (Ramot et al., 2008; adaptations from Manuel Zimmer, personal communication). Parameters such as speed and omega turn frequency were automatically extracted from a primary data set, consisting of the individual tracks of the worms. In addition, I included a script that extracted the average direction of the worms (in one-second intervals) as well as the average positional information along the two cardinal axes (delta X and delta Y). The reasoning behind the former was to enable detection of very sudden directional changes of the nematodes at the stimuli on- and offset transitions. In contrast, looking at the averaged position along a given axis

throughout the assay could reveal potential long-term responses to the altered magnetic field, (e.g. if the worms were constantly moving one direction during one stimulus interval). Including these two parameters into the readout aimed at detecting as many potential behavioural changes as possible.

3.2 Tracking Results

3.2.1 Primary Screen of *C. elegans*

I started the experiment by analysing the behaviour of *C. elegans*. Figure 3.4 A shows the results for the *C. elegans* N2 strain when assessed on the aforementioned behavioural apparatus. The data consists of two separate experiments (two individual trials each). The centre plot displays the primary tracks (of one trial) and shows that the worms indeed displayed an aversive behaviour against the CuCl_2 . Regarding the extracted parameters the following observations were made:

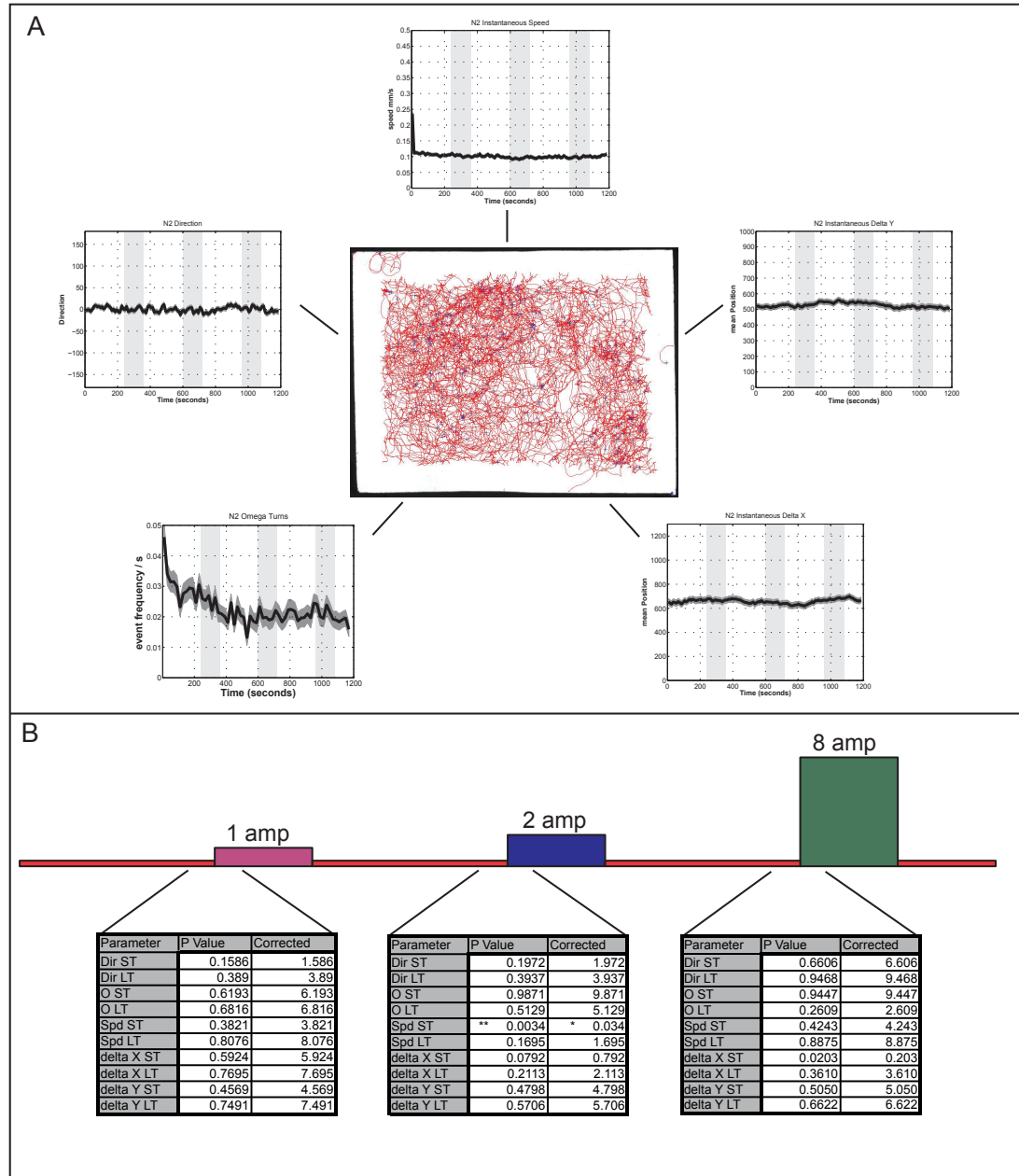


Figure 3.4: Primary screen of *C.elegans*: **(A)** Primary data set consisting of the tracks of individual worms is depicted in the centre. Parameters, such as speed (Spd), direction (Dir), omega turn frequency (O) and positional changes in X/Y (delta X/Y) were extracted using a modified version of the Parallel Worm Tracker (Ramot et al., 2008). The gray bars in the graphs indicate the stimuli intervals as in Figure 3.3. **(B)** Statistical analysis. For each stimulus (1,2 and 8amp), the aforementioned parameters were analysed by two separate t-tests: ST=short term (15 seconds before versus 15 sec after the stimuli-onset), LT=long term (one minute before ST versus one minute after ST) The first column shows the uncorrected p-value for each t-test and the second column the p values after a Bonferroni correction with the correction factor 10 (the amounts of independent t-test employed within one experiment). (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

(1) the instantaneous speed equilibrates at approximately $0.1 \mu\text{m}/\text{sec}$, not showing any obvious changes when applying the stimuli;

- (2) the positional parameters delta-X and delta-Y seem to oscillate around the centre lines (approximately 600 in delta-X and 500 in delta-Y). This derives from calculating the mean position along a given axis and is thus expected;
- (3) the directional parameter was subject to higher variations than the positional ones, as expected. Neither showed significant changes upon stimuli application;
- (4) omega turns, the hallmark behaviour, when compared to the other parameters, contains by far the most variation. Again, no obvious effect was observed during the stimuli periods.

In order to employ a student's t-test to analyse the data generated, I had to circumvent the problem that the worm population is not represented by the same number of tracks over time (because not every worm is tracked in every frame, hence each worm is represented by more than one track). I did so by writing a script that extracted only those tracks that extended throughout a given time interval. The tracks were saved in a separate matrix, which could then be used to perform statistical comparisons such as paired t-tests.

First, I assessed the short-term response (ST) by comparing the 15 seconds immediately preceding and immediately following the stimulus. Second, I analysed the long-term (LT) response by comparing the 60 seconds prior to the ST with the 60 seconds following the ST. The results I obtained are shown in Figure 3.4 B. I treated every magnetic stimulus as a separate experiment resulting in ten independent variables. To correct for multiple testing, it was necessary to perform a Bonferroni correction with a correction factor of 10 (Figure 3.4 B). Although no obvious effects were observed in the primary data set, the t-test comparing the short-term (ST) response to the 2amp stimulus yielded a significant difference in the speed parameter. Examination of the individual trials revealed that this difference was not consistent between trials, suggesting that it is a false positive. The present data suggests that *C. elegans* does not sense magnetic fields.

3.2.1.1 Response to CO₂

In order to validate the capture and analysis system I evoked a well-established behaviour in *C. elegans* by applying a transient CO₂ puff, lasting for two minutes (the intensity steadily decreased over that time). Figure 3.5 shows the behavioural readout.

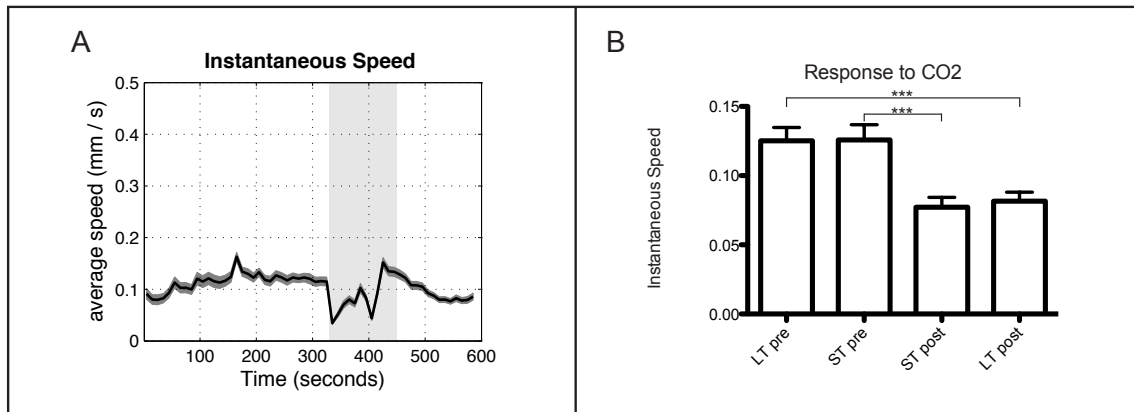


Figure 3.5: Response to CO₂: (A) The Stimulus application consisted of a transient CO₂ puff (grey bar) coming from a fine plastic tubing right above the assay plate. An immediate, approximate decrease of velocity can be observed. (B) Quantification as in the magnetic responses, results in a highly significant decrease (***p*<0.001) in both, the LT (LT pre vs LT post) and ST (ST pre vs ST post) comparisons.

I observed a notable decrease in average speed on the application of CO₂, that was highly significant in both the short- and long-term (LT: *p*<0.0001, ST: *p*<0.0001, uncorrected; Figure 3.5 B). None of the other parameters showed a significant response (data not shown).

3.2.2 Screening other Nematode Strains

3.2.2.1 The Strains

After the establishment of a magnetic behavioural assay in our laboratory, the next step was to screen as many different species as possible in search of a magnetosensitive nematode (The strains of choice are listed in Table 3.1.)

Strain:	Species:	Taxonomy (Order: Family: Genus)	Life history:	Isolated:*
AF13(4)	<i>Oscheius aikoreti</i>	Rhabditida: Rhabditidae: Oscheius	gonochoristic	Andras Fodor, Memorial Park, Madison, Wisconsin, USA.
AF25	unknown	-	hermaphroditic (PP)	
AF272	unknown	-	hermaphroditic (PP)	
AF36	<i>Panagrolaimus rigidus</i>	Rhabditida: Panagrolaimidae: Panagrolaimus	gonochoristic	
AF37	<i>Mesorhabditis sp.</i>	Rhabditida: Rhabditidae: Mesorhabditis	pseudogamy	
AF40	<i>Panagrolaimus rigidus</i>	Rhabditida: Panagrolaimidae: Panagrolaimus	gonochoristic	
AF4733	unknown	-	hermaphroditic/males rare	Isolated in Pennsylvania.
AF5840	unknown	-	hermaphroditic/males rare	Isolated in Pennsylvania.
AF72	<i>Mesorhabditis miotki</i>	Rhabditida: Rhabditidae: Mesorhabditis	hermaphroditic (PP)	Isolated in Pennsylvania.
AF7340	unknown	-	hermaphroditic (PP)	Isolated in Ohio
AF8032	unknown	-	hermaphroditic/males rare	Isolated at Niagara Falls in Canada
AF8130	<i>Pristionchus sp.</i>	Diplogasterida: Neodiplogasteridae: Pristionchus	hermaphroditic	Isolated in Ontario, Canada.
BW287	<i>Panagrolaimus sp.</i>	Rhabditida: Panagrolaimidae: Panagrolaimus	hermaphroditic	
CEW1	<i>Oscheius tipulae</i>	Rhabditida: Rhabditidae: Oscheius	hermaphroditic	Isolated in 1991 by Carlos E. Winter in soil samples taken at the University of Sao Paulo in Brazil.
DF5006	<i>Rhabditella axei</i>	Rhabditida: Rhabditidae: Rhabditella	gonochoristic	Isolated by W. Sudhaus on July 29, 1979 from a compost heap in Esmoulières, France
DF5012	<i>Rhabditoides regina</i>	Rhabditida: Rhabditidae: Rhabditoides	gonochoristic	Isolated by M. Velasquez in the spring of 1989 from a June beetle larva (Scarabaeidae) near Quetzaltenango, Guatemala
DF5013	<i>Pelodera strongyloides</i>	Rhabditida: Rhabditidae: Pelodera	gonochoristic	Originally came from Sudhaus.
DF5018	<i>Oscheius dolichuroides</i>	Rhabditida: Rhabditidae: Oscheius	gonochoristic	Isolated by W. Sudhaus in April 1978 from decaying matter in a hole in a tree in Malindi, Kenya.
DF5025	<i>Pellioditis typica</i>	Rhabditida: Rhabditidae: Pellioditis	gonochoristic	Isolated by W. Sudhaus on Sept. 3, 1973 from feces of an antelope in Tsavo National Park, Kenya.
DWF1108	<i>Acrobeloides sp.</i>	Rhabditida: Cephalobidae: Acrobeloides	hermaphroditic (PP)	Originally from ecological study at University of GA.
DWF1301	<i>Cephalobus sp.</i>	Rhabditida: Cephalobidae: Cephalobus	gonochoristic	Isolated in Fort Collins, CO.
DWF1501	<i>Dolichorhabditis sp.</i>	-	gonochoristic	Isolated in Brazil.
DWF1604	<i>Oxyerculabaudis sp.</i>	-	gonochoristic	Originally from UCR collection.
DWF1701	<i>Zeldia sp.</i>	Rhabditida: Cephalobidae: Zeldia	hermaphroditic (PP)	Isolated in Fort Collins, CO.
N2	<i>Caenorhabditis elegans</i>	Rhabditida: Rhabditidae: Caenorhabditis	hermaphroditic	Bristol, UK
HMephisto	<i>Halicephalobus mephisto sp. Nov.</i>	Rhabditida: Panagrolaimidae: Halicephalobus	hermaphroditic (PP)	Beatrix gold mine, South Africa
MT8872	<i>Panagrellus redivivus</i>	Rhabditida: Panagrolaimidae: Panagrellus	gonochoristic	MT, received from Rothamsted Experimental Station, Harpenden, Hertfordshire, England.
PG-1	unknown	-	hermaphroditic (PP)	Collected by M.GALLO.
PG-2	unknown	-	hermaphroditic (PP)	Collected by M.GALLO.
PG-3	unknown	-	hermaphroditic (PP)	Collected by M.GALLO.
PPC14	unknown	-	hermaphroditic (PP)	"Markiertes Windloch" Cave, Grosser Otter, Austria
PPC74	unknown	-	hermaphroditic (PP)	"Markiertes Windloch" Cave, Grosser Otter, Austria
PS1156	<i>Acrobelus sp.</i>	Rhabditida: Cephalobidae: Acrobelus	gonochoristic	Tamarisk, Palm Desert, 11/82.
PS1163	<i>Panagrellus redivivus</i>	Rhabditida: Panagrolaimidae: Panagrellus	gonochoristic	
SB122	<i>Choriorhabditis dudichi</i>	Rhabditida: Rhabditidae: Choriorhabditis	hermaphroditic (PP)	Found 6/29/95 in rotten wood on the ground between bushes in the Bronx Zoo, New York.
SB146	<i>Choriorhabditis remanei</i>	Rhabditida: Rhabditidae: Caenorhabditis	gonochoristic	Isolated in Freiburg, Germany from compost, associated with pill bugs.
SB193	<i>Rhabditis brassicae</i>	Rhabditida: Rhabditidae: Rhabditis	hermaphroditic (PP)	Found 3.10.87 in turf near a bank, Traben-Trarbach (Rheinland-Pfalz/Germany)
SB408	<i>Poikilolaimus sp.</i>	Rhabditida: Rhabditidae: Poikilolaimus	hermaphroditic (PP)	Swimming, chemoautotrophic mats Movile-Cave Romania

Table 3.1: Summary of nematode strains selected for tracking, showing their strain designation, name, taxonomic rank, life history, and place of isolation (if known). All strains were obtained from the Caenorhabditis Genetics Center (CGC) (University of Minnesota) (except for: *H. Mephisto* from Esta van Heerden (University of the Free State), SB408 from the New York University Rhabditid Collection (NYURC) and PPC14 and PPC74, isolated by myself). Overall, 38 different strains were tracked. 13 were wild isolates (not yet been classified) and the remaining 25 consisted of 18 different genera and 16 different species. * information obtained from <http://www.cbs.umn.edu/CGC/strains/gophstrnt.txt>.

In pursuit of this objective I screened 37 different strains, 13 of which were wild isolates that have not yet been classified. The remaining 25 strains all belonged to the order *Rhabditida* (except for *Pristionchus sp.*, which is a member of the closely related order *Diplogasteridae* (De Ley, 2006b), which constitutes a very distinct order; Blaxter, 2011). These 25 strains encompassed 18 different genera and 16 different species. I wish to place emphasis on the choice of two strains, namely *Halicephalobus Mephisto* (Borgonie et al., 2011) and *Poikilolaimus sp. H. mephisto* was isolated in the terrestrial deep surface of South Africa in a depth of 1.3 km and *Poikilolaimus sp.* was isolated from the Movile cave in Romania, which was found to contain a chemoautotrophically based cave ecosystem (Sarbu et al., 1996). Both of these habitats may have favoured the evolution of a magnetic sense.

In addition to the screening of cultured strains, I isolated and cultured wild cave dwelling nematodes. I obtained soil samples from 5 different caves in the Eastern Alps of Austria, and employed a Baermann funnel to extract nematodes that were subsequently cultured on NGM plates. This approach yielded two novel isolates (PPC14 and PPC74), both found in the "markiertes Windloch". (See Appendix B)

3.2.2.2 Screen Results

All strains that I screened were treated the same way as *C. elegans* (See 3.1.2). They were starved, tracked, the data extracted, followed by a statistical analysis. The results for all 38 strains are summarized in table 3.2. After reviewing the qualitative and statistical results I decided to repeat the strains DF5006, PG3 and PPC14. DF5006 showed a significant difference in its short-term omega turn frequency response to the 1amp stimulus and in its long-term omega response to the 8amp stimulus. In the repeated experiment (repeated experiments are marked with a † in table 3.2), however neither result could be reproduced.

In the PG3 dataset, the fact that it displayed a significant positional change along the X-axis in both, the short- and the long-term response to the 2amp stimulus appeared very interesting. When repeating the experiment, significant differences were again observed, but in different parameters, delta-X ST and LT to the 1amp stimulus and omega-turn LT to the 8amp stimulus. Although interesting, the results from the first experiment could not be replicated. Finally, PPC14 changed its LT omega turn frequency highly significantly ($p < 0.001$), but also this result could not be replicated.

Table 3.2 (previous page): Summary of screening results: The Table shows the results of the 30 t-tests carried out per nematode strain (five parameters: speed (Spd), direction (Dir), omega turns (O), positional change in X/Y (SPX/Y); three stimuli: 1 (1amp, 2 (2amp) 8 (8amp) and two comparisons: ST=short term (15 seconds before versus 15 sec after the stimuli-onset), LT=long term (one minute before ST versus one minute after ST) . For all strains at least 100 worms were tested. A Bonferroni correction for multiple comparisons was performed with the factor 10. (* $p<0.05$, ** $p<0.01$, *** $p<0.001$, ns= not significant) Strains marked with † are independent replicates.

It is concluded that none of the investigated strains showed a detectable behaviour upon the application of a magnetic stimulus. The raw data for all tracked strains is shown in appendix A.

4. Discussion

I have established a behavioural assay to screen for magnetoreceptive nematodes. The stimulus application apparatus was custom-built, following the quality standards recommended for biomagnetic experiments (Kirschvink, 1992). It allows independent control of the intensity and duration of three magnetic stimuli, which were designed to maximise the number of altered parameters in the magnetic environment. The apparatus, furthermore, permits recording of freely moving animals and automated extraction of behavioural parameters. I adapted the existing Matlab scripts to additionally extract positional and directional parameters and to allow further statistical analysis. The proper function of the capture and analysis system was validated by observing a statistically significant slowing response after transient application of a CO₂ puff to the animals.

Following the establishment of the aforementioned apparatus I screened 37 different nematode strains and analyzed their response to magnetic field changes. Based on the hypothesis that troglobite species might have an evolutionary advantage from sensing the geomagnetic field I collected samples from caves in eastern Austria and extracted wild nematode strains, two of which I successfully cultured in the lab.

I obtained significant responses to the magnetic stimulus in the primary screen. These results were then either assessed qualitatively and in some cases the experiment was repeated. However, in none of the reanalysed strains the results could be replicated and it must therefore be concluded that none of the strains could sense the magnetic field changes under the experimental conditions employed in the assay. Two possible explanations might account these negative results:

- The result is a false negative. One or more of the nematode strains is magnetoreceptive but the assay failed to either elicit or detect a response.
- The result is a true negative. None of the screened nematode strains are magnetoreceptive.

The first possibility cannot be excluded although it seems unlikely. The capture and analysis system was controlled and validated, via a CO₂ stimulus that led to a reduction in velocity. Furthermore, almost identical capturing and analysis systems have been successfully used by other groups to detect the same behavioural responses (speed and omega turns) elicited by other stimulus modalities (Zimmer et al., 2009). It should also be kept in mind, that while this study employed a range of magnetic stimuli, it is nonetheless possible that alternative intensities might elicit a response. A further limitation of this study was the absence of a positive control. Since the aim was to find the first magnetosensitive nematode, a true positive control for the experiment was naturally not available and thus, a false negative cannot be excluded. Yet, the data obtained in this study rather indicates the lack of a magnetic sense in the analysed nematode strains. So the question remains: how might one find a magnetosensitive nematode?

Following the current approach, many more nematodes can be obtained from databases and research groups around the world (such as the CGC and NYURC, that were mentioned before) and new species could be collected and isolated from various natural habitats, such as caves. An alternative approach to find a naturally occurring, magnetosensitive nematode is to magnetically enrich the samples taken from sediments. This could be achieved by a bar magnet placed into the sediments that potentially attracts magnetoreceptive species which could then be collected together with the water/mud surrounding the magnet. Although this might increase the chance of finding a magnetotactic nematode, this would still rely on high-throughput screening of as many different species as possible.

The false positives obtained in the screen raised the question whether the paired t-tests, employed for statistical analysis are an appropriate measure to validate results. An alternative approach that would better cope with the high variation encountered in the data may be to analyse random intervals within the data set (not coinciding with the stimuli onset) and use the thereby generated random values as a reference for noise producing false positive readouts.

In addition to the above-mentioned tracking approach, a conditioning paradigm could be employed to screen the nematodes. It has proven successful in the honeybee,

Apis mellifera (Walker and Bitterman, 1985) and the fruit fly, *Drosophila melanogaster* (Gegear et al., 2008). The strength of this approach is that it couples a sensation to a reward and the scoring is thus not dependent on the detection of potentially very subtle, in this case unknown, behaviour.

I have established a screen for magnetosensation in nematodes. To date this screen has been applied to just a small percentage of the nematode species that reside on the planet earth. Should a magnetosensitive nematode exist, the assay described in this thesis will allow its identification.

5. References

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6. Appendix

Appendix A consists of the primary readouts for the screened nematode strains.

For each strain, the strain designation (e.g.: N2), species name (e.g.: *C. elegans*) and the number of trials composing the data (n=4) are depicted next to the speed, omega-turn, direction and positional graphs. Gray bars indicate the stimuli applications as in Figure 3.4.

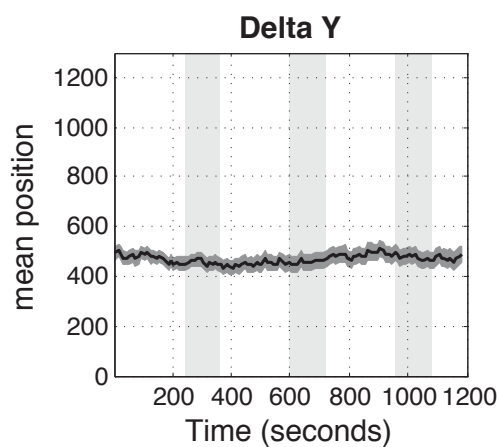
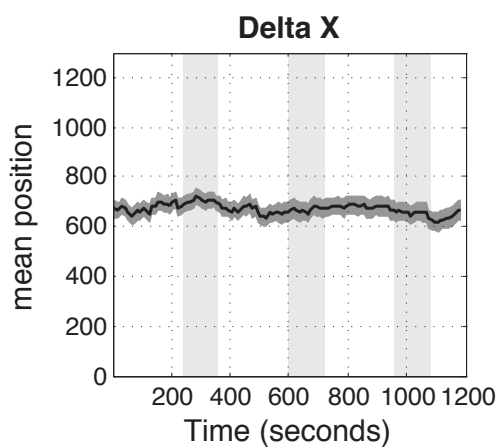
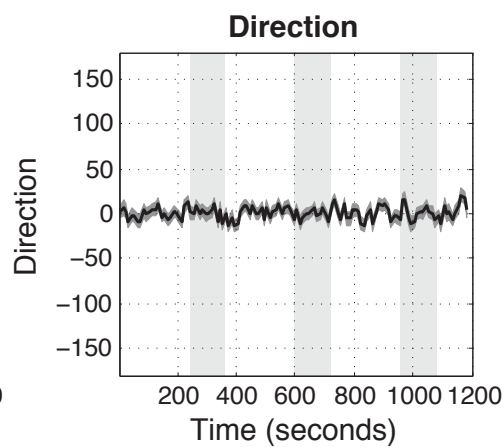
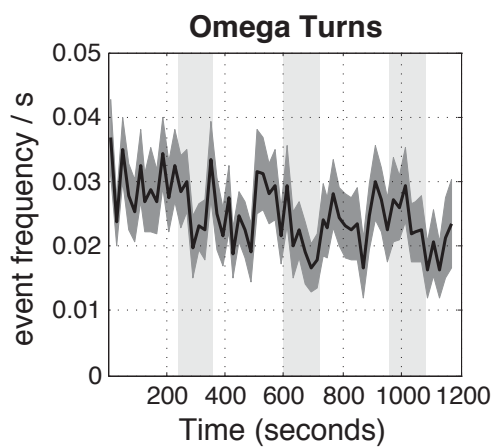
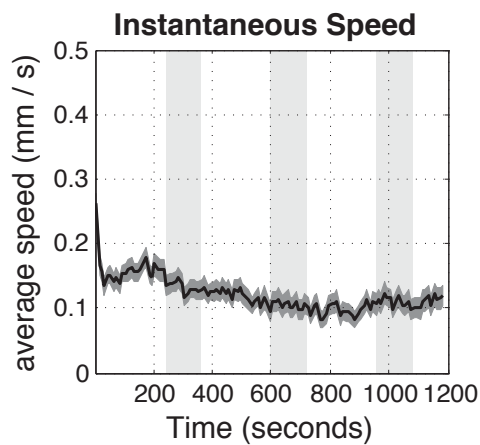
Appendix B contains the information about the caves from which samples were taken, including Sample#, collection date, place of collection, and isolation method.

Appendix A:

AF13(4)

Oscheius akosreti

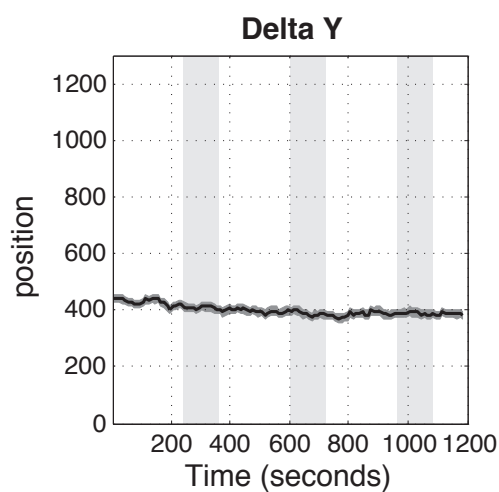
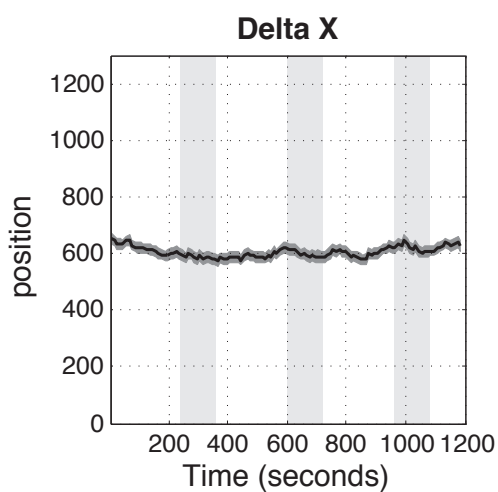
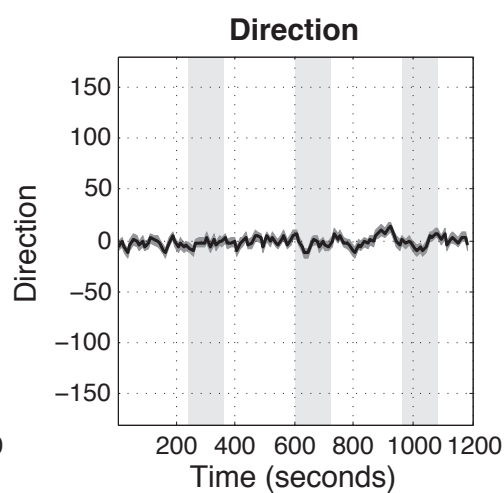
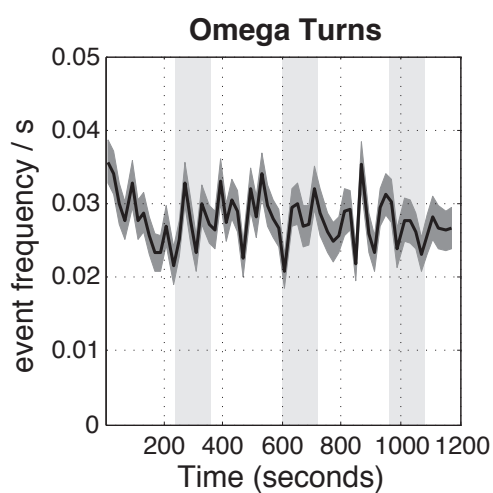
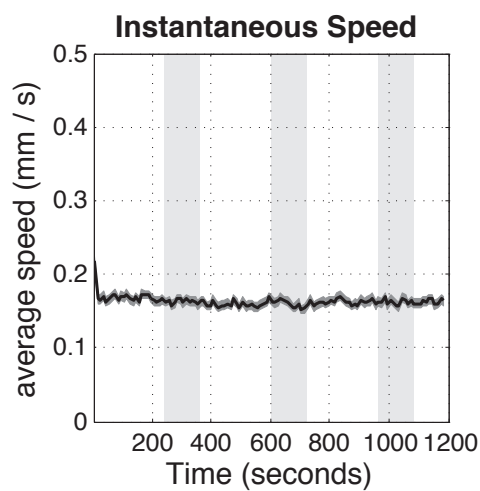
n=2



AF25

species unknown

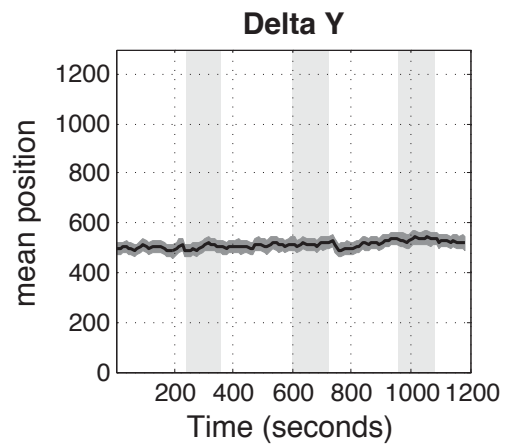
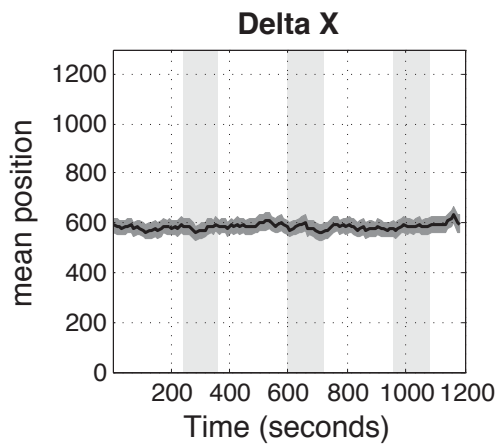
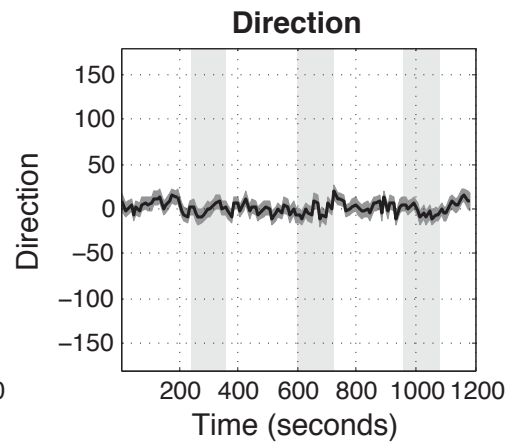
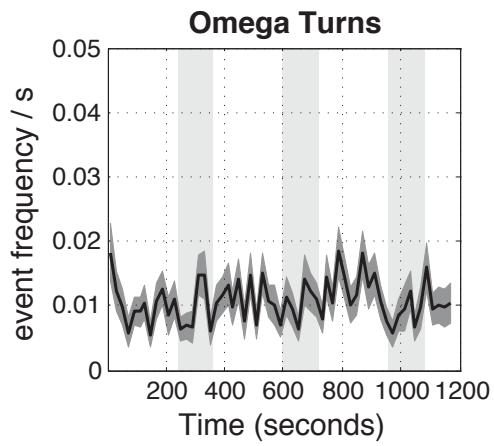
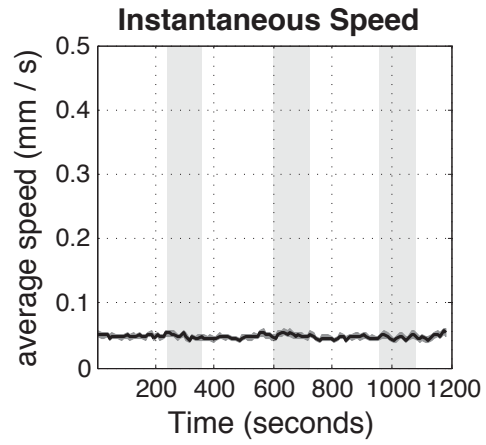
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AF36

Panagrolaimus rigidus

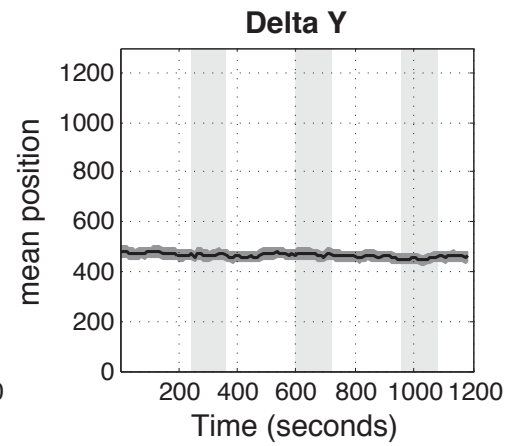
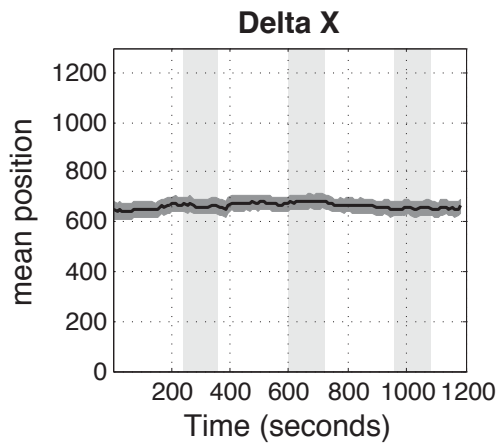
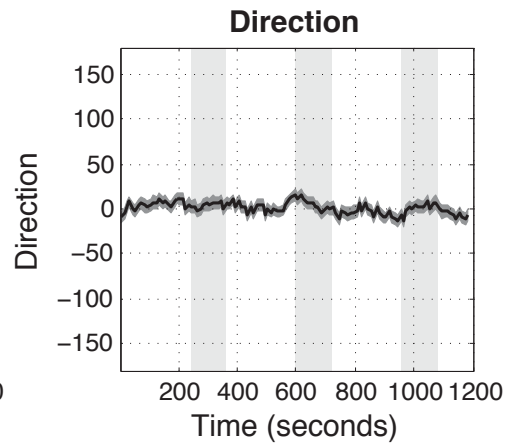
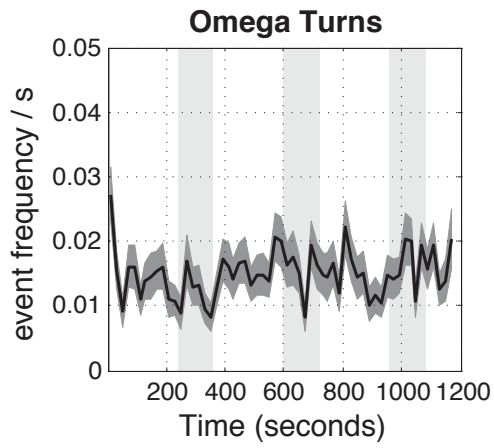
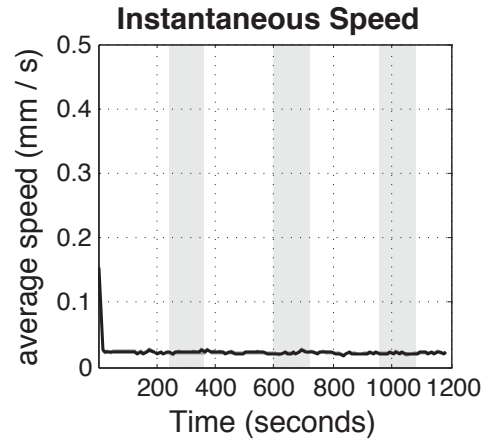
n=2



AF37

Mesorhabditis sp.

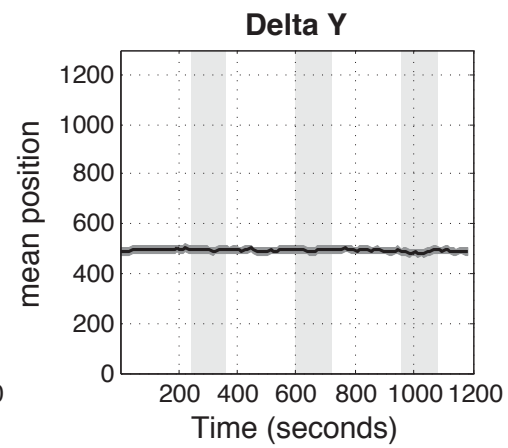
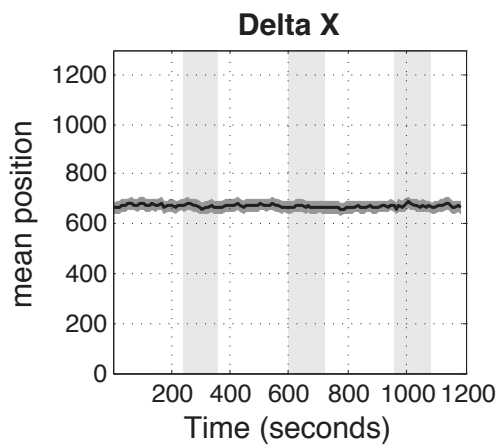
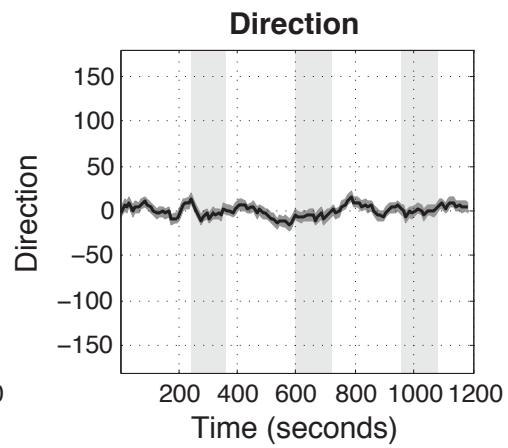
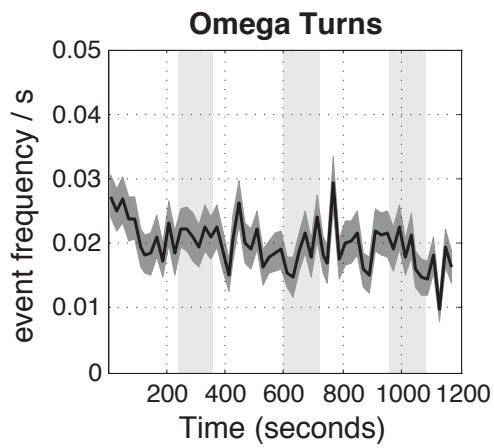
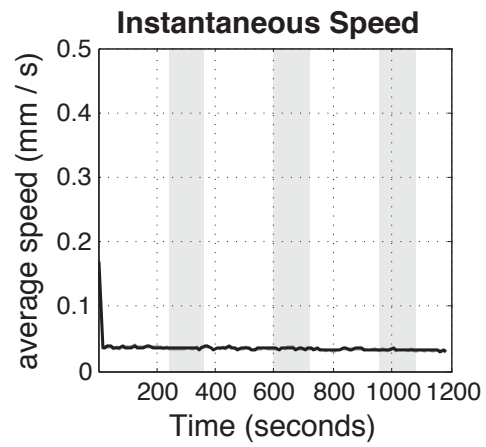
n=2



AF40

Panagrolaimus rigidus

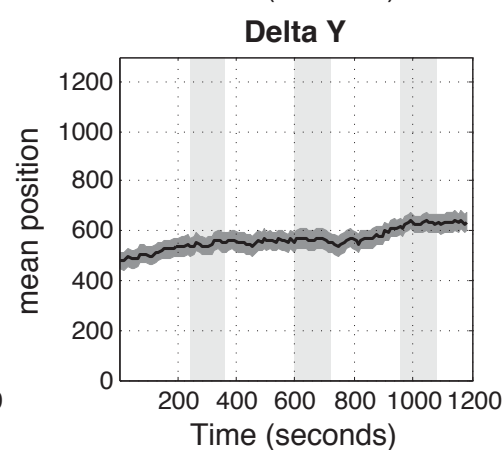
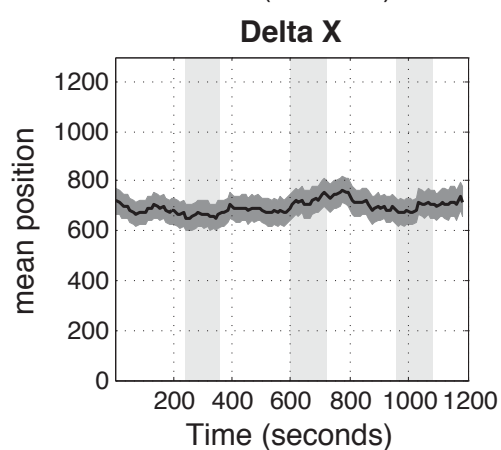
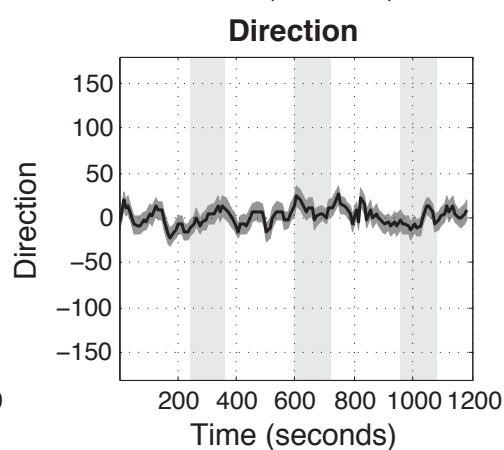
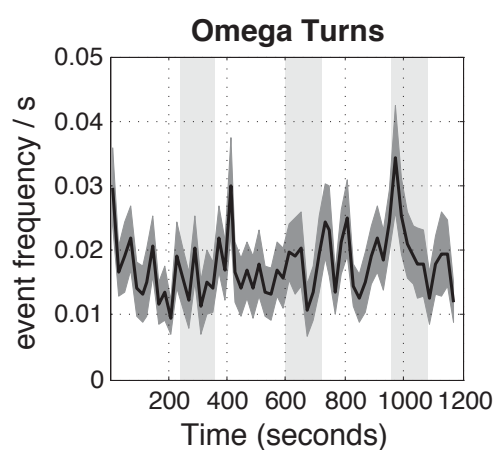
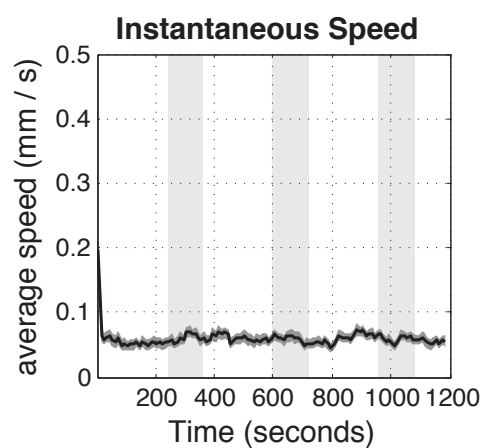
n=2



AF72

Mesorhabditis miotki

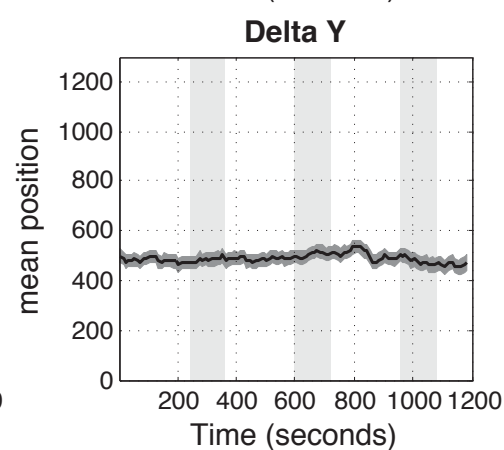
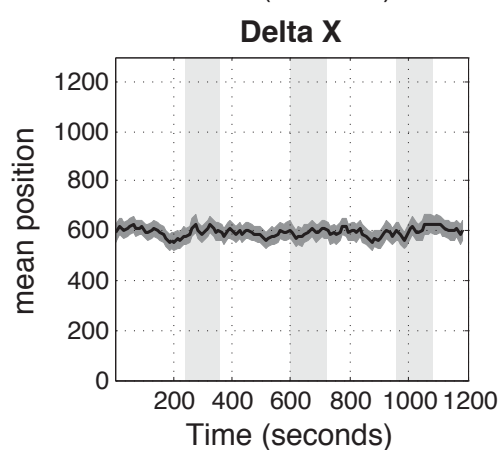
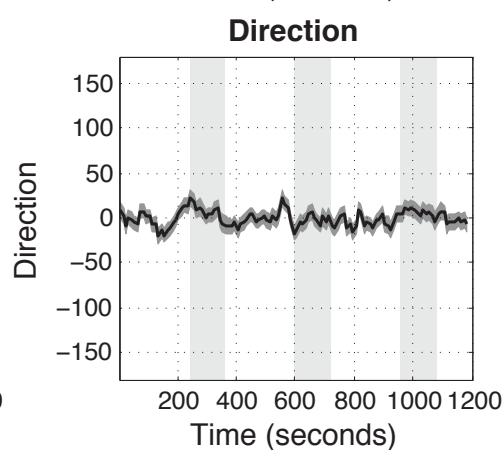
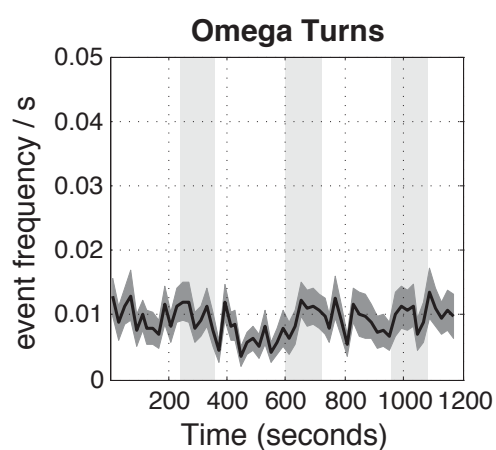
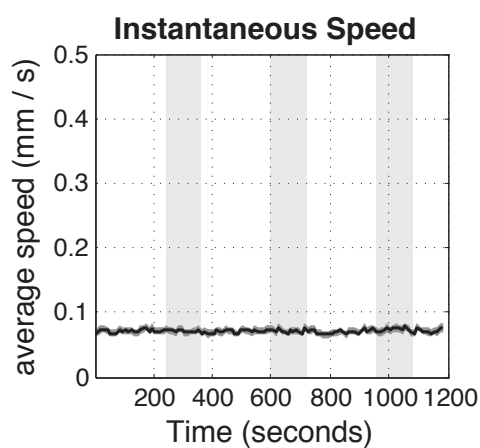
n=2



AF272

species unknown

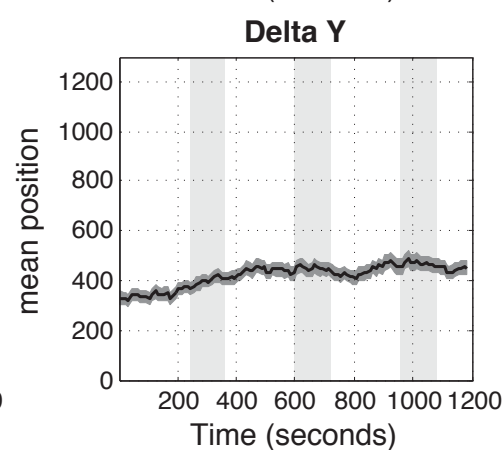
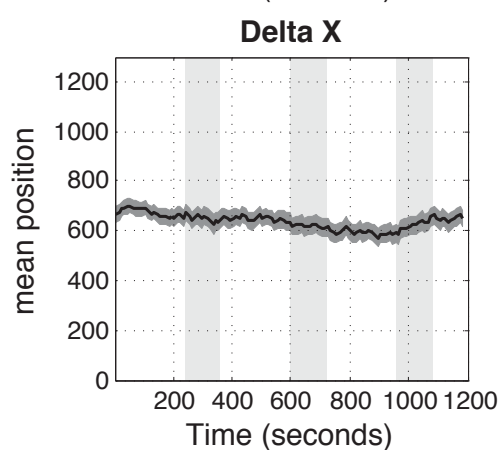
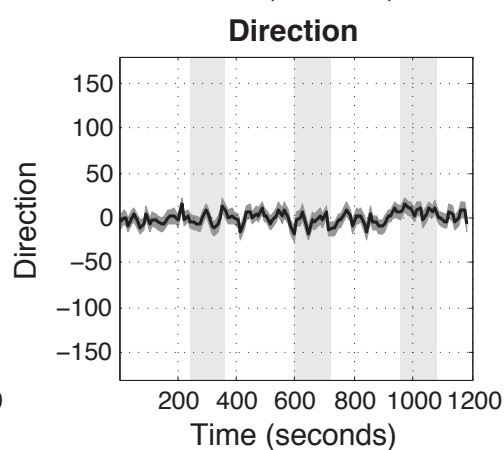
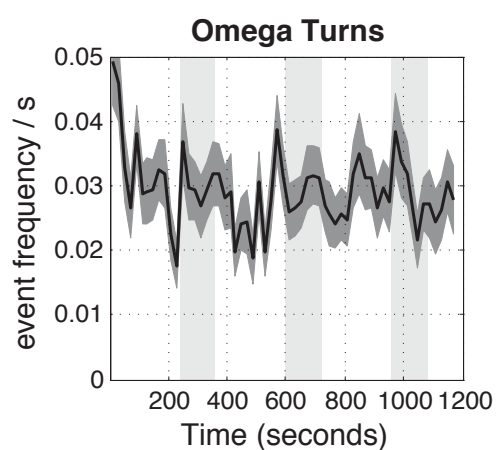
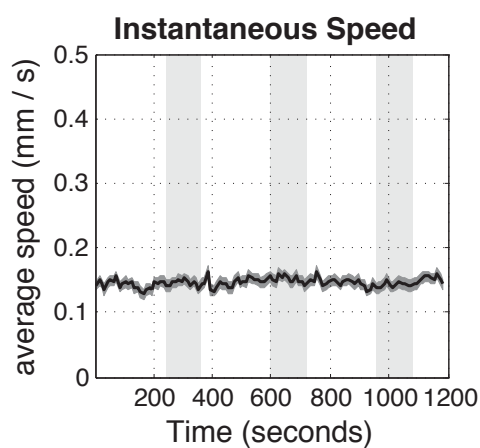
n=2



AF6733

species unknown

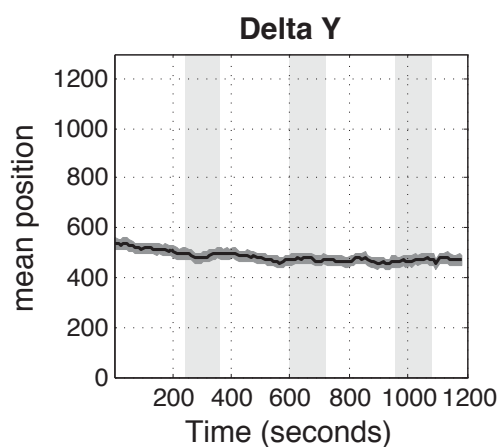
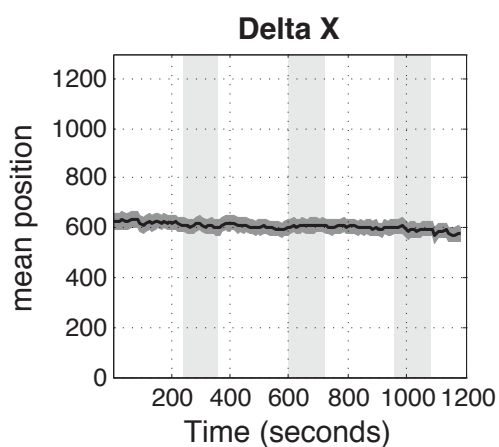
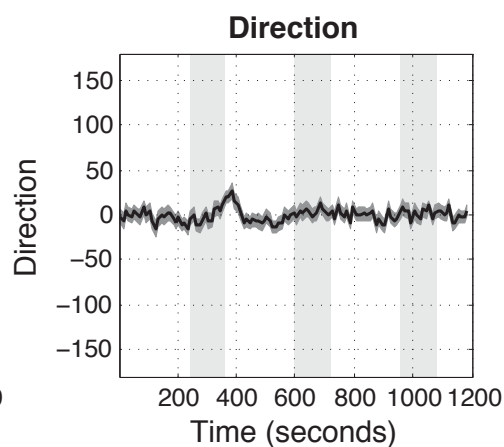
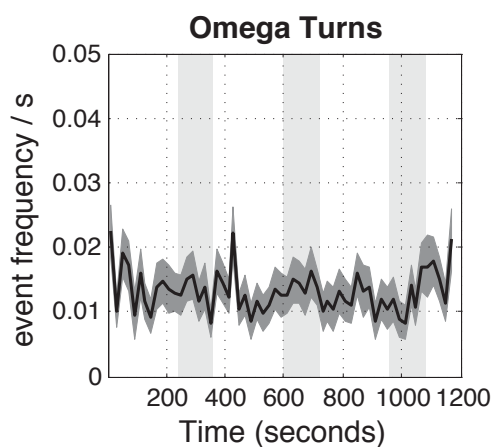
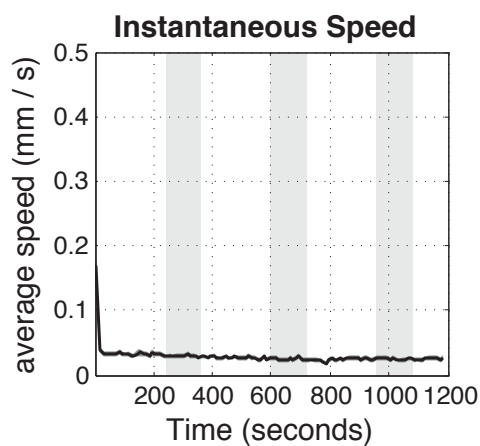
n=2



AF6840

species unknown

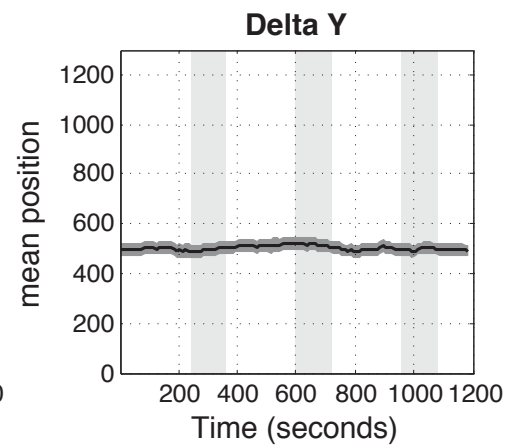
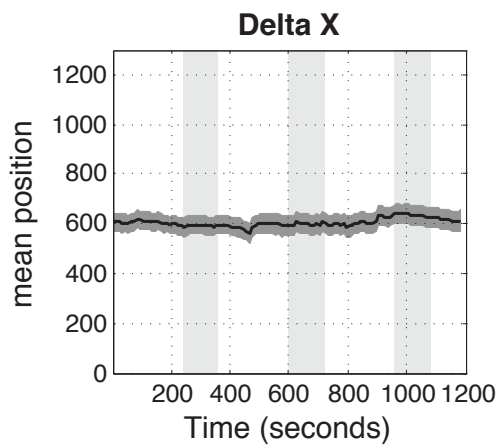
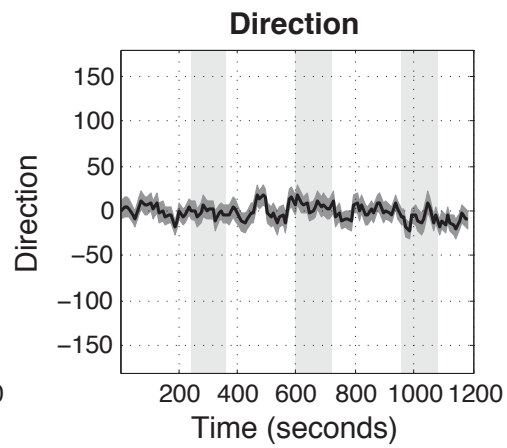
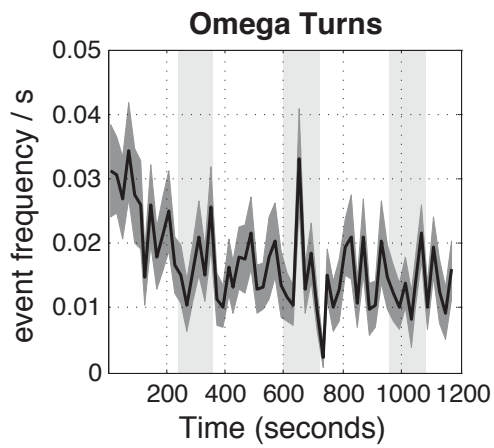
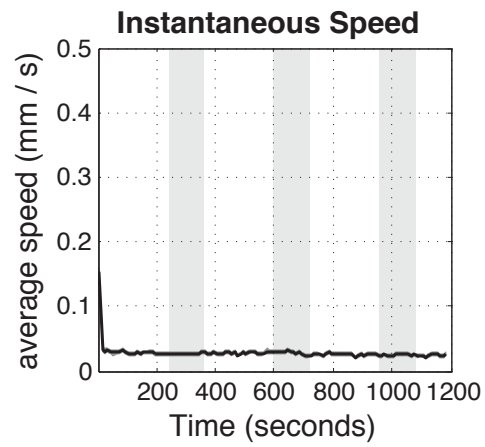
n=2



AF7340

species unknown

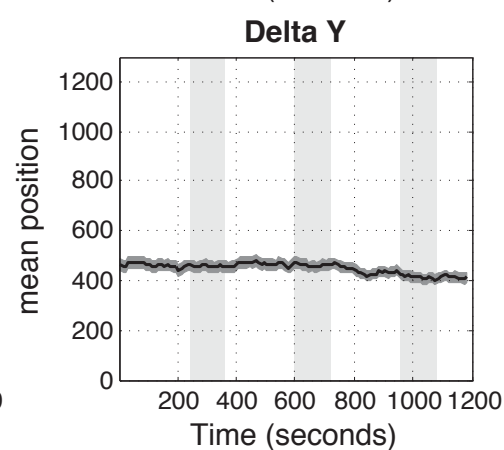
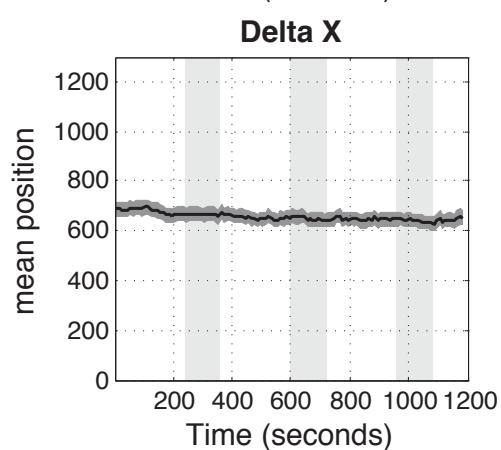
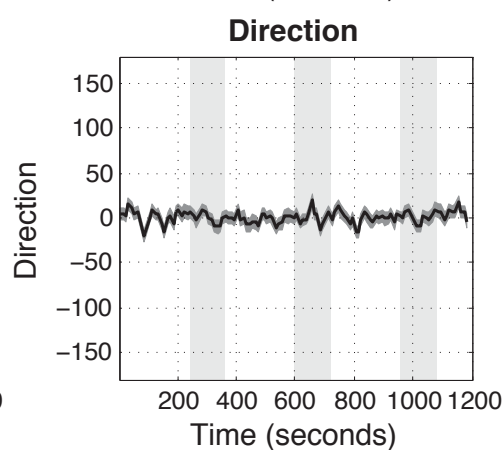
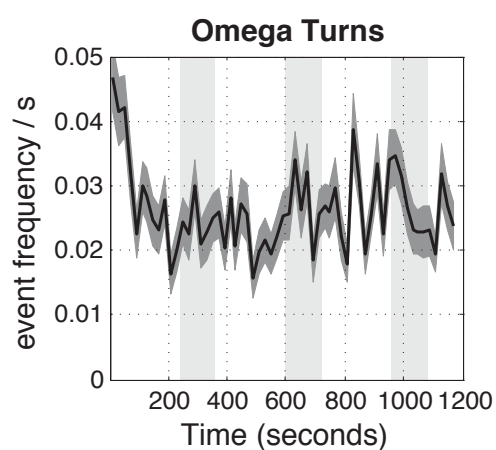
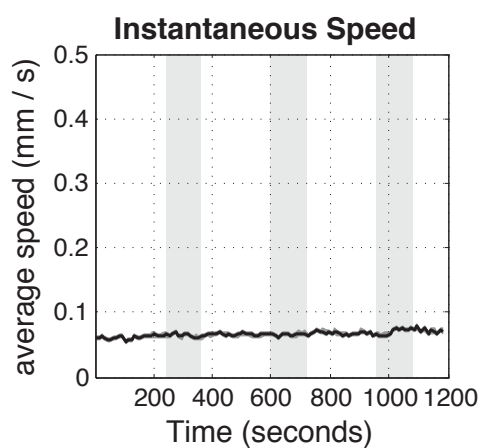
n=2



AF8032

species unknown

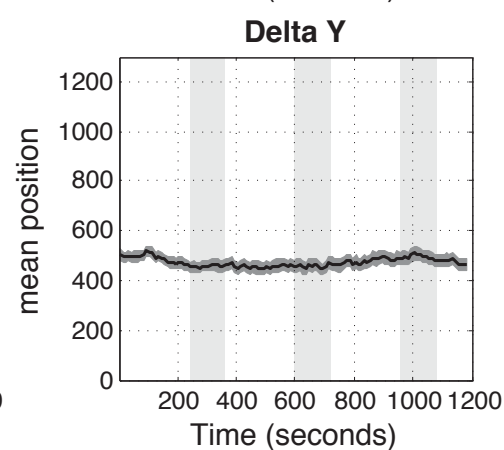
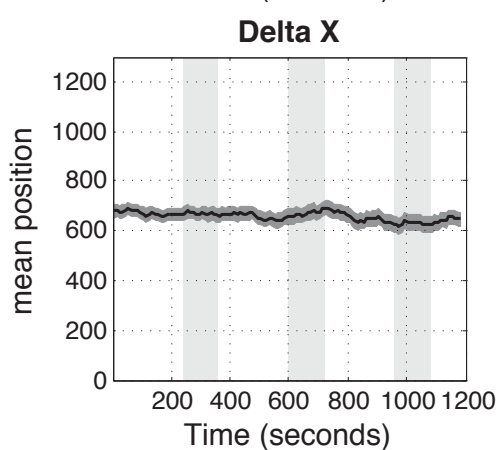
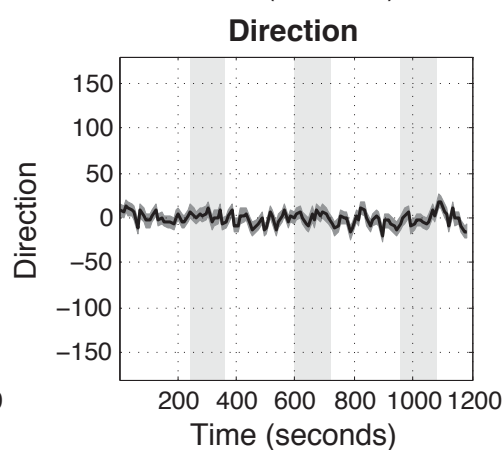
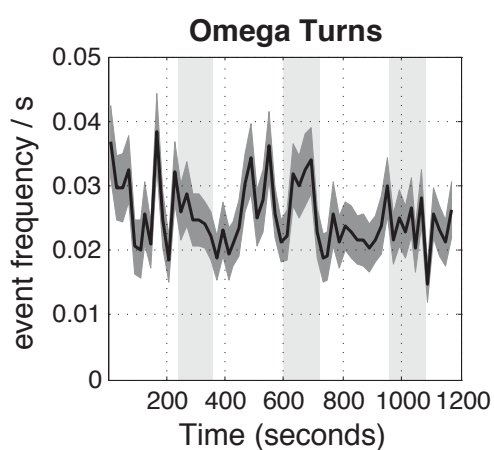
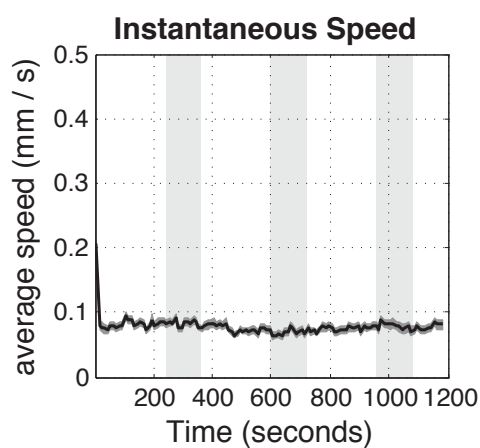
n=2



AF8130

Pristionchus sp.

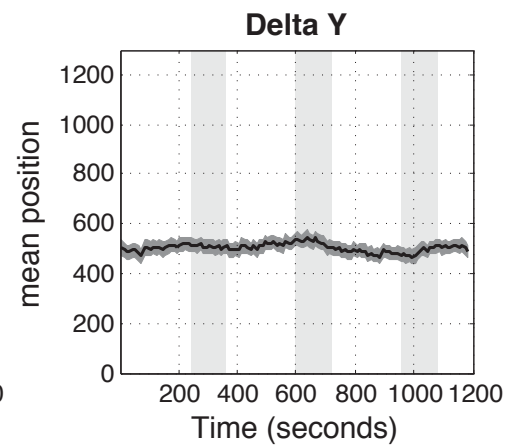
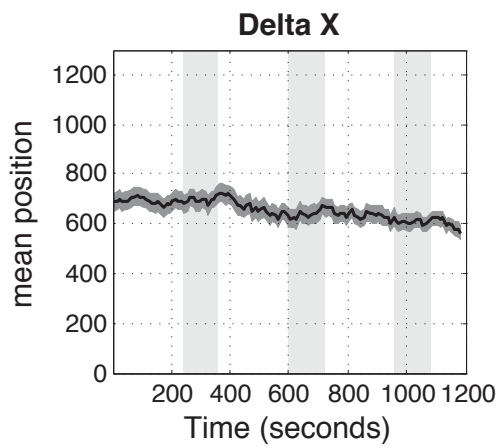
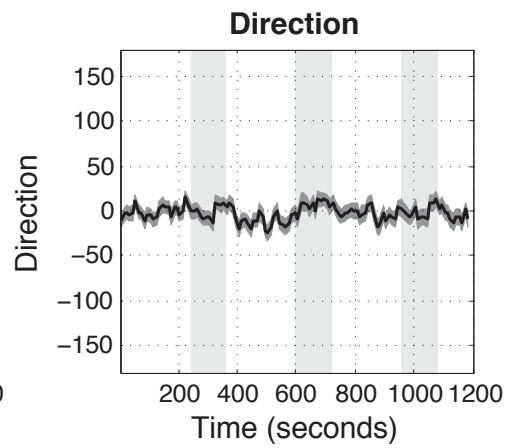
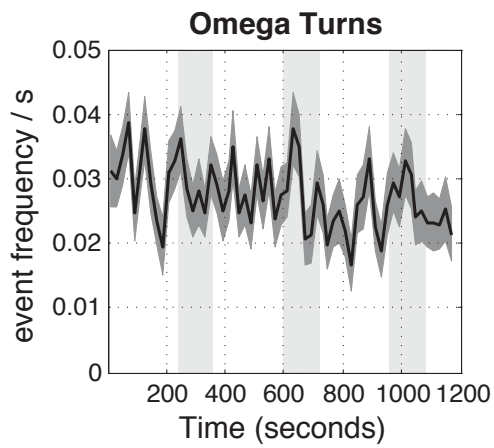
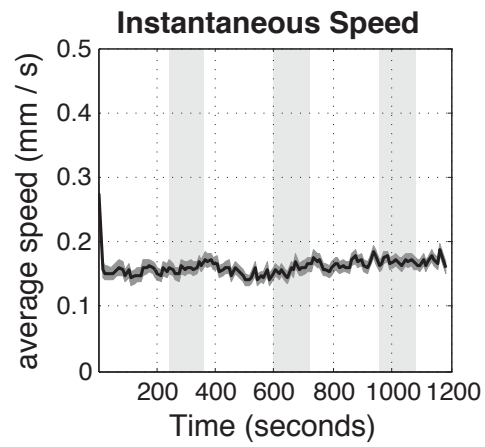
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BW287

Panagrolaimus sp.

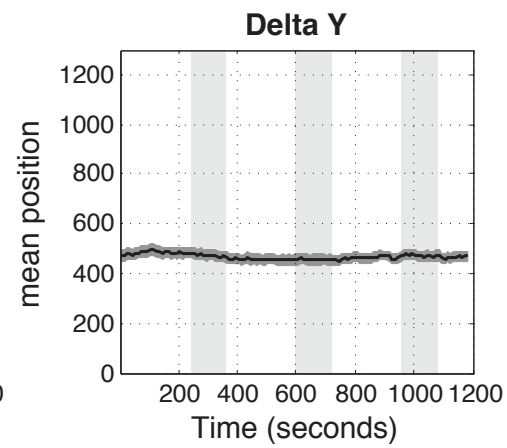
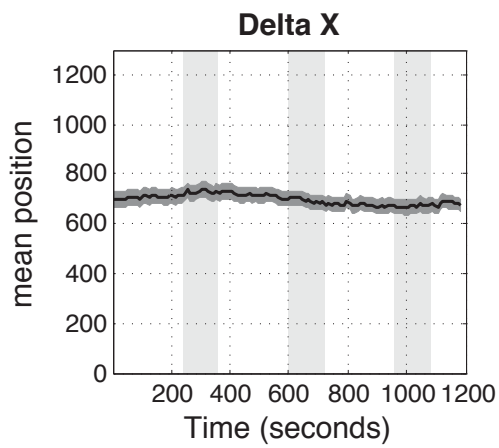
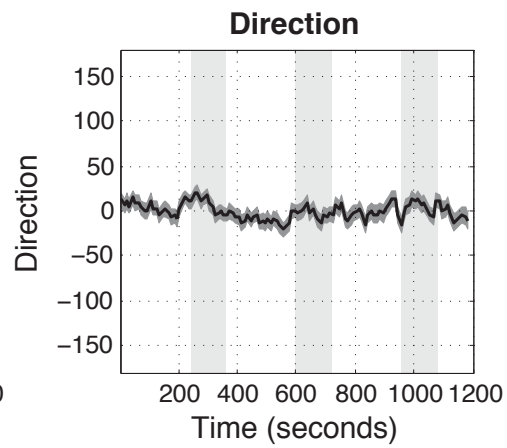
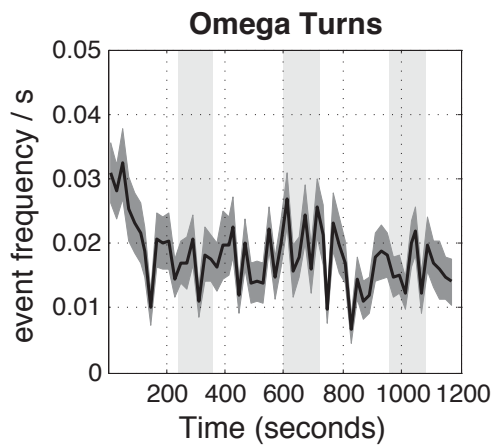
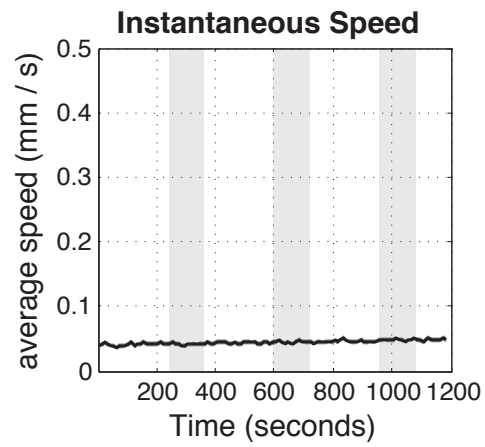
n=2



CEW1

Oscheius tipulae.

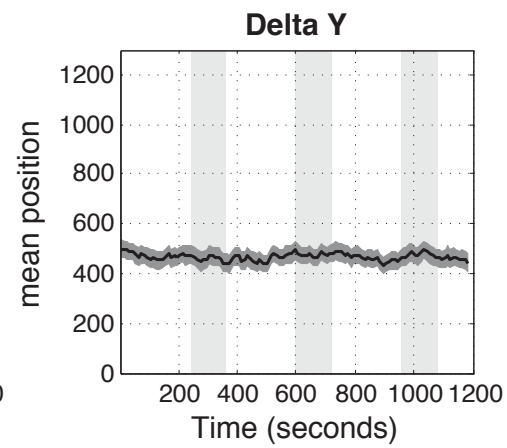
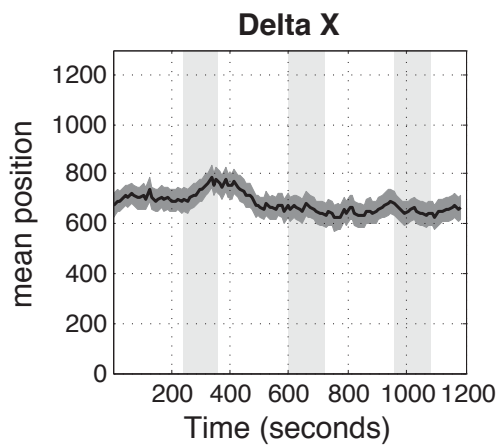
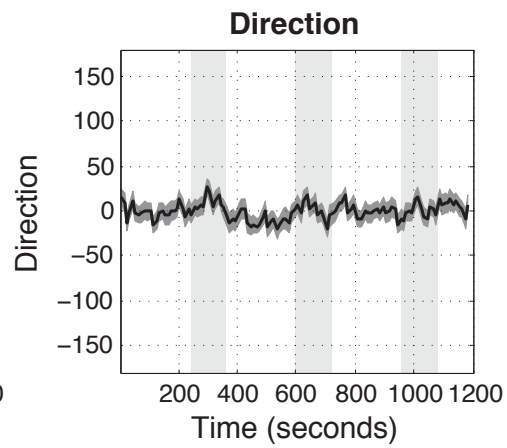
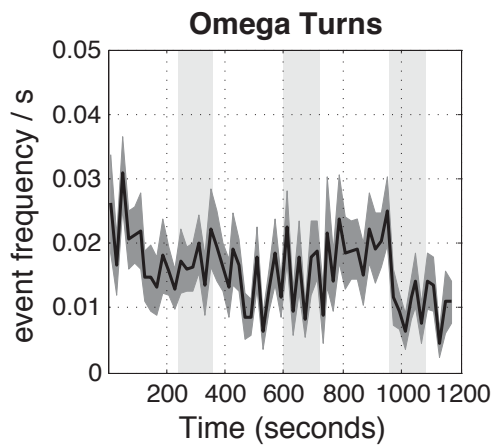
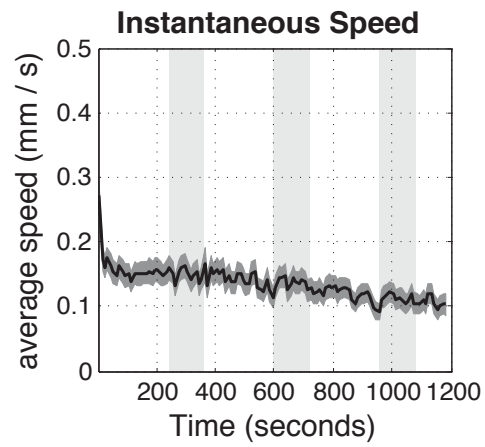
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DF5006_1

Rhabditella axei

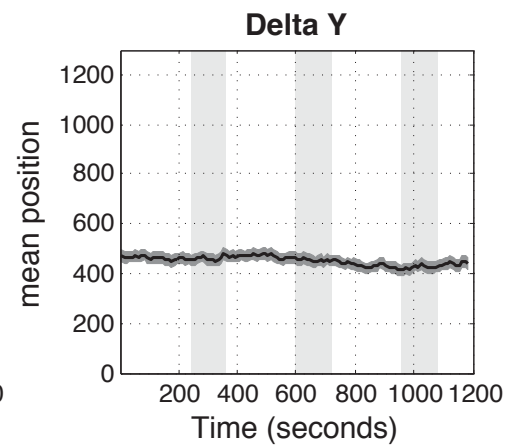
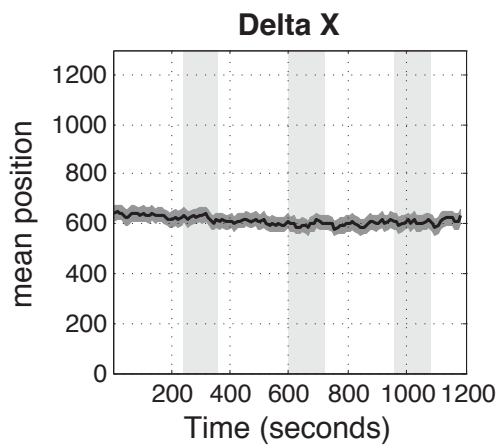
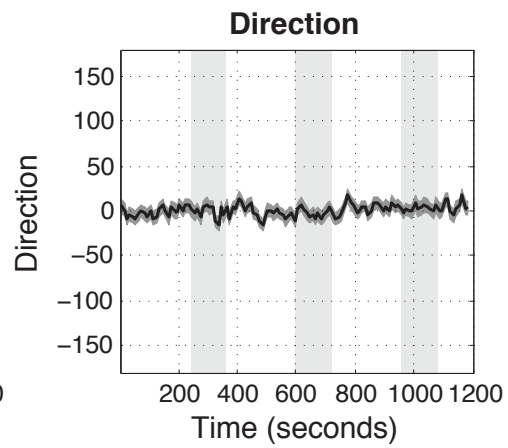
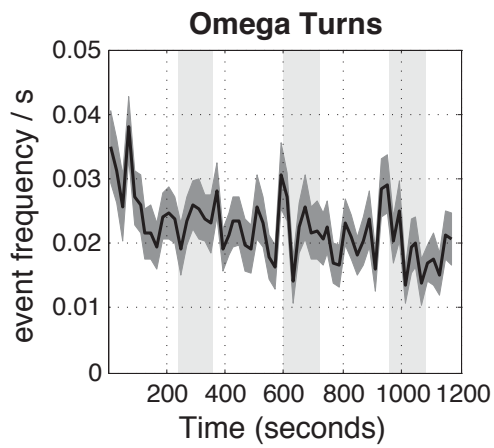
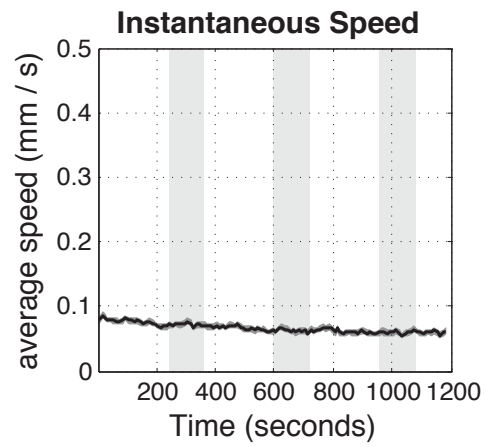
n=2



DF5006_2

Rhabditella axei

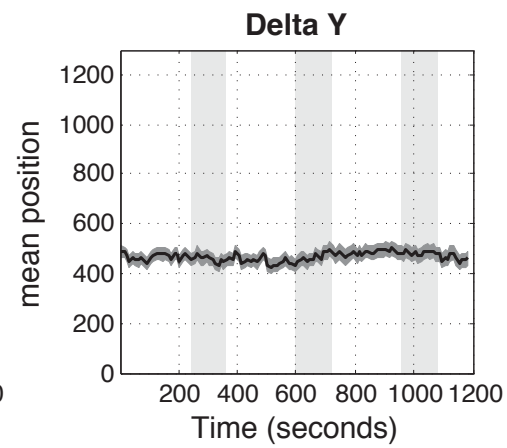
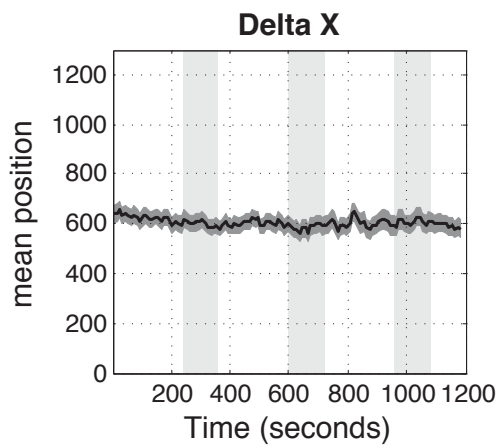
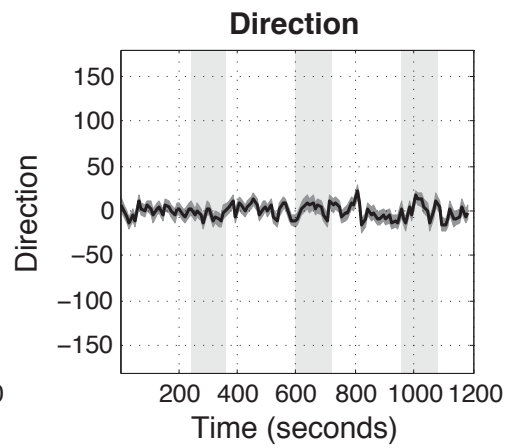
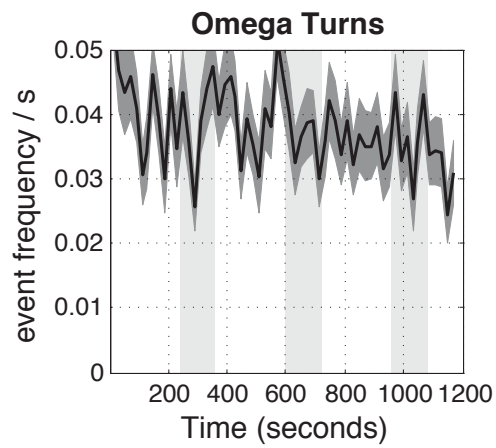
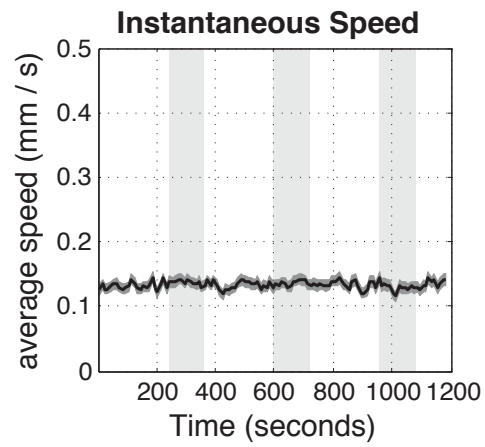
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DF5012

Rhabditoides regina

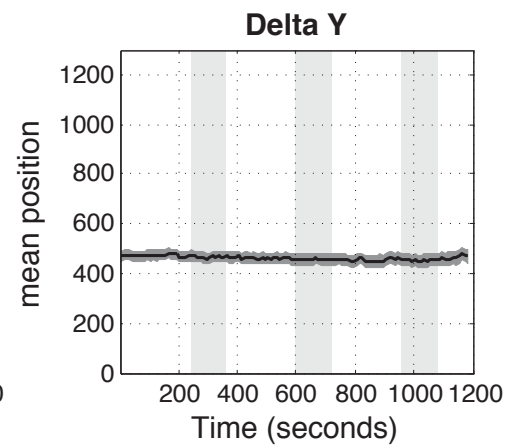
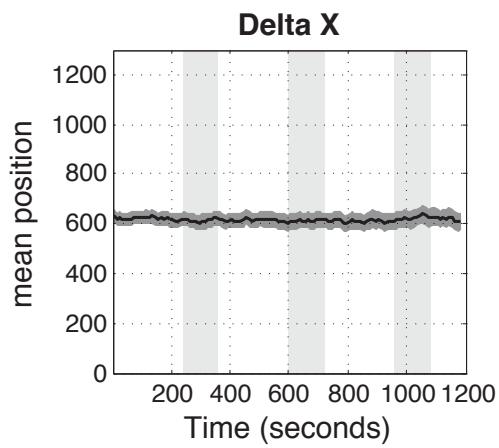
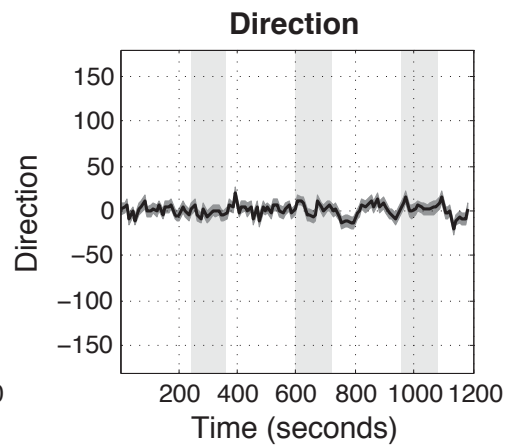
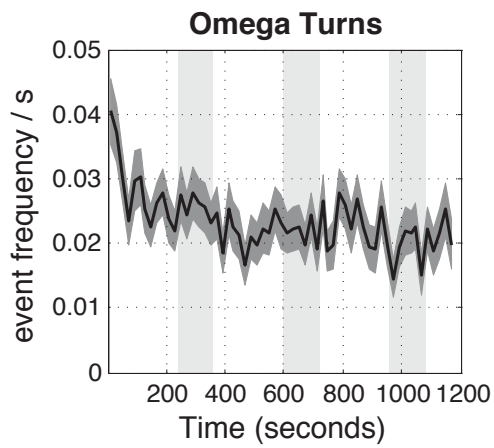
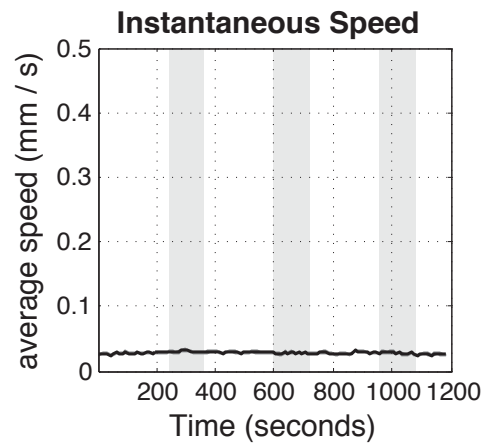
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DF5013

Pelodera strongyloides

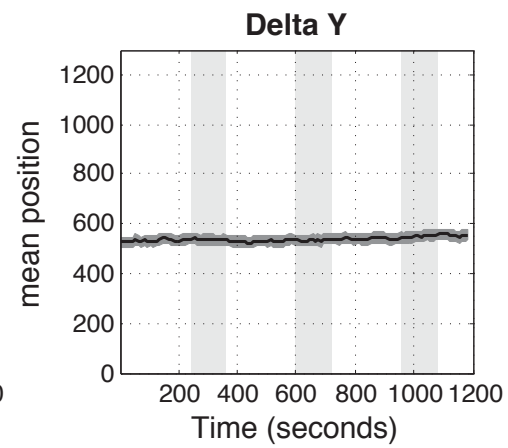
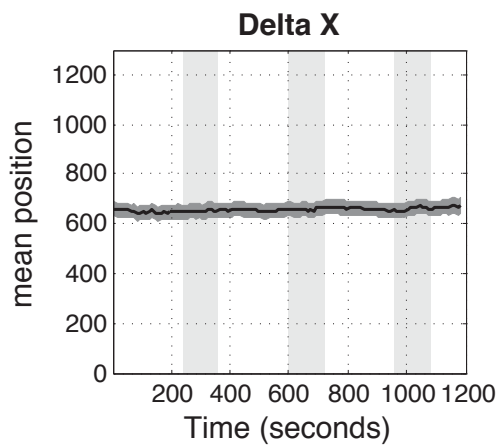
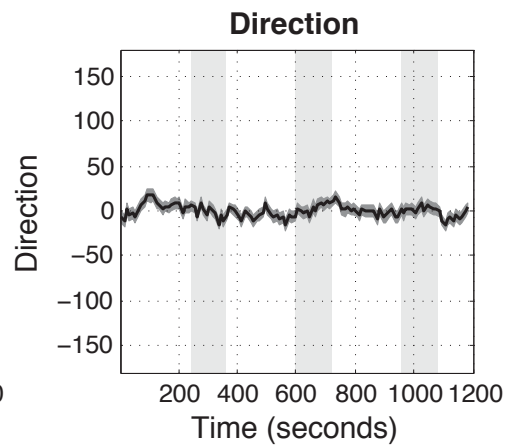
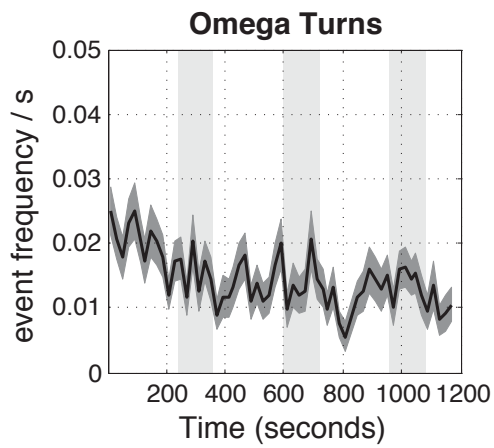
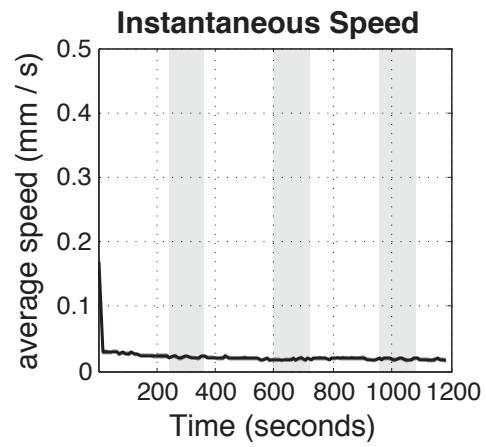
n=2



DF5018

Oscheius dolichuroides

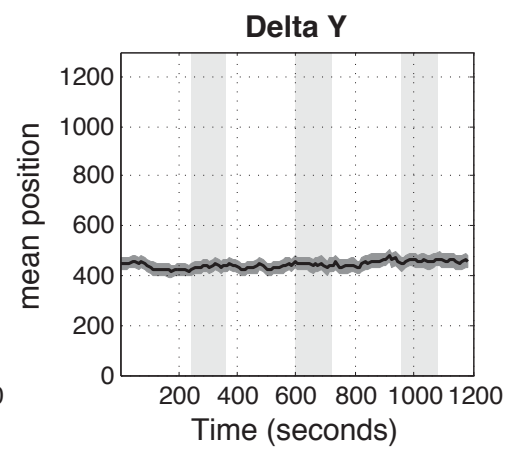
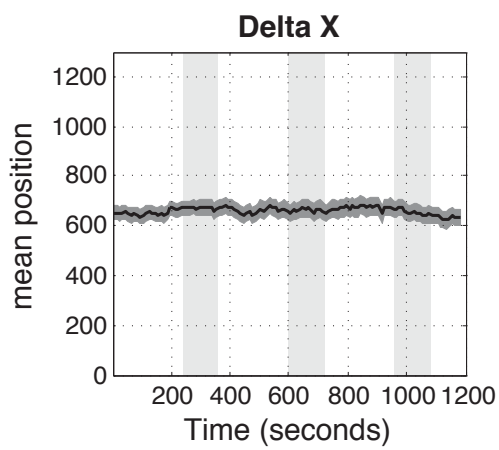
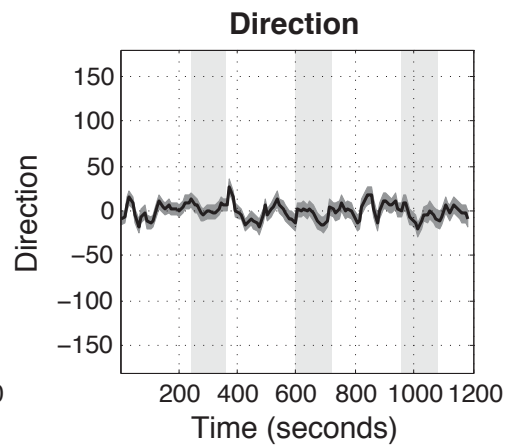
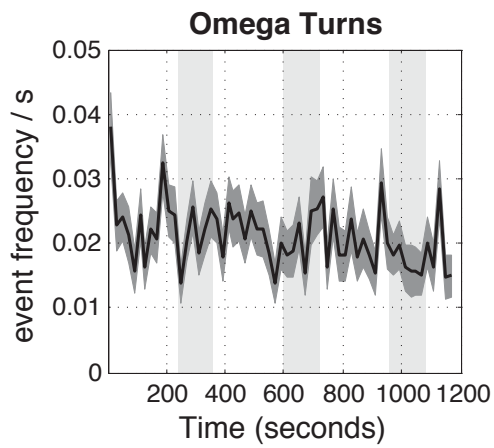
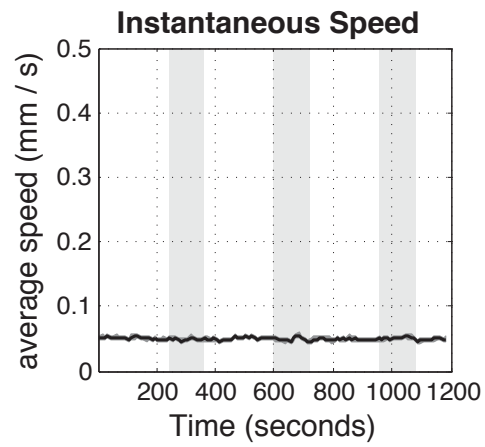
n=2



DF5025

Pellioditis typica

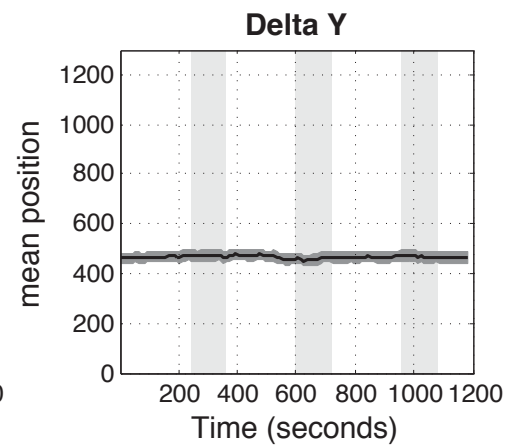
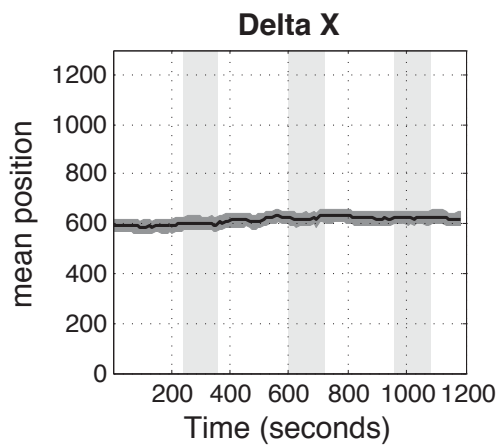
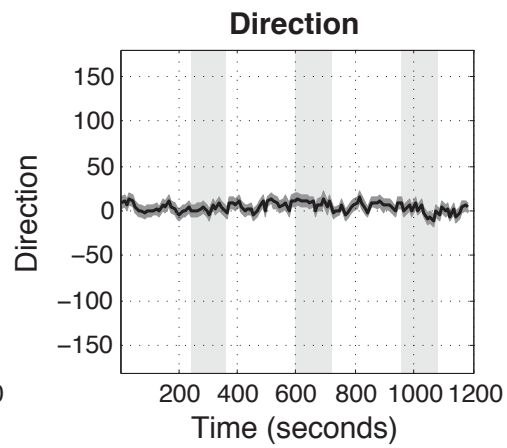
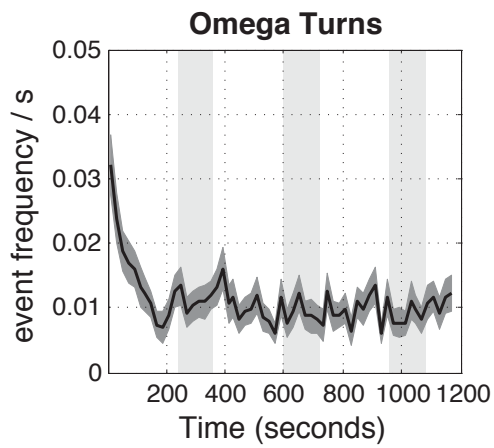
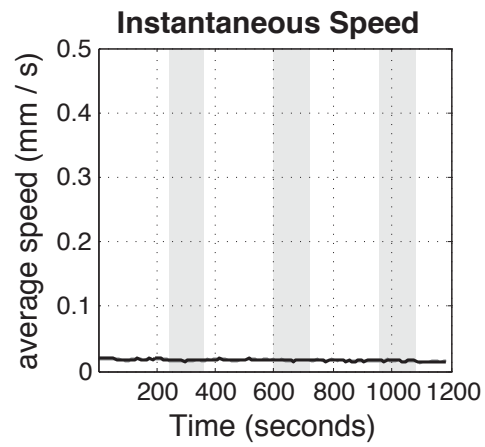
n=2



DWF1108

Acrobelloides sp.

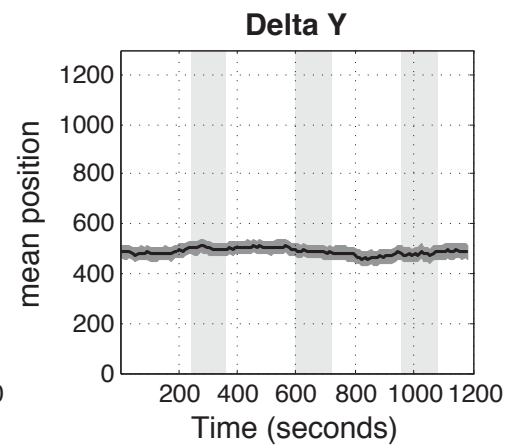
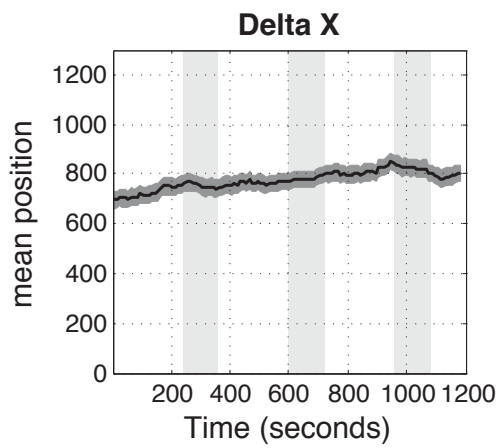
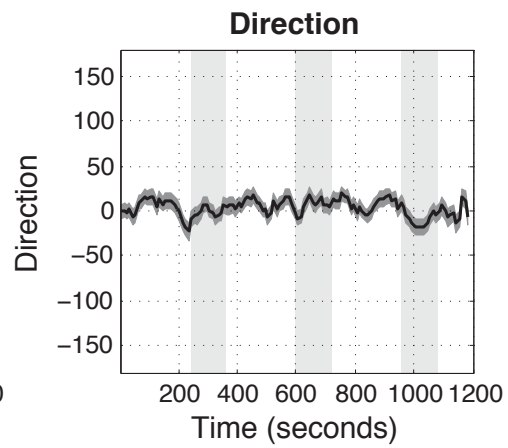
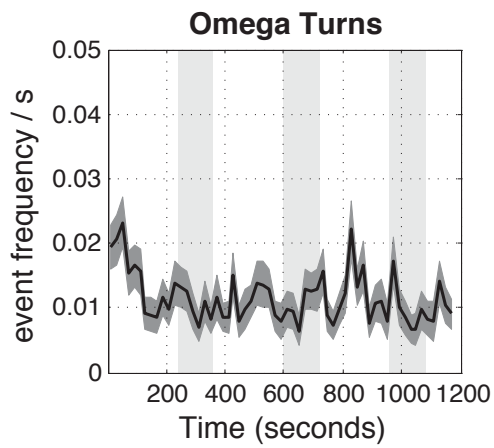
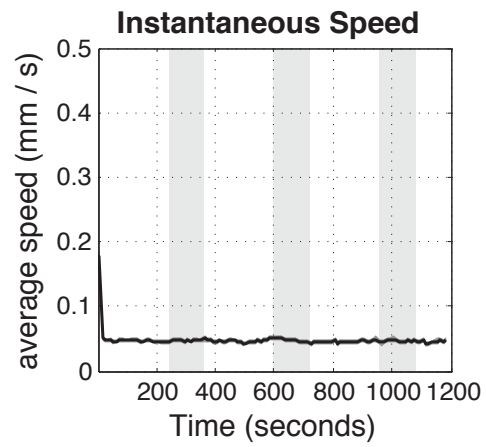
n=2



DWF1301

Cephalobus sp.

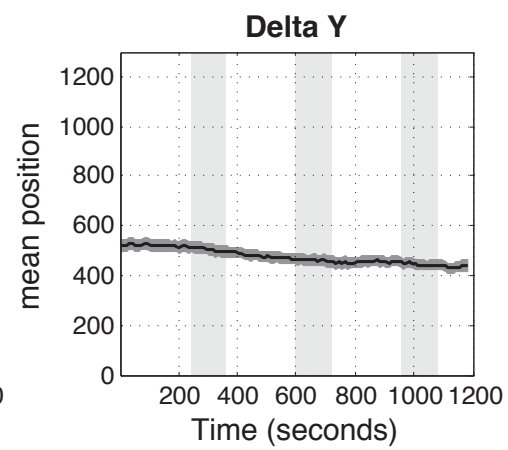
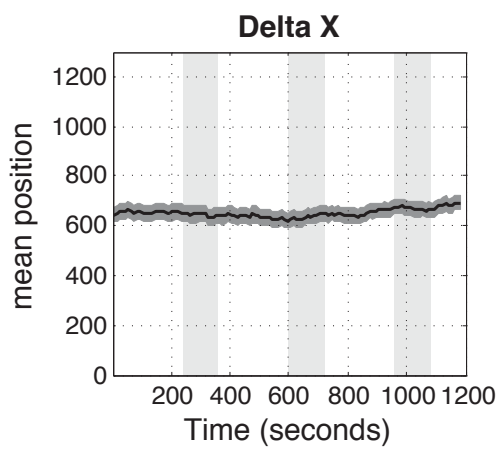
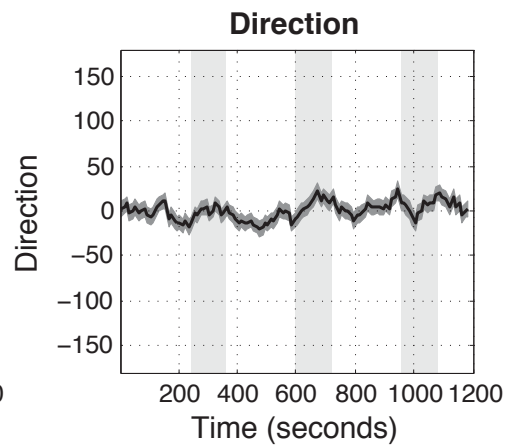
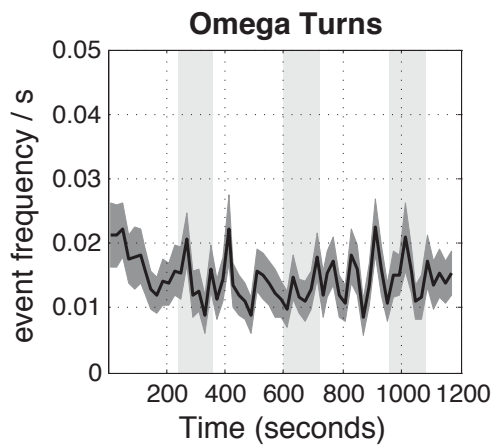
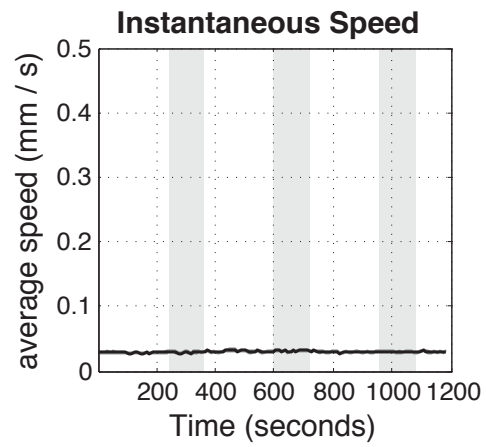
n=2



DWF1501

Dolichorhabditis sp.

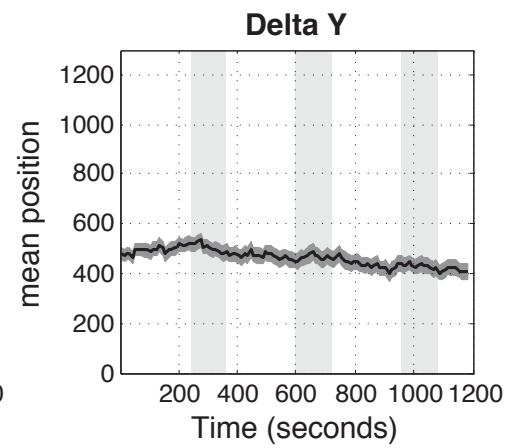
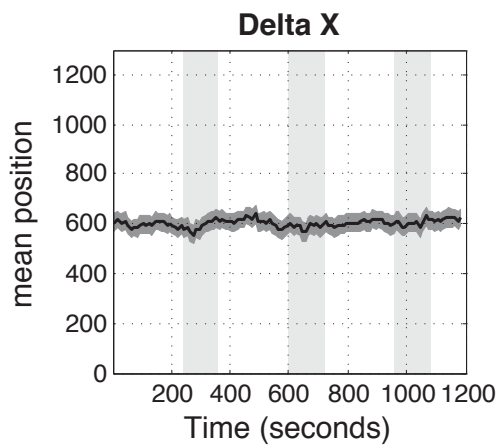
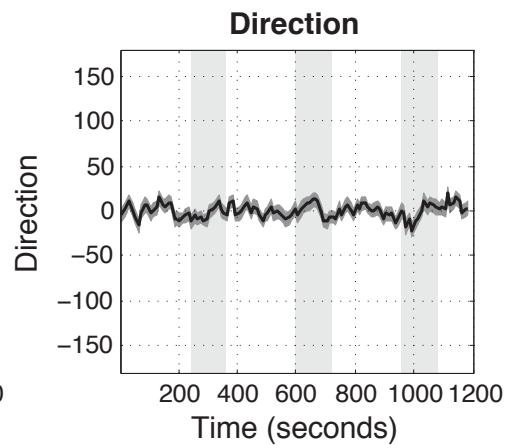
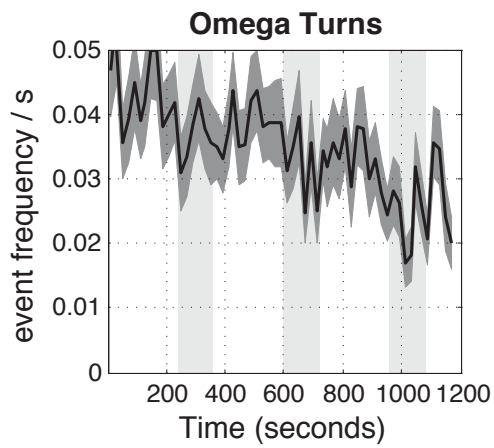
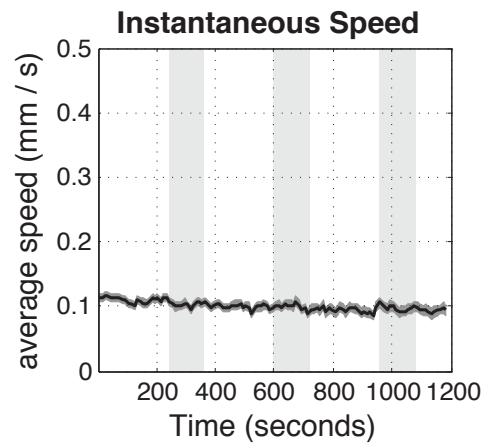
n=2



DWF1604

Operculrhabditis sp.

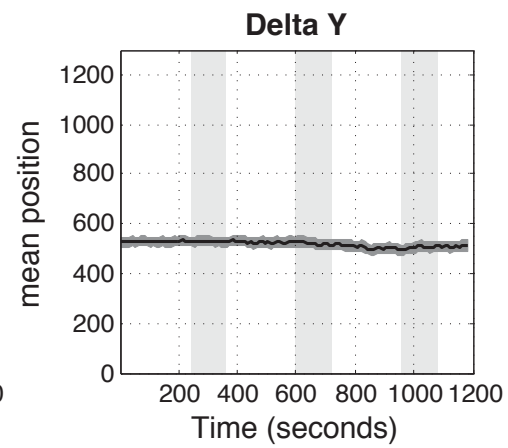
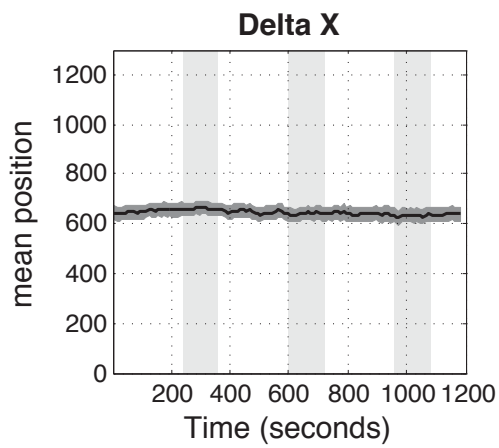
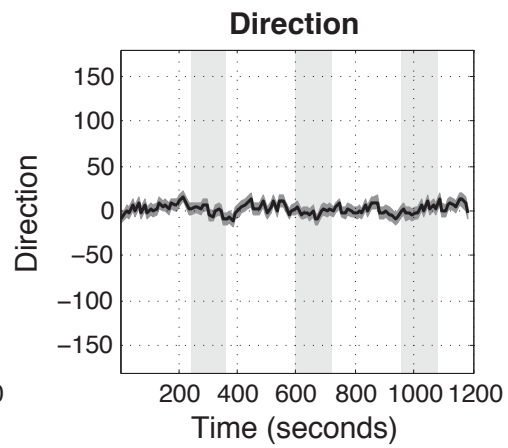
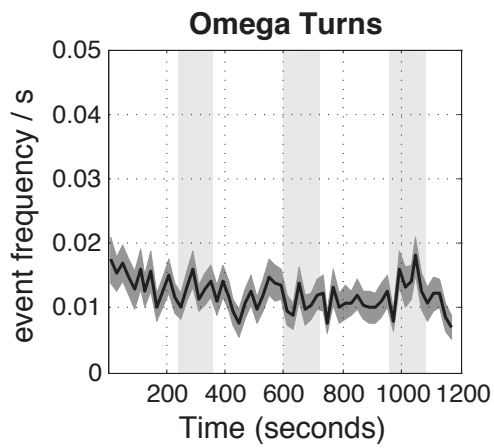
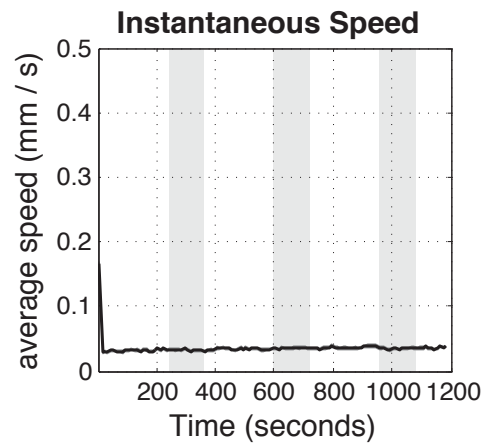
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DWF1701

Zeldia sp.

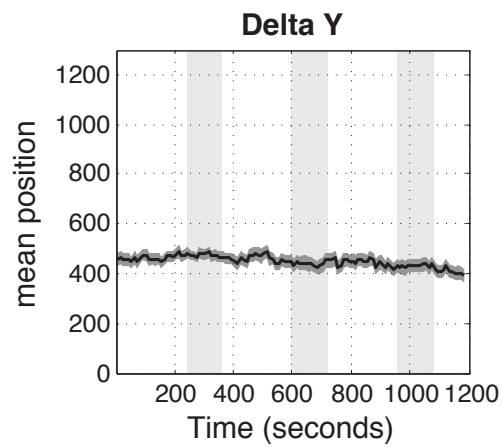
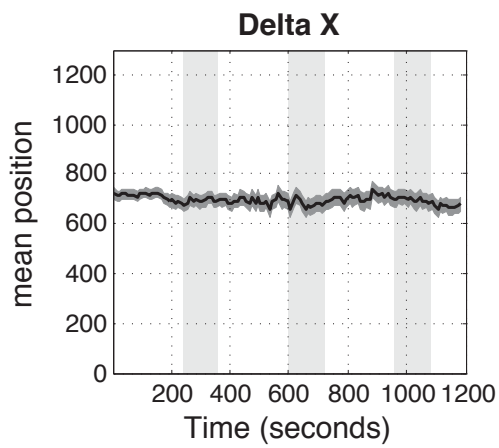
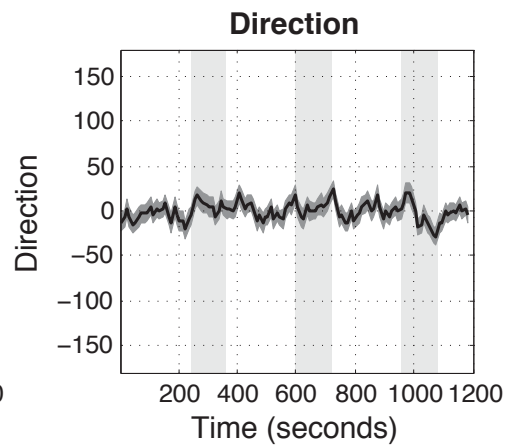
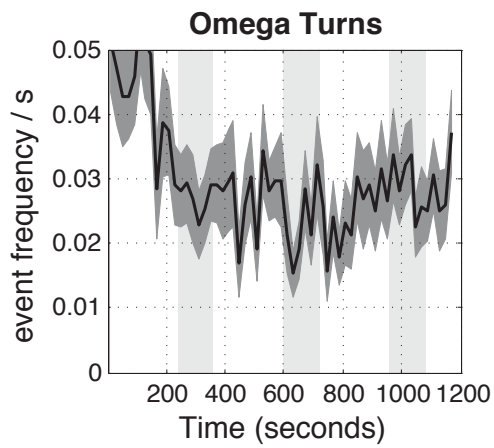
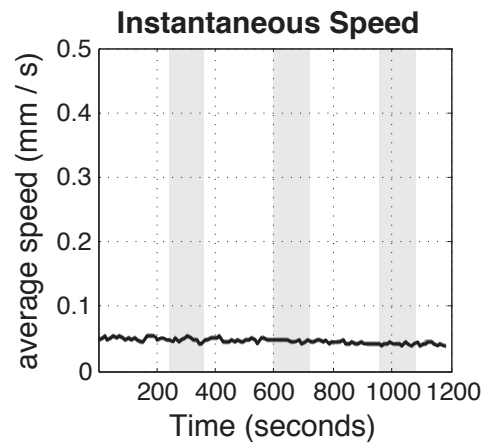
n=2



HMephisto

Halicephalobus mephisto sp.
Nov.

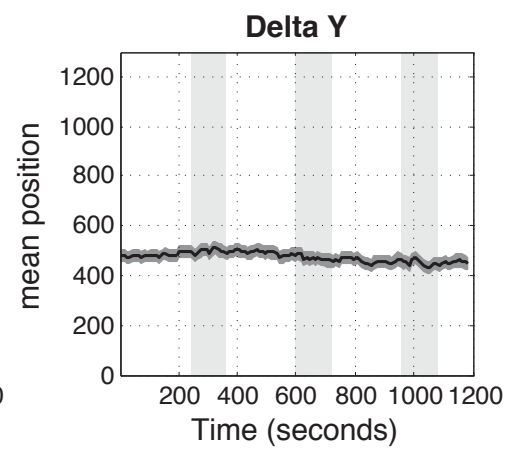
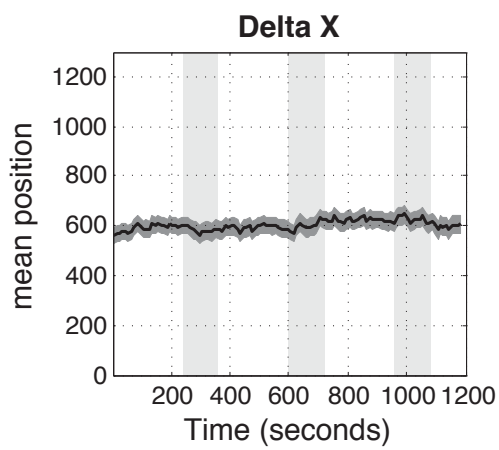
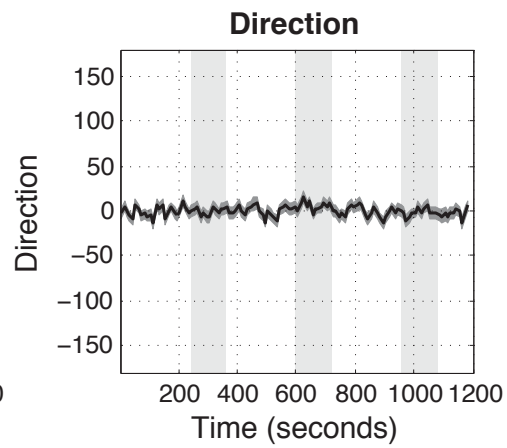
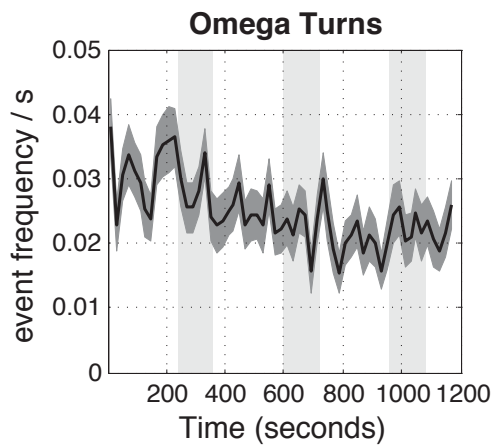
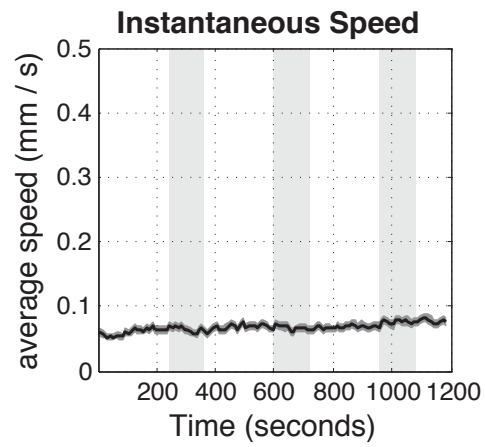
n=2



MT8872

Panagrellus redivivus.

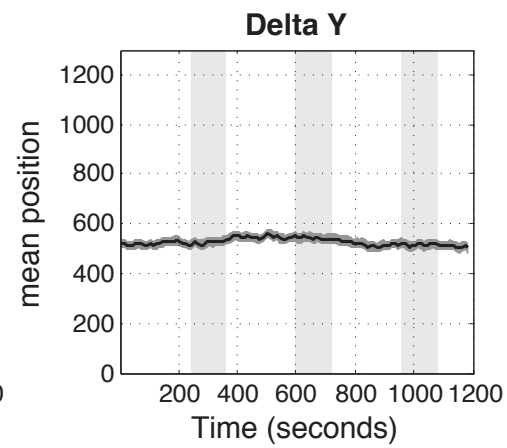
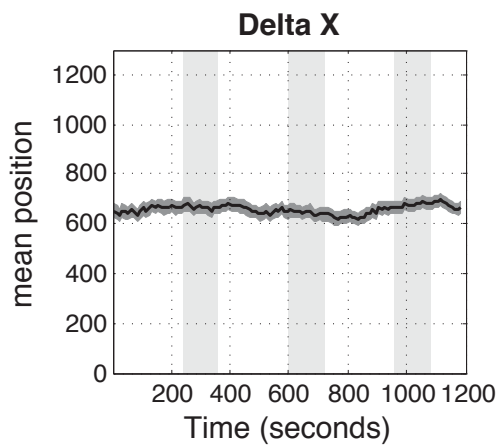
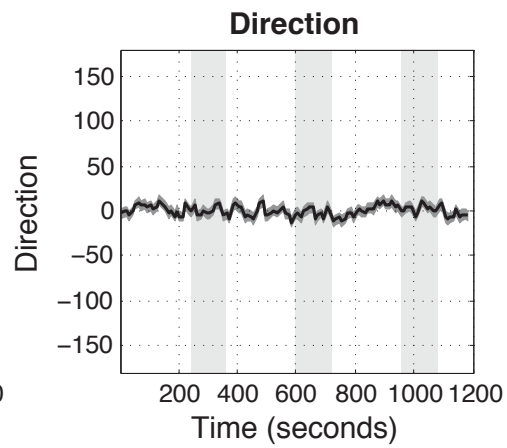
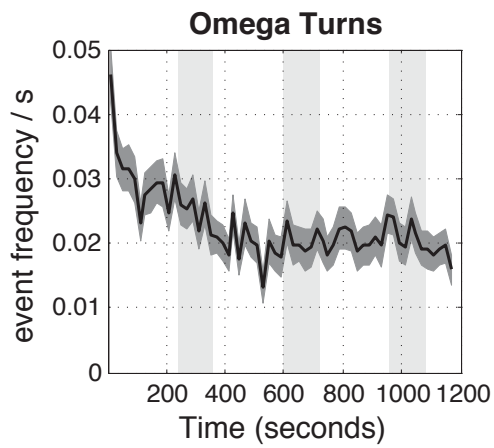
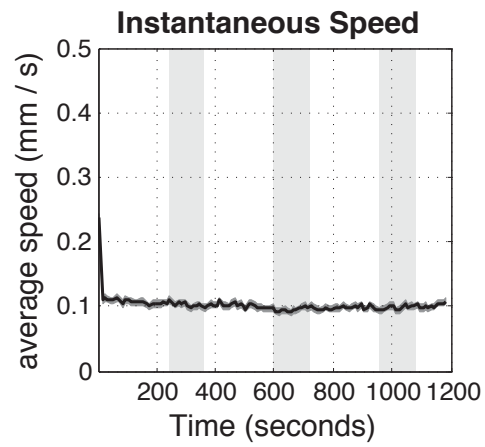
n=2



N2

Caenorhabditis elegans

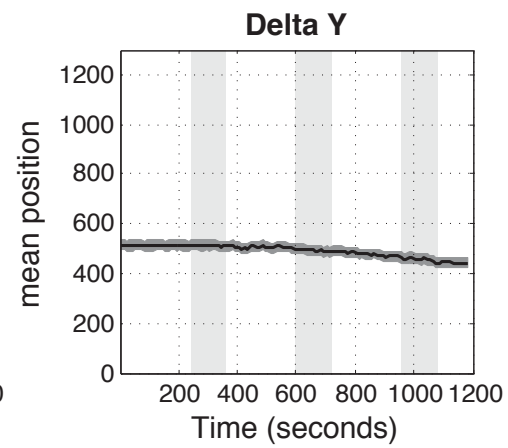
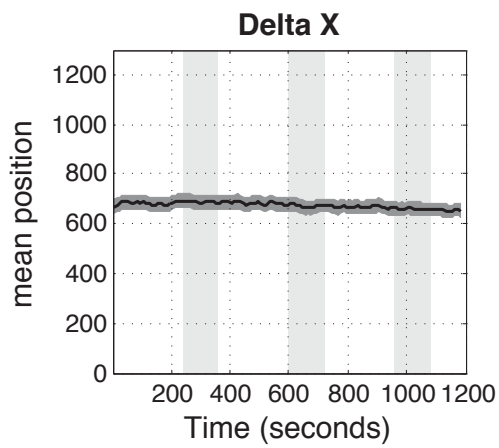
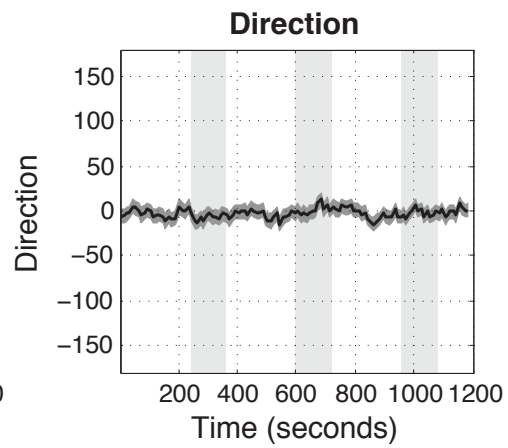
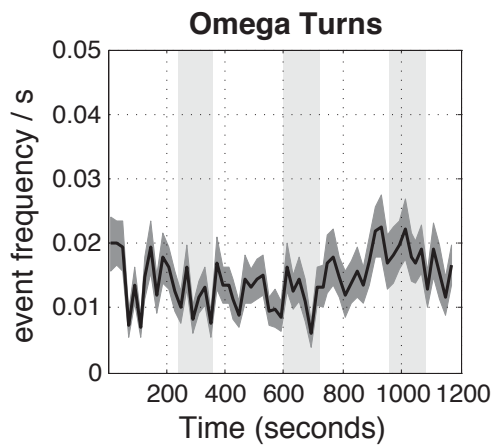
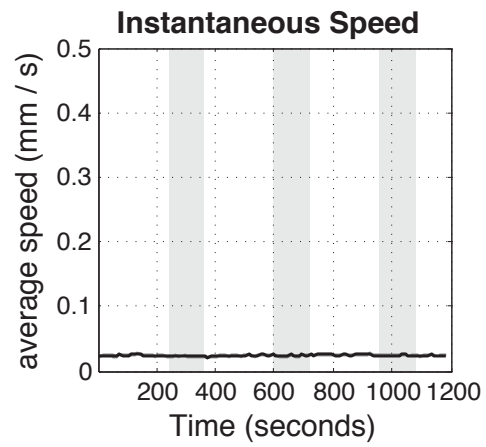
n=4



PG-1

species unknown

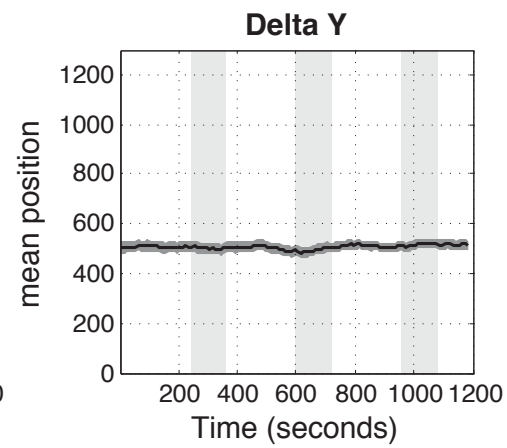
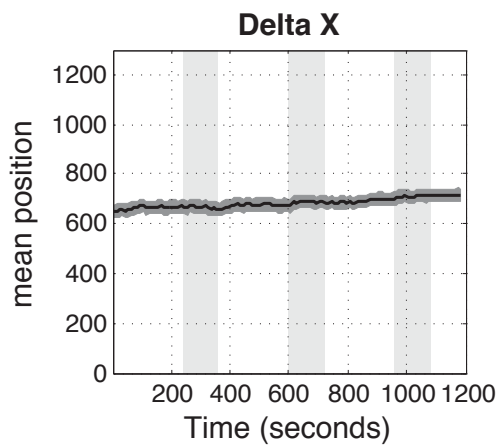
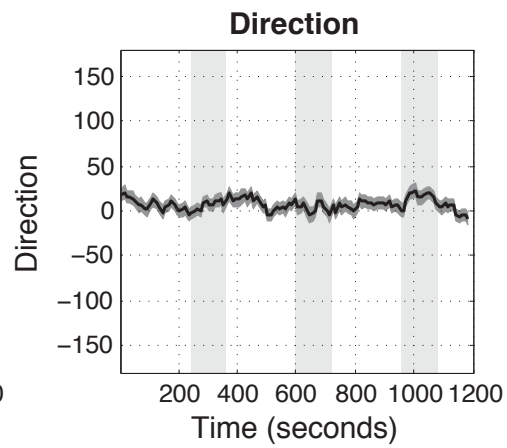
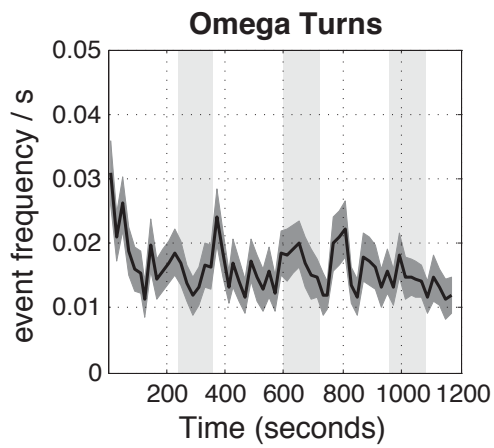
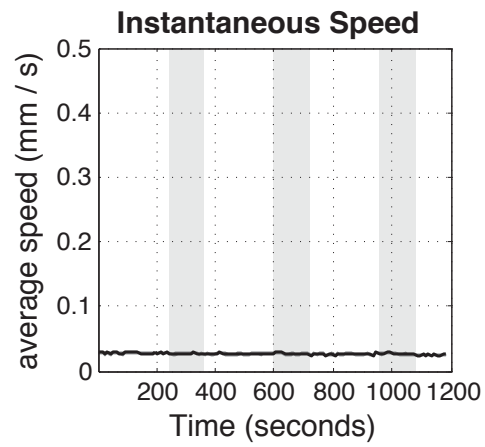
n=2



PG-2

species unknown

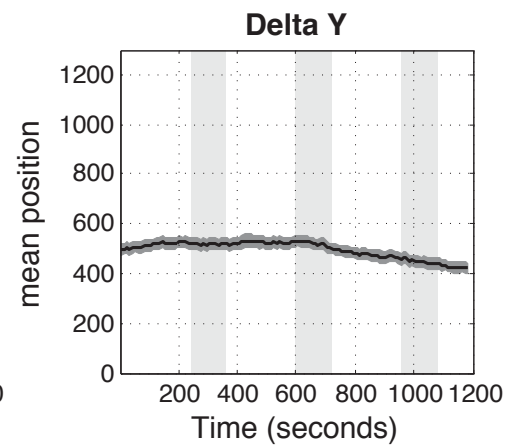
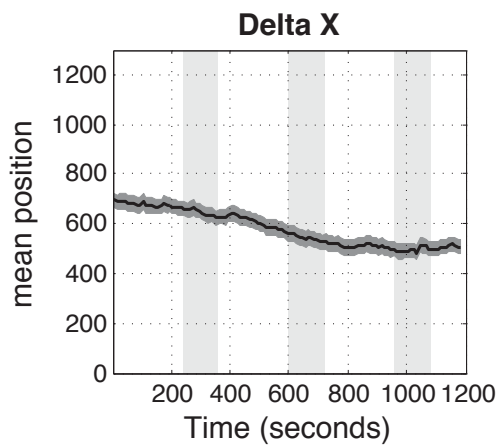
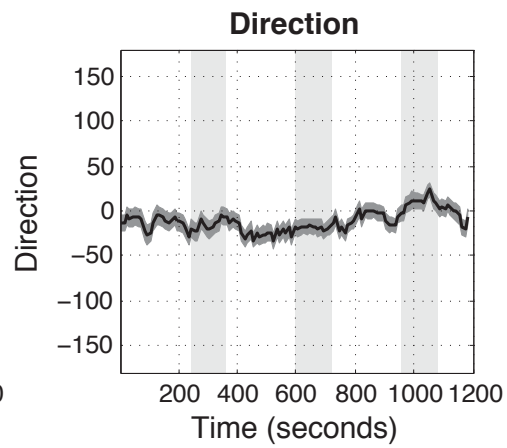
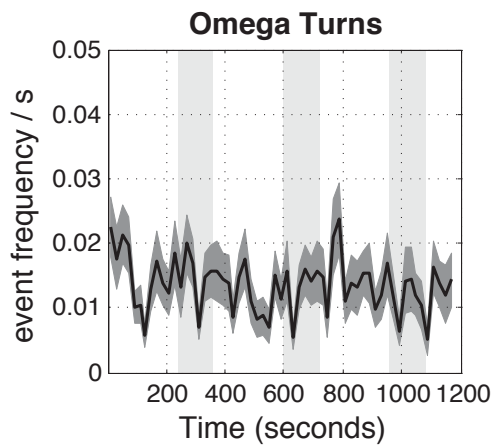
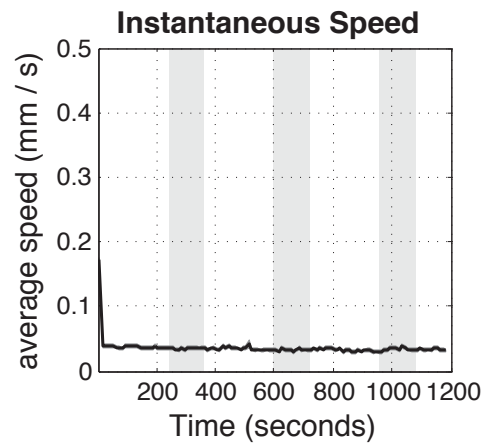
n=2



PG-3_1

species unknown

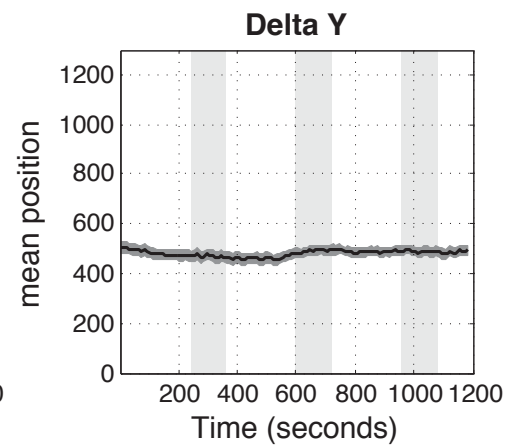
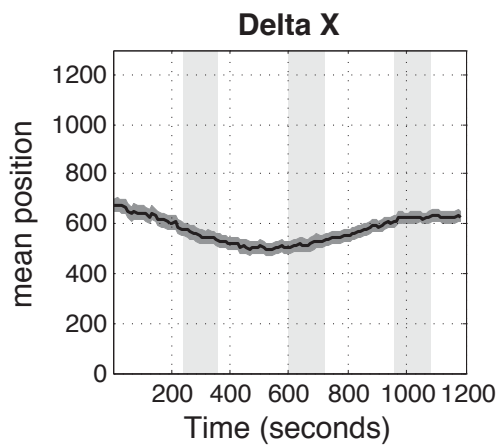
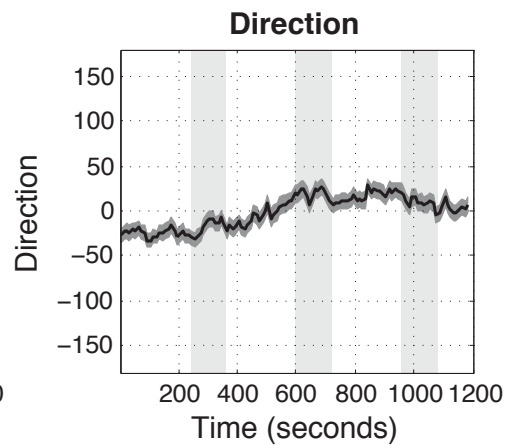
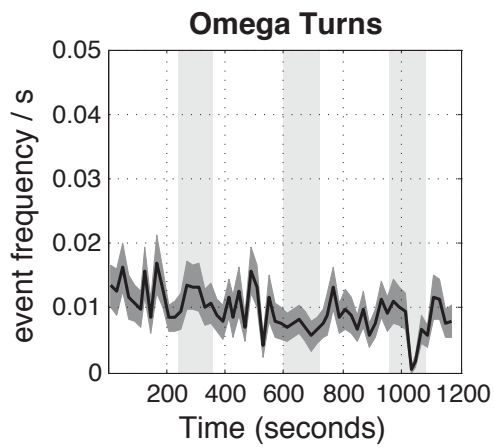
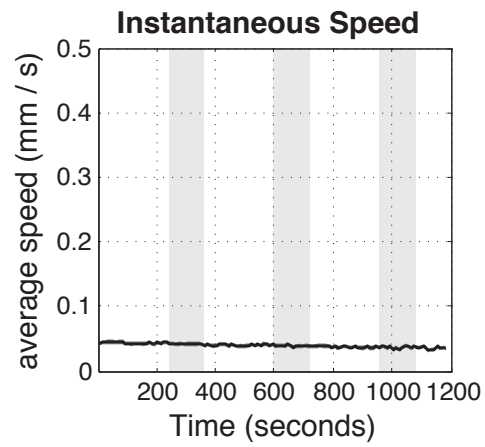
n=2



PG-3_2

species unknown

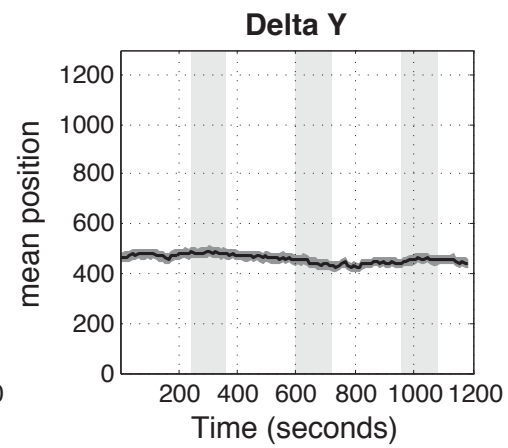
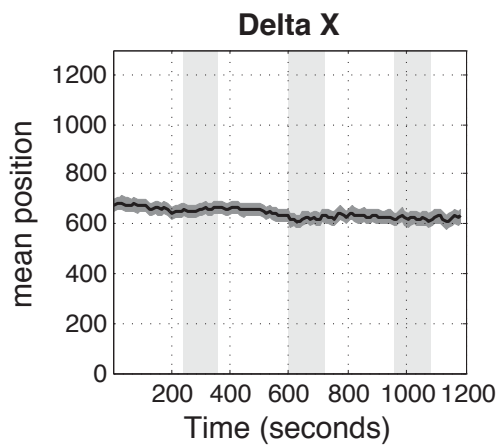
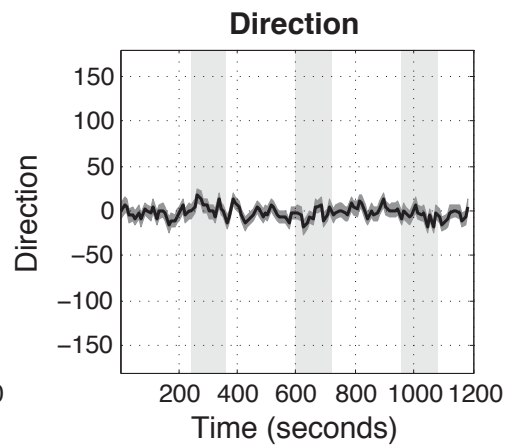
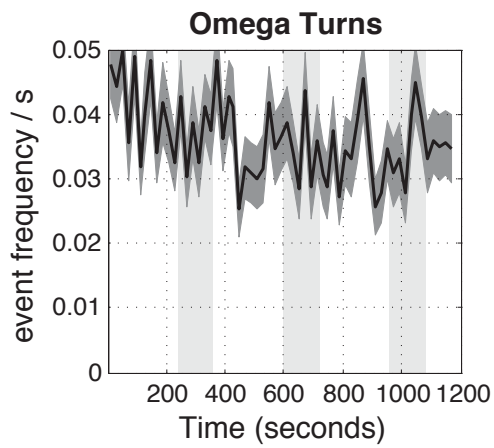
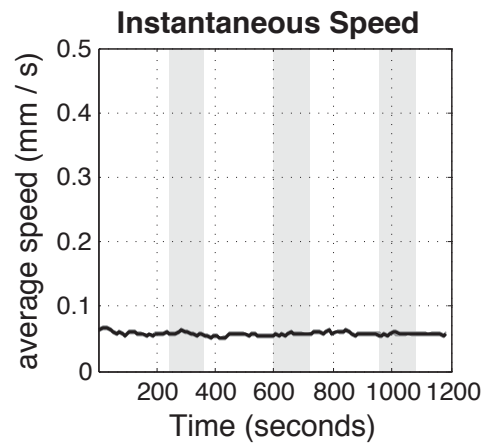
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PPC14_1

species unknown

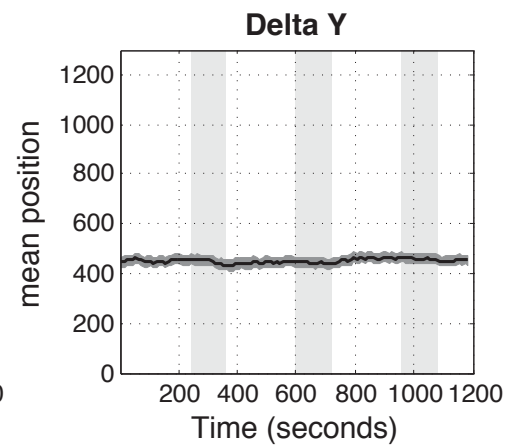
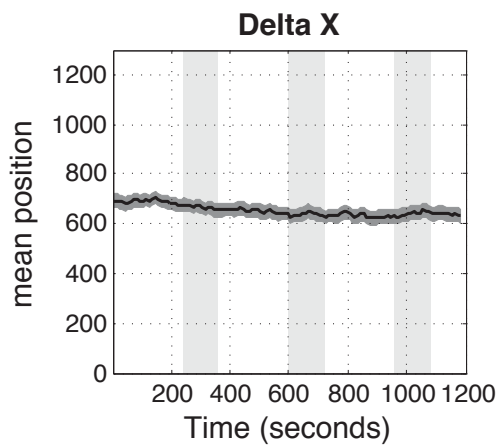
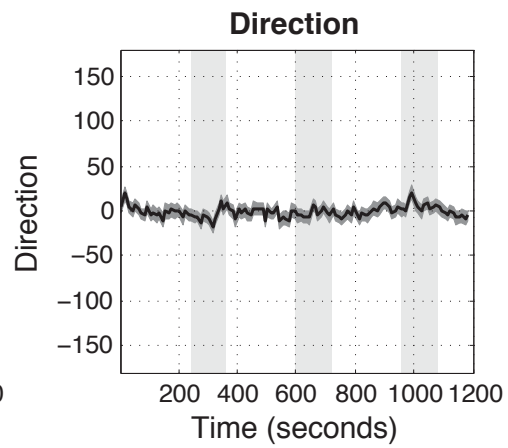
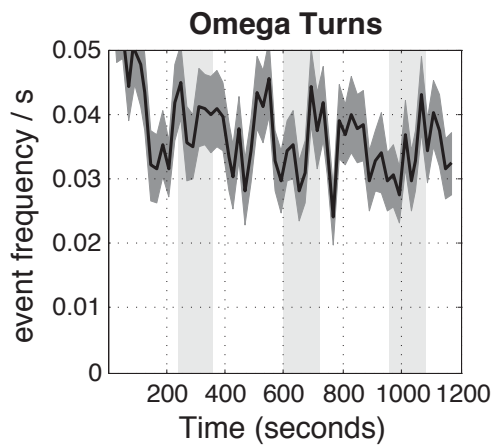
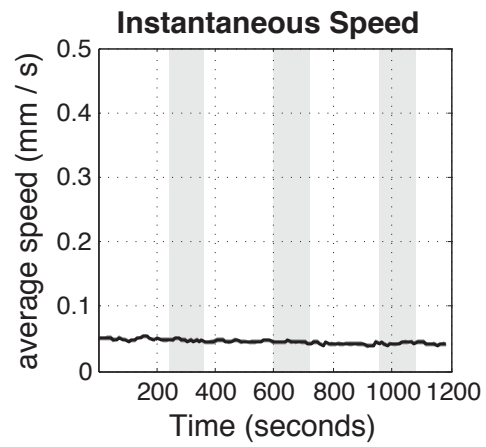
n=2



PPC14_2

species unknown

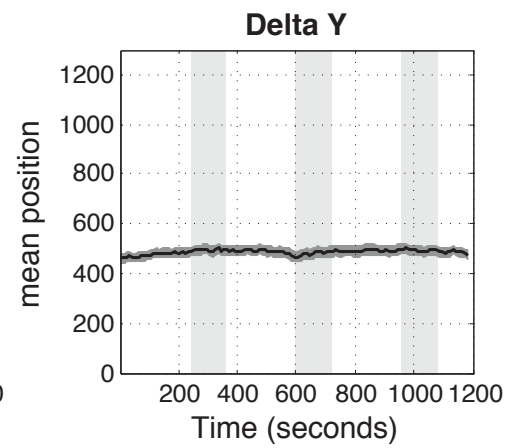
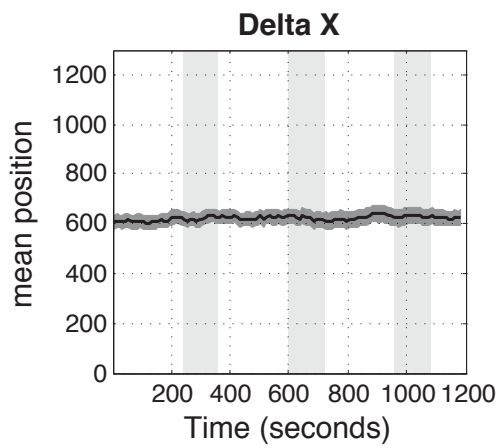
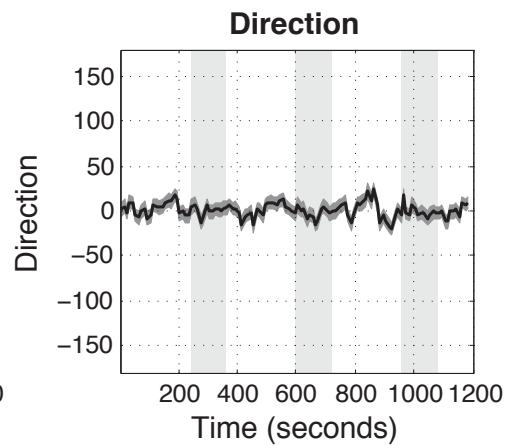
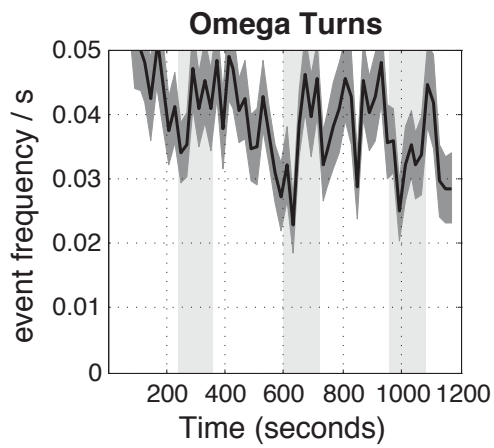
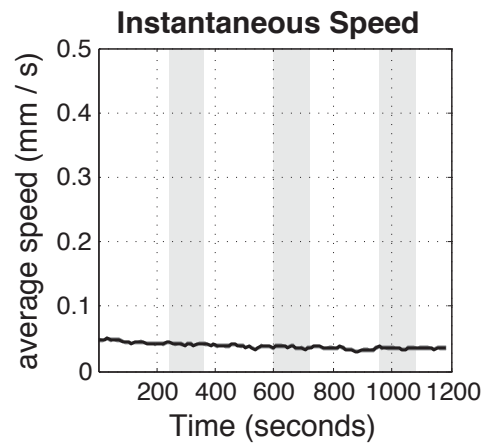
n=2



PPC74

species unknown

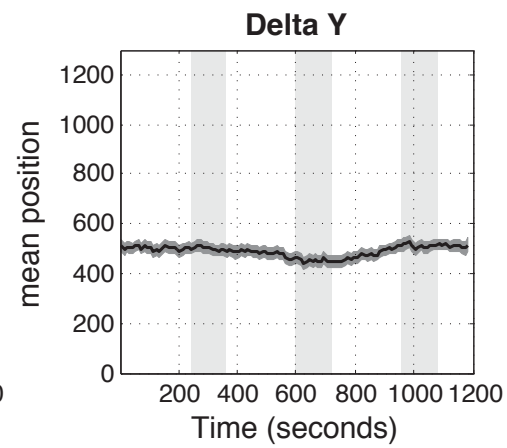
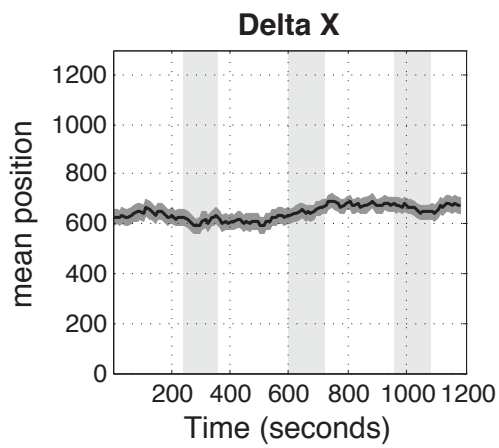
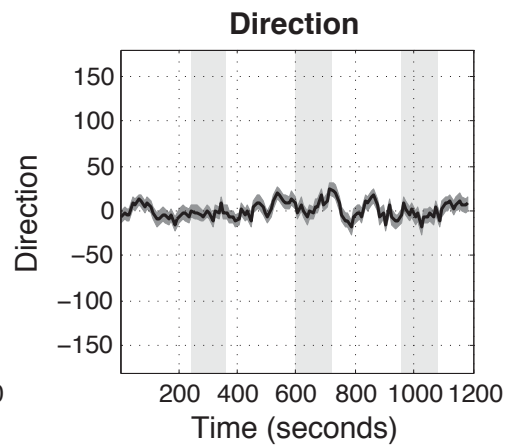
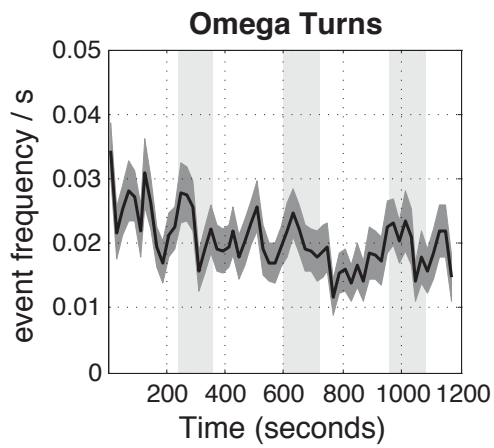
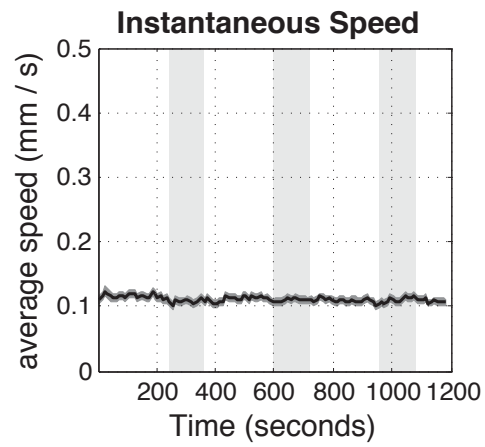
n=2



PS1156

Acrobeles sp

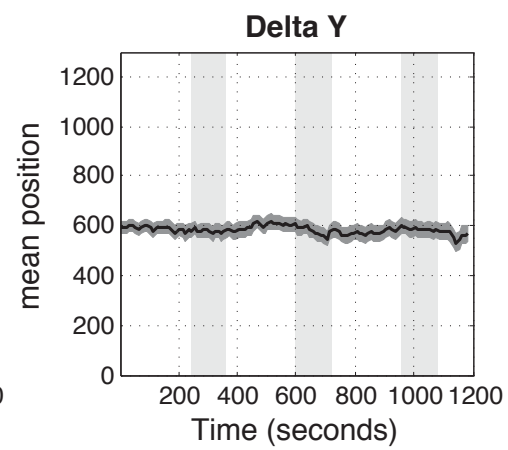
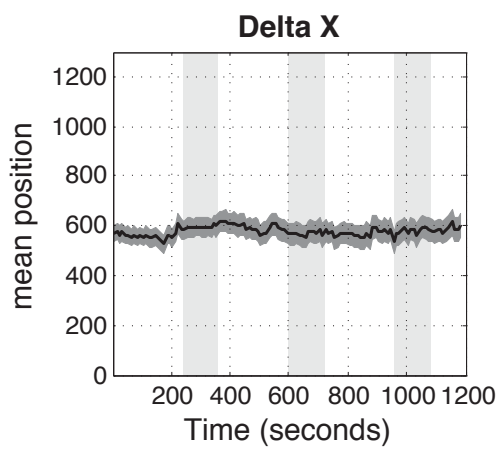
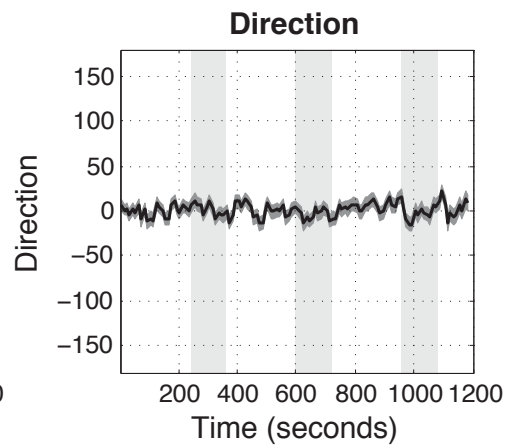
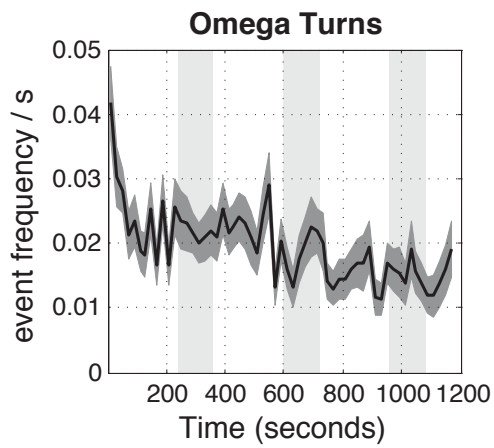
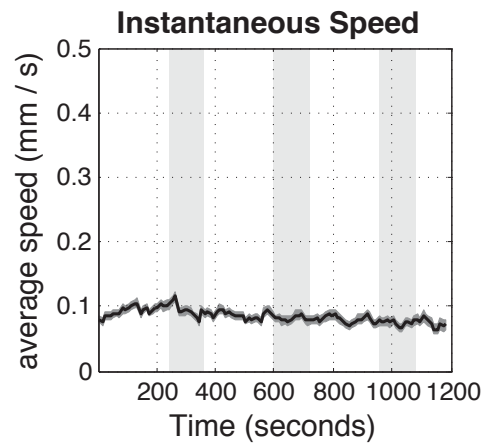
n=2



PS1163

Panagrellus redivivus

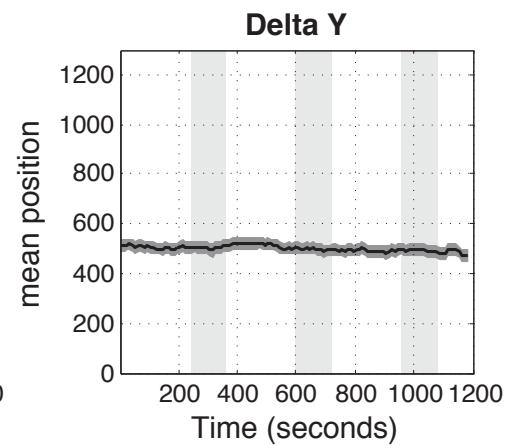
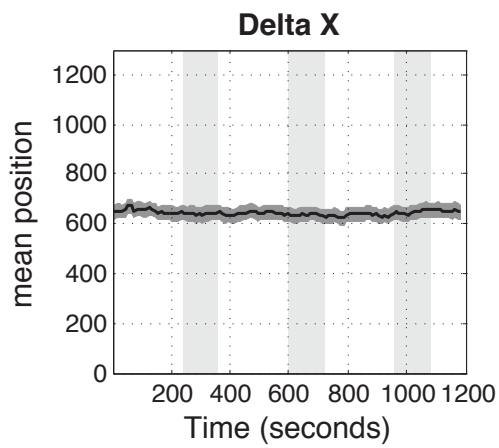
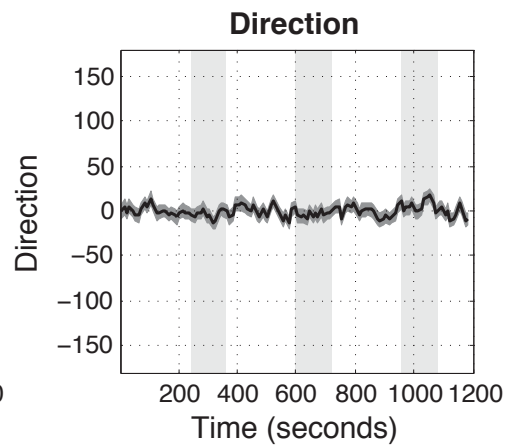
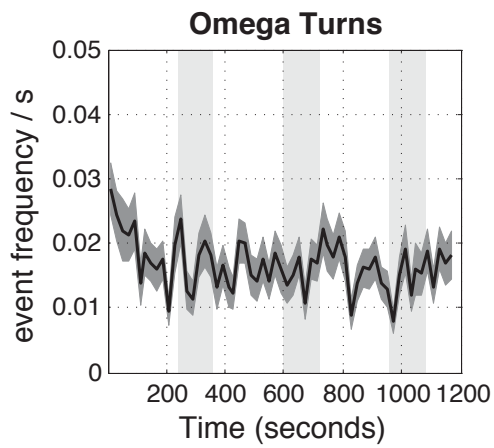
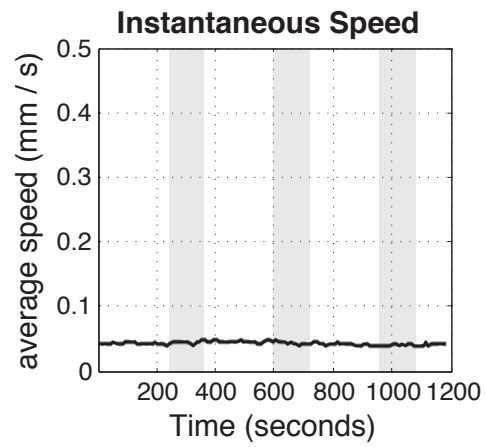
n=2



SB122

Choriorhabditis dudichi

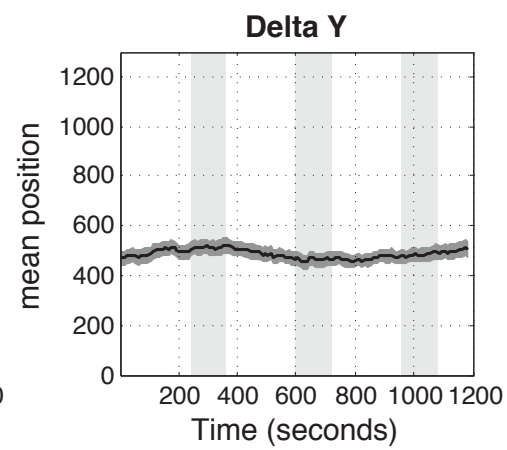
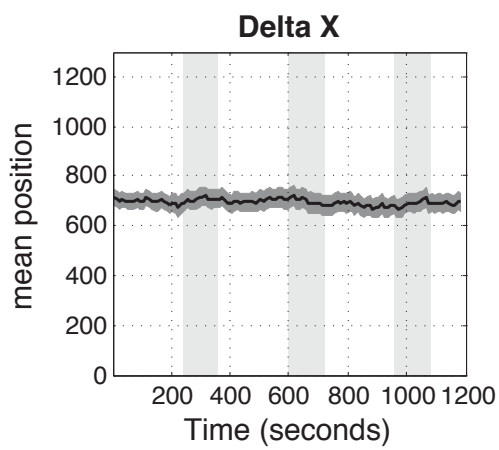
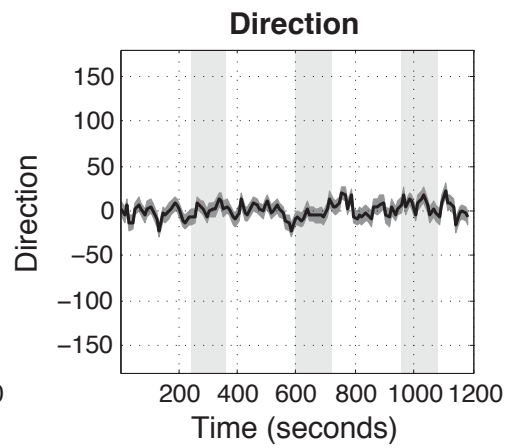
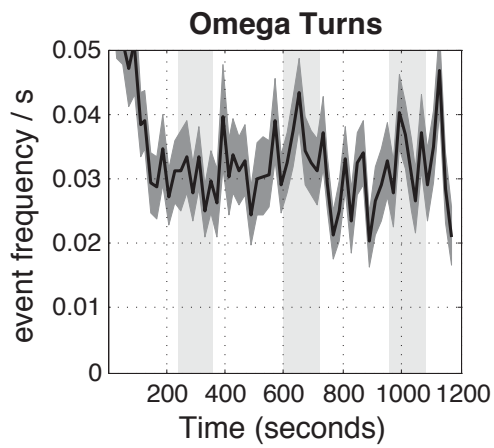
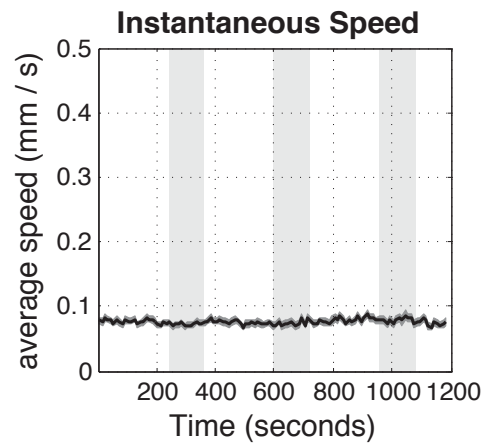
n=2



SB146

Choriorhabditis remanei

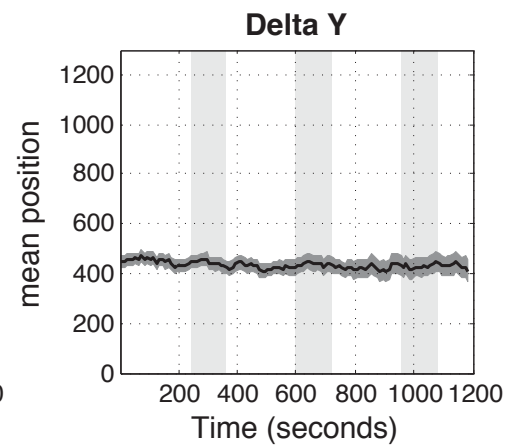
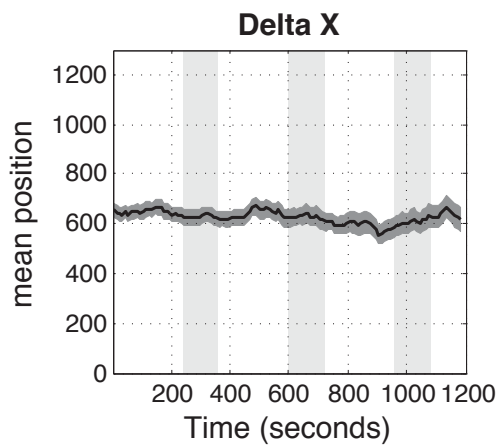
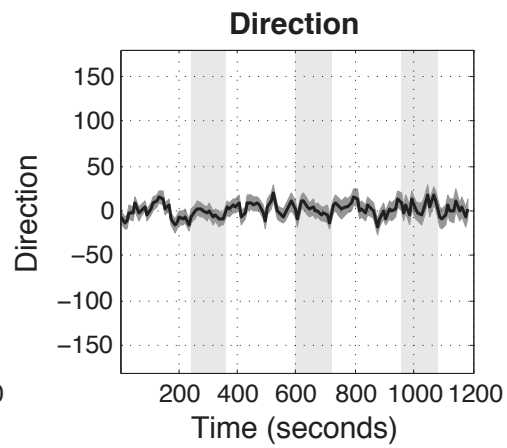
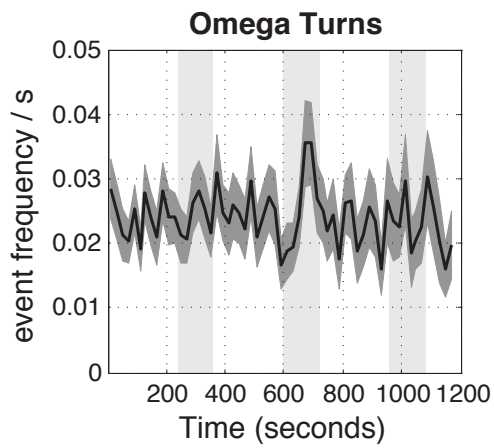
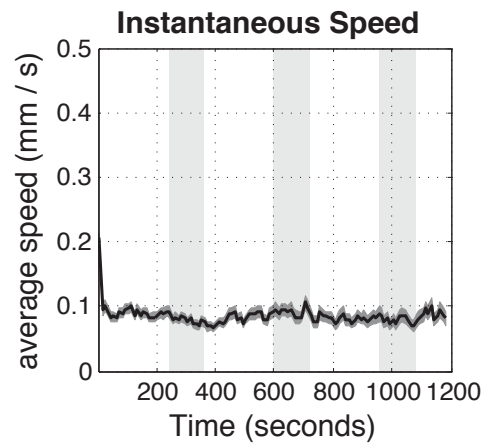
n=2



SB193

Rhabditis brassicae

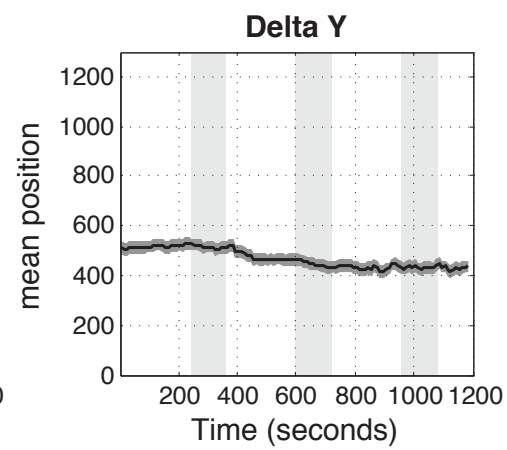
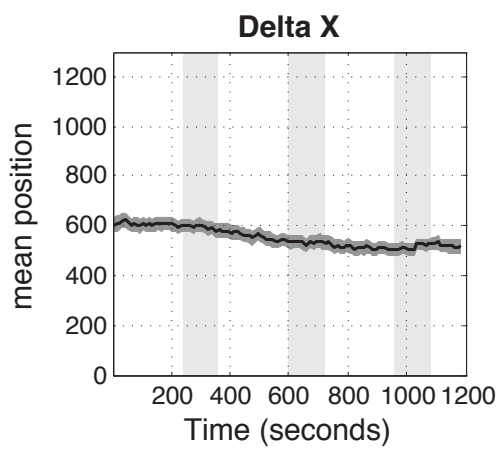
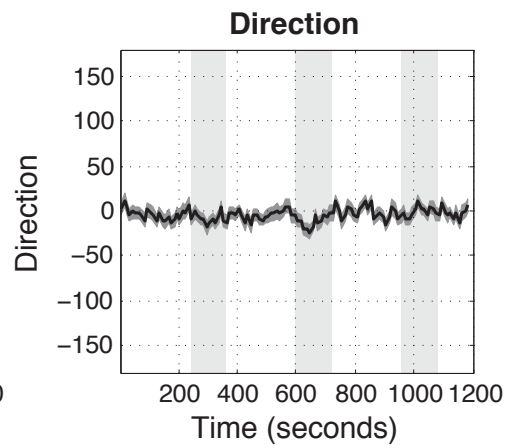
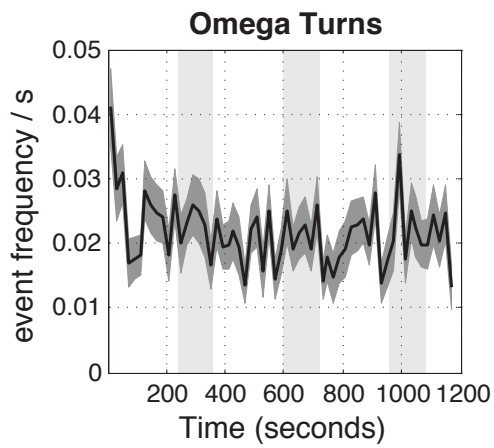
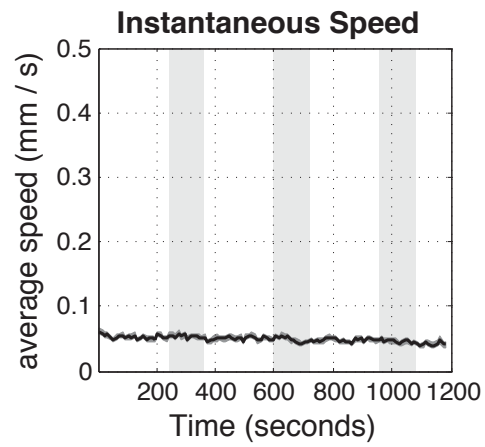
n=2



SB408

Poikilolaimus sp.

n=2



Appendix B:

Sample#:	Collection Date:	Collection Place:			Geodata:	Isolation Method:
S_001	17.10.14	Gams bei Hiefiau	Kraushoehle	small puddle, deepest point	47° 40.095'N 14° 48.215'E	Baermann Funel
S_002	18.10.14	Gams bei Hiefiau	Odelsteinhoehle	??	47° 31' 27.1" N, 14° 36' 42.88" E	Baermann Funel
S_003.1	27.10.14	Kirchberg am Wechsel	Hermannshoehle	"Lake"	47° 37' 1" N, 15° 58' 56" E	Baermann Funel
S_003.2	27.10.14	Kirchberg am Wechsel	Hermannshoehle	"small puddle, imm. right"	47° 37' 1" N, 15° 58' 56" E	Baermann Funel
S_004	08.11.14	Grosser Otter	Markiertes Windloch	Sediment, deepest point	719165/276515	Baermann Funel
S_011	29.11.14	Huetten	Reintal Tropfsteinkluft	puddle, 2nd hall	47.625878°N 16.136545E	Baermann Funel

7. Acknowledgments

I would like to thank David Keays and his amazing lab for the great working atmosphere during the last year; my entire family, in particular my parents, and all of my friends for the constant support; and whoever made it to page 93.

8. Curriculum Vitae

Details

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Education

Since 10/2005	Student of <i>Molecular Biology and Philosophy</i> at the University of Vienna
06/2004	High School Graduation with honor
09/2002 – 06/2004	High School, Erich Fried Realgymnasium
09/2001 – 06/2002	Exchange Student at Eisenhower High School, Russel PA, USA (Sophomore year)
09/1996 – 06/2001	High school, Erich Fried Realgymnasium

Work Experience

10/2010 – 10/2011	Diploma thesis at the lab of Dr. David Keays at the Research Institute of Molecular Pathology (IMP), Vienna
12.07. – 14.09.2010	Department of Neuronal Cell Biology, Prof. Kiebler.
06/2010 – 06/2010	“Tutor” at a Biochemistry lab course (University of Vienna)
01.09. – 01.12.2009	University of British Columbia, Vancouver, BC, Human Vision and Eye Movement Laboratory, Prof. Jason Barton Working title: The nature of upright and inverted face representations: an adaptation-transfer study of configuration.
01.07. – 31.07.2009	BAXTER Bioscience, Orth/Donau
01.04. – 12.06.2009	The Johns Hopkins School of Medicine, Baltimore, MD Department of Neuroscience, Paul Worley Laboratory, Working on the role of mTOR in synaptic plasticity.
01.07. – 31.07.2008	BAXTER Bioscience, Orth/Donau
01.07. – 31.07.2007	BAXTER Bioscience, Orth/Donau
10/2004 – 10/2005	Civil Services, Hospital, Barmherzige Schwestern, Vienna (full-time)

Further skills

IT knowledge	Good knowledge of <i>MS Word</i> , <i>MS PowerPoint</i> and <i>MS Excel</i> , <i>Adobe Photoshop</i> , <i>Adobe Illustrator</i> Basic <i>Matlab</i> programming skills
Statistics	Good Knowledge of basic methods (t-test, ANOVA, etc.)
Foreign languages	English (fluent) French (good)