DISORIENTATION AND ITS USE TO INVESTIGATE THE VESTIBULAR SYSTEM OF *OCTOPUS RUBESCENS*

by
Brandon Bowers

A THESIS

submitted to

WALLA WALLA UNIVERSITY

in partial fulfillment of
the requirements for the
degree of

MASTER OF SCIENCE

JUNE 2025

This thesis for the Masters of Science degree

has been approved by the Department of Biological Sciences

and the Office of Graduate Studies

Walla Walla University

Major professor

Committee member

David L. Cowles

Committee member

Committee member

Dean of Graduate Studies

Observer of the Process - Graduate Representative

Candidate

6/11/25

Date

ABSTRACT

Orientation is a state in which how an animal perceives the relationship between their body and the external world is accurate enough to allow for coordinated behavior. For humans, remaining oriented requires a complex integration of a variety of senses; primarily visual, vestibular, and proprioceptive information. Although octopuses are anatomically and evolutionarily removed from humans, much of their visual and vestibular morphology resembles that of mammals in structure and function. It is unknown, however, if octopuses become disoriented in similar ways to humans or other mammals. Prior investigations into this topic have been largely invasive, so this project sought to develop a more ethical methodology for investigating orientation through disorientation. A spinning tank was built to be able to spin octopuses. Using wild-caught *Octopus rubescens,* octopuses' ability to catch a crab, and movement patterns in doing so, were recorded and measured with or without being spun. Using DeepLabCut, artificial neural networks were trained to label the positions of the eyes of the octopus and the crab in each video, with varying success. Some data was also manually labelled. These labelled anatomical positions were then used to measure movement characteristics that may have been affected by disorientation due to spinning. Ultimately, the results largely suggest that octopuses did not experience disorientation under these conditions. However, refinement of the methodology and data analysis may allow for more accurate understandings of the effects of spinning in the future.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Vestibular Systems

For organisms to effectively behave in relation to an external world, they must be able to construct some relation of themselves to it: an internal model of the world.[1–4] Though the clarity and complexity of this model may vary widely between organisms, this is a foundational aspect to both motile and sessile life on earth. The relationship between this model and the reality of the external world then decides the effectiveness of the various behaviors that may be elicited by an organism's sensory input. In this context, "orientation" is this relationship between an internally constructed model using sensory information and its similarity or effectiveness in relation to the external reality. Specifically, orientation refers to a state in which an organism's internal model of the outside world is able to allow the organism to predictably coordinate its behaviors in relation to the world. Conversely, "disorientation" is an opposite state, in which the internal model of an organism is altered in some way in comparison to the external reality, such that the organism is less able to behave in a coordinated fashion.

The construction of an organism's orientation commonly involves integration of many senses. Of these senses, the importance of the sensation of gravity in an organism's ability to mechanically relate to the world cannot be understated, as it affects the forces produced by nearly any mechanical action. It is likely for this reason that we find these sensory systems represented in nearly every kingdom of the taxonomic tree[5–8].

Microorganisms, such as *Paramecium*, use a perception of gravity to modulate their swimming velocity relative to their sensation of a gravitational vector.[5] For plants, which are sessile, a perception of gravity from the movement of heavy starch crystals allows for the maintenance of posture and directed growth.[5] Gravity sense using the sensed movement of small, internal crystals is also a common sensing strategy with similar sensory structures being found in humans[6,7], mice[7], birds[8], gastropods[9], cnidarians[10], crustaceans[11], some fish [12], and finally cephalopods [13–16]; to name a few.

Sensory structures that use this strategy, that of tracking the movement of small crystallized masses are the utricle and saccule of humans and other vertebrates[6,7], and the statocysts of invertebrates. Inside of the utricle and saccule and statocyst, we find the respective crystallized masses, referred to as otoconia (in mammals) and statoliths (in invertebrates). These structures, alone or in conjunction with other motion sensing structures are referred to as the vestibular system, and their sensory information as the vestibular sense. It is likely, because of the widespread presence of statocysts, that this sensory feature evolved rudimentarily in an ancient ancestor from which many of these separate forms have radiated; this idea being supported by the retention and expression of similar statocyst-forming and related genes across phyla[17].

Some organisms' vestibular systems have developed new features to compensate for more complex body plans and movements. Throughout the animal kingdom, vestibular sense is used to orient the visual field, to balance and maintain posture, and to provide integral data allowing for directed action or movement. Many organisms seem to accomplish these tasks with surprisingly similar strategies. As with the ubiquity of statocyst-like organs, angular-motion sensing organs are found commonly throughout the animal kingdom. In humans, and many other mammals, these angular-motion sensing organs are called semicircular canals. However, we find similar structures employing similar sensation strategies in often-times fairly disparate animals, such as cephalopods.

The ability to combine gravitational information with more dynamic tracking of linear and angular motion through the use of organs, such as the statocysts and semicircular canals, have allowed animals to more accurately orient themselves while performing complex behaviors. The vestibular sense alone, however, is not sufficient for many organisms to build a reliable internal model of the world.

Octopus Vestibular Morphology

As mentioned previously, both human and cephalopod vestibular systems consist of two sensory systems: a gravity receptor system and an angular acceleration receptor system.[18–20] In both cases, detection of linear acceleration may be sensed by both systems.[13–15] In octopodes, as with all cephalopods, the vestibular organ is called the statocyst (Fig. 1). It is held within a cartilaginous sack deeper than, posterior to, and roughly between the eyes.[13] There, like a bean hanging from the interior of an egg, the statocyst attaches to the anterior wall of this cartilaginous sack. It is suspended in perilymph, a colorless substrate, by numerous, fibrous strands and blood vessels that run from the outer wall of the sack to the external wall of the statocyst.[13] The statocyst itself, is roughly separated into two chambers: an anterior and posterior chamber.[13] The anterior chamber contains the gravity receptor system and parts of the angular acceleration system, the rest of which runs into the posterior chamber.[13] The statocyst is filled with a viscous fluid, similar to the perilymph, called the endolymph, which flows and shifts in the statocyst in response to motion.[13]

Movement of endolymph throughout the statocyst is the main stimulatory driver of the angular acceleration receptory system.[18] This system is composed of a sensory epithelium, called the crista, and gelatinous flaps called cupulae, which make up a stimulatory 'apparatus'.[18] The crista sits atop cartilaginous ridges, which roughly portion the posterior chamber into nine sections, each with its own cupula running in the same plane as its respective ridge. Four of these run in the transverse plane and five vertically or longitudinally.[21] The motion of the endolymph deflects cupulae in the direction of movement and thereby excites hair cells polarized in that direction.[21] This then allows for a detection of the yaws, pitches, and rolls of the statocyst, and inferredly the body, based on the detection of the directed movement of the endolymph. The flow of the endolymph is controlled and routed along the statocyst by structures, such as the anticristae, that may help to either direct or slow the flow, and thereby likely aid in the coherency of the perceived motion.[13]

The equivalent human system, the semicircular canals, accomplishes sensation of angular acceleration with a shockingly similar system. Three semicircular canals filled with endolymph run in varying planes to create a hypothetical matrix. Movement of their endolymph subsequently pushes a cupula that stimulates hair cells polarized in the direction of the cupula's movement.[22]

In respect to the perception of gravity, statocysts utilize a system marginally similar to the saccule and utricle of humans, the otolith of teleost fish, and the statoliths of crustaceans, some cnidarians, and some gastropods.[6,9,11,20] Both human and octopus systems use a sensory epithelium called the macula (Fig. 1). In humans, the hair cells of the macula are embedded in an otolithic membrane that is able to manipulate their bend in response to the movement of crystals embedded on the other side of this membrane in response to gravity or inertia. The movement of this membrane directly deforms sensory hair cells within the macula. Conversely, in animals like crustaceans and fish, a sac-contained mass of mineralizations, or statoliths, is utilized to excite these hairs relevant to gravity and at times linear acceleration. In the case of octopodes, this mass of mineralizations is called a statolith, rather than an otoconia in humans [13,20], and can range from a singular "smooth" stone to an unorganized collection of crystals.[15] The statolith is then connected to the macula by long loose fibrils originating from the macula.[15] If it were not connected by these fibrils it would be free to float loosely throughout the endolymph of the statocyst.[15] The macula itself is backed by a thick pad of cartilage and part of the anterior medial wall of the statocyst itself. [13] As the orientation of the vestibular system changes, so does the position of the statolith with gravity. As it shifts across the face of the macula, like a pen on paper or "like a hand on a clock"[14], the shearing force it exerts on hair cells and in what direction these hair cells are excited informs the direction of gravity, or at times inertia.[15] In the case of linear acceleration, hair cells may be excited by the relative "push" or "pull" of the statolith or simply from a rush of endolymph.

Sensory Integration and Sensory Adjustment

Sensory data from the vestibular system is integrated, in many animal systems, with information gathered from the other sensory apparati, most importantly visual and somatosensory information. In turn, this information is used by the brain to better understand how to delineate the sensation of the body's movements from that of the world's or environment's and, further, how to account for or adjust to it, behaviorally and sensorially.

In humans and other vertebrates, vestibular data is regularly used to adjust the movement or behavior of the eyes through the vestibulo-ocular tract[6], a neurological path similar to that of the cephalopod system.[23] This results in the vestibulo-ocular reflex. During any movement that alters the position of the head, the eyes will reflexively move at an, ideally, equal pace in the opposite direction to keep the target in focus.[24,25] In humans, this reflex does not rely on incoming visual data and responds to vestibular activation and inhibition of the rectus muscles of the eyes in response to processed signals from the semicircular canals. Because this reflex is operated by both laterally and vertically attached muscles and in relation to roll, pitch, and yawing sensations, it may occur in both horizontal and vertical components. Further, this vestibular data is thought to simultaneously route through the vestibulo-collic and vestibulo-spinal tracts from the brainstem in order to coordinate the postural elements of the neck and spine in order to further stabilize and control the intake and processing of visual data.[6,26]

In situations when there is visual perception of full-field movement, or a scene is moving while the head remains still relative to that movement, an optokinetic nystagmus is triggered. This reflex keeps the eyes in slow, smooth pursuit of the moving aspects, thereby stabilizing them, while simultaneously resetting in a saccade to keep the eyes centered on what is ahead of the head.[27] In contrast to the vestibulo-ocular reflex, the optokinetic nystagmus can be solely visually activated.

These ocular reflexes have also been demonstrated in a large variety of organisms, including cephalopods[28], providing evidence of the integration of their visual and vestibular sensory information. Budelmann and Young, through anatomical and neurological surveys of *O. vulgaris,* also verified that the oculomotor system was connected with the octopus' statocyst through both direct nerve tracts, and indirect paths through sensory integration centers believed to combine optic, statocyst, and other motion-related information.[29] Both of these reflexes may be reliable ways of behaviourally verifying perceived motion, whether of self or other, and may inform the mechanics of observed organisms' vestibular and visual sensory perception, or more succinctly their sense of motion and orientation.

Integrated with this visual and vestibular information, is tactile and proprioceptive sensory information. In the human body, mechanoreceptors in joints help to organize the orientation of the body's moveable limbs and joints into a coherent picture[30]. Similarly, cues such as muscle activation and strain help to update a "skeletal" layout of the body's position in space. This information is integrated with visual and vestibular sensation in the spinal cord and brain to adjust and control posture, balance, and movement[31].

How all of this information is integrated and each sense's particular contribution is still not well understood, even in the human body. The relationship of these senses and the result of their synthesis is further complicated by the fact that each of their reactions to one of the sensory apparatuses may influence the intake of sensory data by another. An important example of this relationship exists in cephalopods, where removal of their statocyst results in an inability to keep their eye gyroscopically level[32]; meaning that the information being collected by the statocysts directly affects how the eyes can and do collect visual data. Disentangling how each of these sensory modalities contributes to an organism's internal model and sensorial view of the world may help us understand not only more about our own sensory abilities, but more about the nature of these often evolutionarily similar strategies themselves.

Disorientation

While the synthesis of vestibular, visual, and other sensory data helps to inform the internal model of the relationship between the body and the world, it also allows for sensory dissonance: situations where incoming data from different sensory systems is dissonant or delivers conflicting information about the state of the body, world, or how they relate. Within higher processing, dissonant information can cause problems with gaze stabilization and postural control among other issues[33]. Most commonly, however, these miscommunications result in perceptual disorders that, in humans, we call dizziness or vertigo. The distinction between vertigo and dizziness can at times be unclear, with the terms often being used interchangeably and their distinction being one that is mainly subjective and experiential. Generally vertigo is used in clinical settings or in cases of pathological symptoms, and refers to a specific type of dizziness.[33,34] Dizziness, is a sensation of disturbed or impaired spatial orientation[34]; vertigo, more specifically, refers to a type of dizziness either caused by or causing a false perception of one's environment moving or spinning.[33,34]

Vertigo is a specific example of disorientation caused by a mismatch between an internal perception of motion and a stationary external environment. In studying this phenomenon in non-human animals we cannot measure subjective experience, and thus cannot definitively know whether the type of disorientation experienced is one of perceived false motion. However, because disorientation implicitly elicits forms of impaired motor coordination, this gives us a basis to research analogous forms of disorientation stemming from similar causes in animals.

In humans, disorientation, can be triggered peripherally (in the sensory organs themselves) or centrally (in their integration in the higher order processing of the brain).[5] In cases of non-pathological human dizziness, specifically transient vertigo, the causes are generally at the level of integration. For example, in the case of dizziness caused by spinning, when the endolymph of the semicircular canals continues in inertial movement after external movement has concluded it thus continues to elicit a vestibulo-ocular reflex. As the semicircular canals send signals of motion, the eyes are forced to overcorrect for a now still environment, these two senses send incompatible signals that further clash with somatosensory sensations; all of this resulting in disorientation.

It is yet unknown whether cephalopods experience disorientation due to environmental conditions. While disorientation may not have a consistent metric by which it can be studied across species, by nature, disorientation would elicit discoordination of purposeful motion.[35] Thus we may be able to study disorientation by quantifying the success of seemingly directed actions.

Prior Research and Understanding

Understanding of both human and non-human vestibular systems is still fairly poor. Even in some human vestibular pathologies we lack clear understanding not only of cause, but even how to distinguish symptoms from causes. Often, the symptoms of vestibular dysfunction may overlap in general feelings of imbalance and unsteadiness, which can make it hard to translate the subjective experience of the symptoms into something standardized and diagnosable. For example, Cervicogenic Dizziness, a disorder which causes neck pain, headaches, and dizziness, has a pool of research just to differentiate and validate the existence of the condition as recently as 2017.[36,37] Further, its diagnosis consists, instead of with direct evidence of the condition, with an elimination method of the presence of other vestibular disorders.[36] Even the most common vestibular disorder, Benign Paroxysmal Positional Vertigo, still holds mysteries as to its causes and cures.[38–40]

However, research into these disorders and their morphological causes may provide valuable models for sorting out the contributions and integration of the components of our vestibular system. In a sort of multi-front investigation, then, this is true of our understanding of all vestibular systems: to learn about their function, learning why they dysfunction is a powerful model.

The need for research into vestibular systems and vestibular disorders is not only important for our own medical knowledge, but for mitigation of increasing anthropomorphic effects on the planet. Marine animals' vestibular systems may be affected by the significant human created noise in their habitat, most of which is caused by boats and other water vessels.[41,42] Additionally, the effects of ocean acidification have been shown to alter the development of cephalopod statoliths.[43,44] In order to prevent harm from man-made habitat changes to marine organisms we will first need to understand what is affecting them and how.

One research niche has spawned from the captive rearing of "spinner" cephalopods.[45] Investigation into this strange vestibular dysfunction, which has been observed in octopus, cuttlefish, and squid, is marked by an inability to orient in the water column[45] leading to spinning and uncoordinated motion. This condition has been documented only in captivity, but has been hypothesized to have several possible causes, such as a lack of strontium or prolonged elevated pCO_2 during statolith formation [45–47]. However, the overall condition and its anatomical basis is still little understood. Still, research on this condition’s cause has led to greater peripheral understanding of not only statolith formation, but the husbandry needs required when rearing or caring for coleoid cephalopods in captivity.[43,46,48]

In animals, prior research on the function of the vestibular system has been largely invasive.[32,49–52] Invasive procedures, whether it be behavioral evaluation after structural lesioning, applying implants, or simply euthanization in order to investigate internal morphology [32,49–52], can greatly stress the animal and decrease their general well-being. At times, because of the nature of the investigation and limitations of instrumentation, it may be necessary to carry out invasive procedures. However, for the well-being of the organisms studied it is preferable to seek non-invasive methodologies of investigation.

In this study, I attempted to develop a non-invasive methodology that quantifies “healthy” vestibular function through behavior. The goal of this research was to induce transient disorientation through various isolated and natural simulations of spinning in order to differentiate how important the separate sensory perceptions of movement are to an overall orientation. There have been little to no studies that have tried to induce dizziness in study animals through spinning, so this methodology has yet to be evaluated.

Hypotheses

I expect octopuses to display indicators of transient disorientation in response to being spun during treatment trials. This will manifest as a decrease in: their catch rate of crabs, the duration of crab retrieval, and size-corrected speed. Conversely, I expect transient disorientation to cause an increase in the magnitude of directness, bearing-change, bearing-heading difference, and change in bearing-heading difference in octopuses that have been spun compared to octopuses that have not been spun. Similarly, I predict that octopuses that have been spun will be less likely to perform jetting movements, as these are more highly coordinated motions.

METHODS

Octopus Collection

The nineteen ruby octopuses (*Octopus rubescens*) used in this study were collected in July 2024 on three collection SCUBA dives at the bottle field at Driftwood Park, Whidbey Island, Island County, Washington and brought to the surface in sealed plastic bags. At the surface, octopuses were transferred from their bottle into a red, nalgene water bottle, which was then stored in a cooler of fresh seawater oxygenated with several bubblers. Then the octopuses, secured in their nalgenes in the cooler, were transported to Rosario Beach Marine Lab (RBML) in Anacortes, Washington.

On arrival to RBML, octopuses were weighed, sexed, and assigned numbers. Then they were transferred to individual 3-liter holding tanks with flowing seawater, in which they were kept the remainder of their time at RBML. Their respective nalgene water bottles were also kept in the tank as makeshift dens. Once data collection was over, the octopuses were released at their original collection location.

Octopus Husbandry

The octopuses were fed one purple shore crab (*Hemigrapsus nudus)* per feeding session. Before the beginning of experimental trials (7/6 to 7/17), feeding sessions occurred daily around 4 PM. Once experimental data collection started, octopuses were fed during the trials every other day, occurring from 4 PM – 9PM. If the octopus did not catch the crab during the trials, the crab was dropped into the octopus' holding tank when the octopus was returned.

In order to carry out trials, octopuses had to be transferred from their individual holding tank to the spinning tank. Octopuses were retrieved from their tank by hand, generally by scooping them during a jetting maneuver and lifting them into a large glass jar used to transport them. Octopuses were transported back to their individual tanks by similar methods.

Spinning Tank

To test the effect of spinning on octopus orientation, an experimental, spinning tank was designed and built by Walla Walla University Technical Support Systems. This spinning tank, composed of two drums, a smaller nested within a larger, allowed for the separate or simultaneous spinning of the visual reference and the drum which contained the octopus during trials. The larger, outer drum is open at both top and bottom and sits like a tube around the inner drum. The inside of the outer drum is lined with black and white duct tape to create regular, consistently sized, alternatingly black and white, vertical bars. This outer drum has a diameter of 61 cm and a height of 45.7 cm. The inner drum is placed at the center of the outer drum, with 8 cm between their respective walls. The inner drum is composed of clear acrylic and is a continuous hollow cylinder. This inner drum acts as the containing tank for octopuses during trials. The top of the inner drum is removable and covered with red laminate in order to occlude upward visibility of the octopus out of the drum, as octopuses are observationally understood to not be able to see through red materials (Onthank, pers comm). The space inside the inner drum has a diameter of 44.50 cm and a height of 30.48 cm. (Fig. 2) During use the inner drum contained around 6–7 gallons of water, a majority of which was changed between each trial.

Both of these drums sit on top of a cart 81.28 cm off the ground. The outer tank is set upon a track that is connected to spin with a stepper motor, while the inner tank is affixed to a circular platform that rotates with its own stepper motor. Both of these motors are then hooked up to a power source. Each drum can be controlled individually, spun at varying speeds, and spun simultaneously with the other.

Video Collection

Videos were collected with a Google Pixel 6, resulting in 1920x1080p videos. The phone was rigged with a duct tape sleeve and a GoPro Jaws Flex Clamp Mount along the outside of the tank so that the phone's camera was roughly ¾ of the way to the center of the tank. The camera was attached to the outer drum, as this drum was not spun during the study. Before experimental trials began, videos of the octopuses catching crabs in the experimental tank were recorded. During the experiment, for each individual trial, the entire process from the introduction of the octopus to the tank to its removal was recorded. After each batch of trials, videos were sorted and transferred to a harddrive in MP4 format.

Non-experimental videos - In order to train the artificial neural networks used to label the crab and octopus positions in the experimental trial videos, videos were taken outside of the experimental period. These videos consisted of octopuses feeding in the spinning tank under the same lighting and camera conditions as were used in the rest of the experiment. These videos, however, did not include any treatment. The octopus was transferred to the tank, given a minute to acclimate with the red top of the inner tank placed on, and then fed with a single purple shore crab.

Trials

During trials the spinning tank was filled with approximately 5 gallons of water, measured using the same line on a 5 gallon bucket each time. Between every trial the tank would be emptied and refilled with fresh seawater from a seawater flow system within the marine lab. Experimental trials started on July 24th, so the last group of octopuses collected had received about a six day acclimation period, or they had had 6 more days of exposure to the general environment and process of being handled.

<u>Control</u>- Once the octopus was transported into the tank, it was given one minute to acclimate to the tank under the red screen. After a minute, the red screen would be lifted off and a single purple shore crab (*Hemigraspus nudus*) was dropped into the opposite side of the tank or as far from the octopus as possible. The experimenter would move out of view and the octopus was then given one minute to collect the crab before the end of the trial. Throughout this thesis “trial types” will be used to refer to the two trial types within the experiment: control and treatment.

<u>Treatment</u>- The treatment trials mirrored the controls, however between acclimation and the removal of the red screen, the animals were spun for one minute. The spinning trial would begin after the screen had been in place for one minute. For the experiment's duration only the inner drum was spun during treatment trials. The inner drum was spun clockwise and at a target of 60 deg/s, but could range from 58-63 deg/s. After one minute the machine would be abruptly stopped, the red screen removed, and a

purple shore crab dropped on the opposite side of the tank or as far from the octopus as possible. The experimenter would move out of view and the octopus was then given one minute to collect the crab before the end of the trial.

In both the control and treatment trials, after the octopus was given one minute to retrieve the crab. The octopus would then be gathered and transferred back to their holding tank.

DeepLabCut

Artificial neural networks are an increasingly useful tool in academic research, allowing for the analysis of much larger and time-intensive data sets with a relative decrease in manual workload. While the strategy by which artificial neural networks are trained varies, their general use is to identify non-linear functions within a data-set. In order to accomplish this, the neural network must first be trained on an example set of data, from which it can iteratively correct an internal model to most closely align to the provided data. Analysis of the model's efficacy is then completed on a test set of data, where the values being estimated are known. What the model is actively analyzing may change how it behaves. For example, as with this project, if the data set is composed of images, the artificial neural network might attempt to build a model that best fits the the pattern of luminance values in each pixel of the image in order to make estimations about the images content.

DeepLabCut is a suite of tools that allow for the training of neural networks on markerless animal poses. DeepLabCut allows for the training and use of an artificial neural network to place pose estimation markers on specified locations of an animal in collected videos. This process requires first data collection of the videos to be analyzed, the manual creation of a training data set, evaluation of the neural networks performance, and lastly its use to analyze and mark videos.

In order to prevent skews in training that might occur with use of the videos that are to be analyzed being part of the training set, non-experimental trial videos were recorded prior to the beginning of the trial period and subsequently used as the training set. This meant that the videos that would be used for training were all recorded before the first experimental trial was performed. Further explanation of the data collection of videos is covered in more detail later in the methods.

The manual creation of a training data set for DeepLabCut is accomplished with built in functions to extract and label frames from the desired videos. These extracted frames are then manually labeled at points relevant to the features of the animal one wants to track, such that every frame is labeled with one point for each respective label (e.g. a marker for the label “Right Eye” exists once in every frame where this feature is visible). DeepLabCut also allows for the creation of a “skeleton” or map of connections between the placement of labeled points that ensures the model keeps a consistent shape of how these points are arranged in the form of an animal, if one exists. Though this feature is not necessary for the use of DeepLabCut. During training, a portion of the labelled frames from the training data set are set aside to iteratively test how well the model is able to estimate marker positions in data it hasn’t been trained on.

While the goal for a model is to ultimately achieve similar or more accurate standards of labelling than how it might be gathered, modeled, or collected manually, how we approximate this ability varies. While training, two types of “loss” give estimations of the efficacy of the model developed by the neural network. Loss refers to the difference between the estimation of a value by a neural network and the real value. "Training loss" describes the accuracy of the model's current estimations against the data it is being trained upon. "Validation loss" describes the accuracy of the model's current estimations against the known values for the test set of data. Both of these losses, in most contexts, are calculated using the mean square error, or the squared difference between the model's estimation and the known value. While training loss can help to track progress of the model's training, validation loss describes how well the model can generalize to data that it has not been trained on. While the raw values of these metrics don't provide a sure-fire interpretation of the behavior of the model, ideally a model should be trained until the change in both of these losses has largely plateaued. In other words, additional training does not result in better performance of the model. For a successfully trained model, both of these values should decrease throughout training and ideally have fairly low values. However, the magnitude of these values is also context dependent as to what the artificial neural network is analyzing.

It is possible for these metrics to behave out of sync. If training loss is decreasing, but validation loss is increasing, this points to the model overfitting the training data. This means its model is attuned to intricacies of the training set that are not generalizable upon other data, such as the test set. Or, if training loss isn't decreasing, while validation loss continues to decrease this may point to an underfitting or a poorly trained model. This means that while the model is able to generalize onto the test set, its model may be coincidentally "fit" to the test set and be giving a misleading impression of accuracy and generalizability, while still poorly predicting the patterns of the training set. In order to tackle these issues, should they arise, increasing the variability and size of your data set can help with either under or overfitting, and preventing excessive training can prevent overfitting.

DeepLabCut's model training has been shown to require as few as 50 manually labelled frames to provide a reliably accurate neural network.[53] The minimum amount of training data needed to train a neural network is not necessarily calculable, however there are some factors that can greatly affect the minimum amount of training data required. In the case of DeepLabCut, video quality, such as its pixel resolution, the size of the subject in relation to the size of the frame, and the lighting of the video may all require more, and more varied, training data when the resolution is low, the subject is relatively smaller in-frame, or the lighting produces low contrast between the subject(s) and the background. DeepLabCut has also been commonly used on animals that have rigid body structures that allow models to more reliably predict some locations based upon the layout of the more visible and easily identifiable elements per frame. While octopuses' eyes are set in place in relation to each other, the lack of a rigid skeleton and the stunning flexibility of their mantle and arms to stretch and contract, as well as to orient in myriad ways, complicates the ability of a neural network to construct accurate patterns for assessing orientations in each image. While only the left and right eye needed to be labelled and tended to be visually consistent and conspicuous in the images, the ability of the neural network to assign left and right would rely on the patterns that could be attributed to the rest of the image. In order to give a stable structure to left and right, a third point was labelled on the center-top of the octopus' mantle. In DeepLabCut a node-skeleton was then assigned connecting the left and right eye to each other, and each to the mantle-point making a triangle.

Our octopus-labelling model trained on 2071 frames, compared to 2237 frames in a similar application of DeepLabCut in Weeterman (2022). Weeterman's (2022) model achieved a root mean square error of 2.7 mm. In Weeterman (2022), a neural network was trained to label both eyes and the mantle tip of octopuses. The frames being used contained more than one octopus in a flume with nearly 15 times the area of this project's spinning tank.

DeepLabCut provides several types of "blank", pre-built neural networks that can be trained on the previously created training sets of labeled frames. The number of epochs (generations of iterations that the model runs through the training data set), batch size (number of iterations per generation), and saved snapshots (saved versions of the neural network at moments during the training) can all be adjusted before training. As the artificial neural network is trained, DeepLabCut calculates training and validation loss and continually updates the current best model to be saved, alongside however many other snapshots you specify to save during training.

Once an artificial neural network has been trained, its accuracy can be evaluated with the same data set you've been calculating the validation loss with. This evaluation calculates the root mean square error (R.M.S.E.) for the model when applied to both the training and test set. DeepLabCut models also calculate the likelihood that each estimated marker is accurate. Using these likelihoods, the R.M.S.E. for both these sets, only including markers placed with a high enough likelihood, is also calculated. Lastly, the evaluation calculates the mean average precision (mAP) and the mean average recall (mAR). These two metrics refer to how accurate the model is proportionally, rather than in distance from the target. Mean average precision, refers to the proportion of correct positive predictions, to the total number of predictions. Mean average recall refers to the percentage of correct positive predictions compared to the number of existing positives.[54] Worded differently, it is like the number of eggs found versus the number of eggs hidden in an easter egg hunt. A well-trained model should attempt to maximize both of these values, as ideally they would both be 100%.

The crab-labelling neural network was able to label the crabs' positions accurately and consistently and so was used to label all experimental trials. The octopus-labelling neural network labelled far more inconsistently and inaccurately and would have required extensive data quality checks to verify the accuracy of all the labeled positions. Because of this, all of the labelled positions for octopuses were manually labelled.

Data Analysis

Video Processing

The experimental trial videos were also sorted based on whether the octopus had retrieved the crab in the allotted time or not. Only experimental trial videos in which octopuses had retrieved crabs were used in the data analysis of their movement. However, an analysis was performed on the success of an octopus in retrieving a crab in relation to trial type and date.

Experimental trial videos in which the crab was successfully retrieved were cropped to only include the time from when the crab was first dropped into the water, until it had been retrieved. To standardize the beginning of the clips, the start of the clip was marked as the moment the crab hit the surface of the water. The end of the clip was then marked by a consistent behavior exhibited by the octopuses I have labeled “contraction”. Consistently, octopuses would pounce on their prey by spreading their arms wide out around them, which would then be followed, upon successful catch of the crab, by their arms being pulled centrally inward as they balled up around the crab. The beginning of this “contraction” motion, when their arms first started moving in, was then used as the end point of the clip.

Next, from these clips, a python script, using the “CV2” package[60] (version 4.10.0.84), was used to convert the clips into folders of the corresponding frames, resulting in 60 frames per second for each clip. For the training of neural networks, all non-experimental videos were also converted to frames. These batches of frames were used in both manual labelling and labelling by the trained DeepLabCut neural networks.

For manual labelling, the set of frames for each trial was filtered for every tenth frame, such that the resulting set contained frames at a rate of 6 per second.

For labeling by DeepLabCut, the frames were processed using the PIL package (version 10.2.0) in Python. The contrast of the frames was converted to a factor of 1.4, they were converted to greyscale, and had their luminance inverted. This was done to create frames that were easier to more consistently label, as well as to possibly reduce noise in the image for analysis with DeepLabCut. The processed frames of the non-experimental videos were used to train the DeepLabCut neural network that would identify the position of the crab in each frame. When using that neural network to identify the position of the crab, the processed frames were used.

Frame Labeling

Using the extracted folders of frames, the positions of octopuses' left eye, right eye, and center of mantle were labeled manually. These labels were stored as (x,y) vectors in pixel units.

The position of the crab in the experimental trials was labelled using another neural network trained to label the center of the crab's carapace. This neural network was trained with Deep LabCut using 387 frames. The neural network was trained for 500 epochs on batch size 1. This neural network labeled every processed frame. For data analysis, these DeepLabCut labelled frames were then pared down to the lower frame rate (6 frames per second) when compared to the manual labelling.

In order to assure labelling consistency all manual labeling was done by me, and a consistent labeling technique was devised. Labelling of the mantle center was the least exact or rigorous as this label only functioned as a landmark which would serve as a landmark or determiner of left and right for the neural network. If the mantle wasn't labelled, it may have led to far more incorrect labellings of left and right, as the determination between the two would be much more visually ambiguous. In order to remain consistent with labeling the eyes, they were labeled one at a time for all frames of a video before moving to the next eye. The eye was labeled at the highest, center point of the eye (Fig. 3). Cues such as the distinct eye-bars and eye rings that *O. rubescens* displays also helped to inform the placement of these points.

Mantle Indices

Mantle indices were calculated by taking length and width measurements of the mantle of each octopus. In order to account for the flexibility of the mantle and how often it changed length and width throughout the videos, ten frames were chosen for each octopus that contained a variety of positions and states of movement. From these ten frames, length and width were calculated in pixels using ImageJ. Length was calculated from the center of the eyebar to the tip of the mantle. Width was calculated as the width at the widest section of the middle of the mantle; width being parallel to the eyebar. The length and width were then added together and divided by two to calculate the mantle index. This index was then used to correct calculated speeds of the octopuses, to handle the possible size-based variance of speed.

Quantifying Octopus Movement: Full Video Analysis

Using the positions from every frame in the video, a variety of metrics tracking the nature of the octopuses' movements were calculated. Some of these metrics were then used in direct analysis of the full video, while others were then analyzed in subsets of discrete movement types.

First, the following metrics were calculated using labelled positions from every frame of each video: duration, size-corrected speed, directness, direction of movement, the difference between bearing and movement direction, and the change in the difference between bearing and heading.

Duration was measured, as a disoriented organism might be likely to take more time to complete the same actions and may spend time attempting to orient or wait through disorientation. Duration was determined by multiplying the total number of frames by the number of seconds per frame. In this case, the collections of frames being analyzed were a subset made up of one out of every ten frames in a 60 frames per second video. This means every 6 frames accounts for a second, or that each frame included in the data set represents 0.167 seconds.

In order to calculate metrics relating to position of the octopus, a standard position on the octopus was defined as the center point between the points already marked on the left and right eye (see Frame Labelling). This provided a single reference point relatively close to the statocysts from which to calculate other metrics from. Using the Euclidean distance formula, distance traveled throughout the video was calculated with each frame and the frame before it. These frame-by-frame distances were used to calculate speed, which was expressed as pixels per frame. In order to standardize this speed in relation to the size of the octopus, the speeds were divided by the previously calculated mantle indices per individual.

Heading is the direction of movement of the octopus between each frame and the next. It was calculated using the arctangent of the vector from the first frame to the next. Bearing is the direction the anterior side of the octopus' eyes pointed. Its value is roughly 180° away from the mantle. Bearing was calculated as the angle positively perpendicular (clockwise) to the vector from the right eye to the left. The original eye-to-eye vector was also calculated using arctangent.

Measuring changes in bearing captures how much an animal is turning the tracked point. A high average change of bearing would indicate that the animal is either consistently turning or moving erratically. Increased movement can be energy costly for organisms, and in many organisms oriented movement includes relatively low and/or consistent bearing changes.[55] Therefore, I calculated bearing changes for each frame by subtracting the current frames bearing value from the value of the following frame.

The relationship between bearing and heading may change when an organism is disoriented. For example, while humans generally have the ability to move without respect to the heading of our eyes, our movements generally fall within a smaller range around our general heading. While the difference between bearing and heading consistently changes, this general range of difference between the two directions can serve as a stable measure of oriented movement, especially in relation to movements directed towards a goal. When disoriented, it is possible that this range of bearing and heading differences might increase. Bearing-heading difference per frame was found by subtracting the heading from the bearing. The change in bearing-heading difference was found, then, by subtracting the bearing-heading difference of the current frame from the next frame's bearing-heading difference.

Size-corrected speed, bearing change per frame, and the bearing-heading difference were averaged across the entire video to produce singular averages for each recording. Duration, average size-corrected speed, average bearing change per frame, and average bearing-heading difference were all then used in statistical analysis of the full videos.

Quantifying Octopus Movement: Path Analysis

From the original clips, batches of frames were subsetted from each video in order to analyze directed movement towards the crab. A "directed movement" was defined as a movement of the center point of the octopus in the direction of the crab directly preceding the retrieval of the crab. These movements were marked as starting when the octopus first began moving in the direction of the crab in what would become an obvious attempt to catch it. The movement was marked as ending when the crab could no longer be seen due to the octopus' web, unless the movement carried the octopus past the crab and only caused the view of the crab to be temporarily obstructed. Directed movements were categorized as “jets” or “crawls”. A “jet” was a directed movement that consisted of the use of the octopus’ siphon for propulsion. This movement was characterized by the visible output of water from the siphon (splashes and currents at the surface in line, but opposite to the movement of the animal), higher speeds, and a loose, trailing appearance to the movement of the arms (Fig. 4). The octopus also generally did not touch the bottom of the tank from the beginning of this movement to the end. A “crawl” was any other directed movement.

In order to analyze these motions, the metrics calculated from the full set of frames were subsetted from beginning to end of each directed movement. In the case of average size corrected speed, average bearing change, average bearing-heading difference, and average change in bearing-heading difference: all of these metrics were simply pulled from the calculations done for the full video and then averaged for each specific subset of frames.

Directness describes how directly the octopus was moving towards the crab at any moment. Directness was calculated as the difference in angle between the octopus's heading, or movement direction, and the angle from the octopus’ center position to the crab in each frame (Fig. 5). Directness difference was calculated and then averaged for each movement of both types.

Statistics

In order to account for the random effect of variations in individual octopus behavior and variation possibly caused by when in the study the trial took place, the data was modeled using linear mixed effects models. Linear mixed effects models were constructed using the nlme package[58] in R (version 3.1-164). Type III ANOVA tests were then used to determine p-value.

For non-significant results, a power analysis was used to determine the minimum mean difference between treatments needed to detect a significant difference given the observed variance, an alpha of 0.05, and a statistical power of 80%. To do this the observed mean difference between treatments was increased incrementally by adding 10% of the original mean difference to each of the treatment data points. Then, the LME model was refitted and power calculated using the Pwr() function from the nlmeU package[59] (version 0.70-9). The smallest mean difference at which statistical power reached 80% was recorded as the minimum detectable mean difference and compared to the observed value.

Because of a lack of data for jetting movements occurring after spinning trials, this subset of data was not analyzed. Therefore, the analysis only includes directed crawling movements.

Data Availability

All raw and derived data and the code used to find is available on Zenodo at: https://doi.org/10.5281/zenodo.17088608

RESULTS

Full Video Analysis

The positions of octopuses during experimental and control trials were used to measure various aspects of their movement. The "Full Video" data-set, included all frames during a trial, from the introduction of a crab to its retrieval. The values found for each metric were averaged across all frames per video and these averages were then used in overall analysis of differences between trial types over time. Trial type was found to have a significant effect on average bearing-heading difference (Table 4). Spinning trials were observed to have an average bearing-heading difference 17.84° less than in control trials. (Fig 6) No significant relationships were found between trial type and any of the other metrics calculated for the full video data set. (Table 4) The date on which a trial occurred was also found to have no significant relationship with any of these measurements. (Table 4)

Path Analysis

In order to analyze directed movements, ranges of frames were subsetted from the beginning to end of each directed movement. These frame ranges were then used to pull the respective values for each metric to be averaged only across that subset of frames. These single averages, per metric, per directed movement, were then used to analyze differences between trial type and movement type. For crawling movements, a significant interaction between trial type and date was found for bearing change. (Table 5) Because of a lack of data for jetting movements occurring after spinning trials, jetting movements were not analyzed for significant effects of trial type or date.

DISCUSSION

DeepLabCut

I attempted to train two separate neural networks for this project. The first neural network, trained to label the center of a crab's carapace, attained fairly reliable evaluation results after training. With a test mean average precision of 81.00%, it showed a high accuracy for labelling crabs correctly. Further, it had a mean average recall of 85.96%, meaning it was sufficiently accurate at finding a crab in images when one was visible. When reviewing the model's labelled positions for quality, its lowest accuracy or mis-labels tended to occur once the crab was blocked by or being grabbed by the octopus.

Though a neural network was trained to label the eyes and mantle of the octopuses, its performance was less reliable and therefore it was not used to analyze the data. With some quality assurance and possibly further training, however, it may provide higher resolution and more reliable data. While my training data set of 2071 frames was around 200 frames smaller than in a comparable application of DeepLabCut by Weeterman (2022), other factors more likely led to the lower reliability of my model. Surface glare on the water and a general low contrast of the trial footage both may have contributed to the neural network's difficulty in producing an accurate model.

Interpretation of results

The majority of octopuses, regardless of trial type, were able to catch the crab provided in their trial with no significant difference being found between trial types (Table 1). An octopus' "success" or "failure" in catching the crab could be explained by many different factors. The time allotted to catch the crab, the appetite or temperament of an octopus, and the crabs' own movement patterns could affect how often octopuses were able to catch the crab. The time allotted to catch the crab, if too short, could artificially increase the amount of “failures”, even for octopuses that are not disoriented. In providing a full minute to catch the crab, this is unlikely to have been a limiting factor. The average duration of full videos for spinning trials was also only 7.46 seconds (Table 5), which suggests that one minute might have actually been too large of a window to give the octopuses. Instead of limiting the number of “successes” with too short of a catch window, I may have actually allowed what would have been “failures” to catch the crab to become “successes” in this larger window.

If octopuses did experience some amount of disorientation, it’s also possible that the allotted time to retrieve the crab allowed them to reorient after being spun before attempting to catch the crab. This “reorientation”, were it occurring, would have likely resulted in an increase in full video duration after being spun, which was not observed (Table 4; Fig. 6). If the recovery from some experienced disorientation was quick enough to have allowed for “reorientation” and catching of a crab in under eight seconds, the disorientation being experienced may be too brief to detect.

Trial type was shown to have an effect on how octopuses chose to move towards their prey. After being spun, octopuses completed proportionally less jetting movements compared to crawling movements, with 52.5% of directed movements being jets in the controls compared to only 12.5% after being spun (Table 1; Table 3).

It is important to note, though there were significantly less jetting movements, the lack of data that prevented full analysis of jetting movement metrics, came from a lack of trials, rather than a legitimate proportional lack of jetting. Because of mechanical issues with the machine, spinning trials were cut short part way through the experimental trial.

Out of 32 recorded direct movements in spinning trials, 4 of these were jetting movements (Table 3). In future experiments, with greater amounts of spinning trials, it is likely that the amount of jetting movements, even reduced by trial type, would be sufficient for data analysis.

A decrease in jetting movements after being spun could be explained by the octopus being disoriented. Jetting movements are generally faster and likely require higher levels of coordination. If the octopus was disoriented, it would be less likely to jet, just as a disoriented person would be less likely to jump. This sharp decrease in the proportion of jetting movements after being spun suggests that the spinning trials had an effect on how octopuses chose to move, however this result cannot be definitively attributed to disorientation without other evidence that either supports this interpretation or rules out other explanations.

It is equally possible, based on this result alone, that the animals performed less jetting movements due to states of fear or stress caused by being placed in the tank and activation and movement of the tank. The motor of the spinning tank produced a vibration and whirring upon turning on and the spinning of the tank moved the octopus involuntarily. Both of these sensations could have alarmed octopuses and made them unsure of their environment. The decrease in jetting movements in spinning trials would then be explained by apprehension and a reluctance to move in the current environment, rather than disorientation. This interpretation may have been supported by lower average size-corrected speeds throughout the full video, as if stress prevented jetting movements, it would have likely lessened overall movement as well. Interestingly, however, no significant effect was found for trial type on the average size-corrected speed in the full video data set (Table 4; Fig. 7). This absence of a significant difference in the size-corrected speeds of full videos means that octopuses were not significantly limiting their movement, compared to controls, while still favoring crawling over jetting movements after being spun.

As with the results of the effect of spinning on size-corrected speed in the full videos, the size-corrected speeds of crawling movements were also found to not have been significantly affected. (Table 5; Fig. 15) This is contradictory to what I would expect if the octopuses had been disoriented by being spun. Again, it is possible that they simply waited to make a directed movement towards the crab until they were no longer disoriented, however this would have likely resulted in an overall slower size-corrected speed in the full video. This change in full-video duration was not observed. (Fig. 6) It's also possible that the level of disorientation was such that they could still crawl at a "natural" speed, similar to how if you are only a little dizzy you can still likely walk. Another possibility is that while crawling movements are comfortable enough to retain a "natural" speed while disoriented, as was observed, jetting movements would be on average slower after being spun because of a higher required coordination. An analysis of jetting movements would be needed to determine whether this is occurring or not. Understandably, the duration of crawling movements was also not significantly affected by trial type. (Table 5; Fig. 19) An unaffected duration and speed means that their crawling movements did not change considerably in average speed, duration, and, implicitly, distance.

A significant effect of spinning on bearing change was not found for the full videos (Table 4; Fig. 13), or for crawling movements. (Table 5; Fig. 11) One complication in the use of bearing change as a metric of disorientation is that as activity increases, bearing change also implicitly increases. For example, my bearing change when unmoving would be effectively zero. While moving, though, all of the minor movements of my head, as well as the implicit bearing change of turning in my environment, could naturally lead to a higher average bearing change. I would expect to see increased activity, or movement, in the control compared to spinning trials, and so would expect to see relatively decreased bearing changes in spinning trials. This, however, directly contradicts the increase in bearing change I would expect in the movement of a disoriented octopus due to an inability to orient. It is possible that we were unable to detect a significant increase in bearing change because these two opposing trends averaged together. According to a power analysis of the average bearing change in full videos, a mean difference of ~3.33° per frame would be necessary between trial types to detect a significant effect. (Table 6) The observed mean difference was much lower (0.333° per frame), suggesting that there is still a fairly wide range of change in behavior that may be detected in future experiments. (Fig. 8) Because analysis of directed movements only included movements, I expect that this feature of the metric was likely less impactful on the results of crawling movements. For directed movements, if there was an effect of trial type we would have likely found it. Using a post-hoc power analysis, it's predicted that a mean difference of up to ~26° per frame would be required to detect a significant difference at a power level of roughly 0.8. (Table 6) This highlights another possible issue with how bearing change is calculated. Differences between average bearing change do not account for the possibly random nature of whether an octopus' average bearing change is clockwise or counter clockwise, meaning that rather small bearing changes in opposite directions would act additively when calculated as a mean difference. This may be applicable if octopuses bearing changes in a consistent direction relevant to the direction they are spun, but without confirmation of this dynamic it may simply add uninterpretable noise. Even then, an average bearing change of 26° per frame might still be biologically unreasonable and simply not provide data relevant to disorientation, as this would require average bearing changes between trial types to be up to 156° per second apart. In order to detect more biologically relevant mean differences, future experiments would require higher N values, and therefore simply more trials. Disorientation due to spinning may have caused changes in bearing change relative to the direction of spinning, meaning that if spun clockwise, bearing change may shift more counter-clockwise to counteract the perceived spinning. This effect still would not explain such a drastic change, and it is unlikely to me that this change in bearing change could be consistently observed in spinning trials. It is instead more likely that changes in average bearing change due to disorientation would be relatively small and/or erratic. In future analyses and projects, it may be worthwhile to somehow compare time-moving or inactivity with other averaged calculations such as speed and bearing change, to determine whether average bearing change was influenced by a counter increase or decrease in activity. Similarly, testing for consistent changes in the direction of bearing change, to determine if the direction octopuses are being spun is affecting the direction of bearing change could help to determine whether this metric is applicable to the trends resulting from disorientation.

Though trial type and date were not found to have a significant effect on bearing change in crawling movements, a significant interaction between trial type and date was found. (Table 5) This interaction means that while spinning did not significantly change how much octopuses change the directional facing of their eyebar, how “wobbly” they are, the date of the trial had a significantly different effect on one trial type compared to the other. In this case, the date caused a steeper decrease in bearing change in spinning trials than in controls. (Fig. 10) This hints at some amount of acclimation to the trial itself, as the octopuses may have simply shown a higher bearing change because of increased stress or were possibly able to adapt to the feeling of the spin like one would adapt to riding a bike. This also seems fairly natural, as I would not expect octopuses to have to acclimate to catching a crab without being spun. To support claims of acclimation, I would again need to have longer term data for the spinning trials compared to the controls.

If octopuses did not experience disorientation, then it would likely be harder to detect any amount of acclimation to spinning. The general vibrations and motion of the machine could still affect the movement of octopuses when first exposed to it. This sort of acclimation would likely manifest in less movement, which would seemingly result in a lower average bearing change, a lower size-corrected speed, and likely a higher duration. Along with not seeing any significant differences in size-corrected speed or duration of full videos, the trend of the “acclimation” to spinning trials observed in the average bearing change of crawling movements starts with higher average bearing changes and decreases over time (Fig. 10). This likely rules out the chance that this decrease was due to stress, and may support the idea that the octopuses experienced some amount of disorientation.

If they do acclimate to the trial, the rate at which they do could affect the results. For example, an unacclimated octopus may be more startled and less food motivated at the beginning of the experimental period. Generally, as they would become comfortable and acclimate it is likely they would behave more exploratively, which could have implications for duration, average bearing change, and average size-corrected speed of full videos. Similarly, if the octopus begins to associate the trials with food, it's possible that their choice of movement type might change. Anecdotally, this is supported by the behavior of the animals throughout the experiment. In the first half of the experiment, octopuses became less easily startled and more easily handled. Most notably, several of the octopuses even began exhibiting behaviors that may point to an expectation of the food in relation to being transported and to being placed in the tank. For example, several of the octopuses began gnawing at the glass of the transport receptacle used to move them to the spinning tank; and many of the octopuses began attempting to pull my finger and or whole hand down into their web, at times with surprising strength and effort. Octopuses also began and continued to display an upside-down “searching” behavior when placed into the tank that consisted of their arms stretching out over the surface of the water, their beak facing the air, and their suckers palpating the surface of the water. While it is unclear what the goal of this behavior definitively was, it demonstrated a very active and vulnerable attitude from the octopuses, and supports that the octopuses were acclimated to the environment. Much of this behavior, especially the upside-down “searching” behavior, did not occur until around halfway into the month-long experiment, which suggests that there was some type of adjustment or association period for the octopuses. As the octopuses possibly became more acclimated, and specifically if they had been conditioned to associate food with their transport, the spinning tank, or some other element of the trials, this may have led to changes in their patterns of movement.

If bearing change measured how “wobbly” octopuses might have been, or how much their general direction was changing, directness measured how much their movement aligned with the direction of the crab during directed movements. No significant difference was found in crawling movements between trials. (Table 5) Analyses were not run for full videos, as directness of movements is irrelevant if the octopus is not trying to move towards the crab. While we cannot assuredly say that directed movements were performed directly to catch the crab, only movements that directly resulted in catching or interception of the path of the crab were recorded and it is unlikely that octopuses were not aware of the crab or not intending to catch it. This is well supported by the surprisingly low average time to catch crabs during spinning trials. Between spinning trials and controls there was a -4.872° difference in average directness in crawling movements (Fig. 14), meaning that the average difference between the direction of the crab and the direction of the octopus' movement was slightly larger in the spinning trials than in the controls. However no significant effect of trial type was found. (Table 5) While I would expect directness to decrease in crawling movements, it is likely that crawling movements contain much more noise than jetting movements relating to the general change in the direction of movement. While crawling may naturally contain many small changes and deviations in direction throughout the movement, jetting movements consist of a largely singular, directed thrust that decides the character of much of the rest of the movement. For this reason, the directness of jetting movements could contain much more relevant directness data, as it may estimate, how far off the octopus' jetting direction was from the location of the crab. Directness could, naturally, be impacted by the movement of the crab as well. To remedy this for jetting movements, a single angular difference could be measured between the direction of the octopus' jet and the direction of the crab from the octopus at the start of the jet. This same idea does not apply to crawling movements which can adjust themselves with the changing position of the crab.

Octopuses' average bearing-heading difference, or the difference between the "forward" facing of their eyes (the angle perpendicular to the vector from the left to right eye) compared to the direction of their movement, was found to be significantly smaller in spinning trials of the full video data set. (Table 4; Fig. 9) During spinning trials, the average bearing-heading difference of full videos was on average ~17.80° less than in controls. Looking at the average bearing-heading differences in each trial type: spinning trials had an average bearing-heading difference of 27.28° and controls an average of 45.12°. Both spun and control trials averaged positive bearing-heading differences, meaning that the bearing was, on average, counter-clockwise of the heading (as heading was subtracted from bearing). Another way to phrase this, is that the octopuses, on average, moved in a direction clockwise, of their bearing. Interestingly, in both trial types of crawling motions and controls of the full video data set, octopuses had an average bearing-heading difference of ~50° (Fig. 9, Fig. 16). Because the bearing was calculated to lie perpendicular to the general facing of the eyes, this ~50° difference means that octopuses were moving mainly in the direction of their right eye's field of view, which might suggest some amount of lateralization in eye use. Lateralization describes differential or preferential use of separate sections or hemispheres of an organism's brain to complete varying tasks. This differential use then affects how organisms use other elements of their body. For example which arm they choose to grab something with or which eye they choose to face prey with, if applicable. Lateralization can vary both on an individual level and population level. In *O. vulgaris,* lateralization of eye use has been observed during tasks that involved prey capture, but was not observed, in the same test group, when exploring a maze[56]. Specifically, it was found that octopuses showed preference for approaching prey items with one eye over the other, yet varied by individual on which eye was preferred. Similar preferences have been observed in common cuttlefish (*Sepia officinalis*)[57] and oval squid (*Sepioteuthis lessoniana*)[58]. The consistency of a clockwise heading in reference to bearing, may suggest that the octopuses in the trials were likely to align their movement with, rather than our calculated bearing, the direction their right eye was facing. If the bearing-heading difference was completely random, we would expect the overall average to hover around 0°, as this would be the general center of the random distribution. An average of ~50° demonstrates that octopuses were primarily leading their movement with their right eye, and moved in a direction anteriorly forward (away from the mantle).

This is a rather strikingly consistent result, especially as other species of cephalopods have generally shown individual variation in their lateralization, which if the case here, again would have likely averaged out to a bearing-heading difference of 0°. Because of octopuses' flexible body plans and ability to move well in nearly any direction, it would be more than possible that the bearing heading-difference would not be required to remain in defined range. Yet, though having large monocular fields of view, octopuses have laterally facing eyes, and the existence of a binocular field of view has not yet been found.[59] This thereby may be a limiting factor and explain some of the reason why, aside from lateralization, octopuses are moving in consistent directions with the “true facing” of their eyes (rather than my calculated bearing) to begin with.

The fact that no significant difference in bearing-heading difference was found when only analyzing crawling motions (Table 5; Fig. 16), may be due to strong lateralization during motions directed at prey capture (as seen in *O. vulgaris)*[56]. If the significant decrease in average bearing-heading difference in spun trials for full videos was due to disorientation, it would likely be due to a generally more random distribution of bearing-heading differences per octopus, rather than necessarily smaller bearing-heading differences. This was generally observed (Fig. 9).

Because full videos include motion that may not be directed at prey capture or a specific, isolated goal, bearing-heading difference may have been more affected by disorientation as the octopus may have not been as strictly preferring one eye over the other. Counter to this point however, the bearing-heading difference observed in full videos show stronger signs of a preference of movement toward the left eye (Fig. 10) than does the directed movement data (Fig. 17). The bearing-heading difference distribution of crawling movements shows little difference in the general distribution. However, compared to the full video bearing-heading difference, crawling movements show a more even distribution between about -100° and 100° (Fig. 17). Whereas, both of the full video datasets are distinctly skewed towards positive bearing-heading differences up to about 100° (Fig. 10).

For future projects, it will be important to thoroughly think through the implications of bearing-heading difference, and how to ensure biologically relevant data is being analyzed from this metric. Given my sample size and variance, a post-hoc power analysis estimates a mean difference between average bearing-heading differences of ~86° would be required to obtain a $p<0.05$. This would likely require the effect of spinning to essentially flip the direction of movement (in reference to the bearing) between control and spinning trials. While there may be some consistent effect from being spun clockwise, it is unlikely that this disorientation would lead to a change in heading that is this severe and consistent. Therefore, future work should aim to retest this hypothesis with a larger sample size.

While I expect that disorientation could change the bearing-heading difference because of animals being less able to coordinate their movements, I would not expect this change to be consistently at or above 90°. Even disoriented organisms usually would not move if their efforts to move in one direction resulted in movements directed nearly a right angle away. This may not be as important of a consideration for an octopuses' body plan and movement abilities, however I still feel changes would likely be less consistently severe, making it more likely that mean differences would result in much smaller ranges. Similarly, as I've noted, more random distributions of bearing-heading differences would likely result in an average closer to zero. This would make it hard, even if spinning trials did cause octopuses heading to switch to a nearly mirrored direction to ever have a large enough difference, as they approached zero. Care should also be taken when interpreting the results associated with bearing-heading difference. A negative mean difference does not implicitly mean that bearing and heading are coming closer together, as negative degree values only communicate that one direction is more counter-clockwise than the other. Because of this, it is important to combine the raw bearing-heading differences with differences between trials' bearing-heading differences in order to accurately construct a model of what is occurring. Further, the metric itself hides a lot of data. For example, the same average bearing-heading difference could result from a wide and evenly distributed range, a range heavily weighted at opposing ends, and a range tightly grouped around the found average. Bearing-heading difference also provides a static image of how the bearing and heading are related. While it's possible that the bearing-heading difference may be changed due to disorientation, it may not change in consistent ways and thus it may be hard to find differences in averages alone. Analysing the average spread of an octopus's bearing-heading difference, may help to carry more data from the raw values through to the averaged metric, such as eye preference and general ranges of coordination between bearing-heading difference.

The measure of the average change in bearing-heading difference provides a view of how much the octopus was changing the coordination of its bearing with its heading. Simply, it measures whether the difference between the bearing and heading was getting larger or smaller each frame. By nature, this metric should average out close to 0°, because it is unlikely that octopuses would change the coordination of their bearing and heading consistently in one direction, without at some point changing it back. A significantly positive or negative average change in bearing-heading difference, would essentially mean that their movement direction is actively rotating in relation to their bearing. Practically, this would imply that the animal was essentially walking in spirals. It would seem reasonable that octopuses' bearing-heading difference may naturally be more or less comfortable in a specific range. This is supported by the finding that on average octopuses' bearing-heading difference was consistently ~50° in both full video and crawling movement controls. If a range around a “comfortable” coordination of bearing and heading were to be maintained, it would require an equal amount of increases and decreases in the change of bearing-heading difference.

Maintenance of this range of coordination of an octopus’ bearing and heading, may also help to limit the “computational” load of sorting out changing sensory inputs. Vision plays a key role in octopus movement and the relative posture of the axis of the eyes and their orientation in relation to their surroundings has been found to be severely affected by removal of the statocysts.[32] Levy et al. (2017) found that octopuses keep a consistent posture roughly parallel to the ground while moving around an environment. They suggest that this stabilization of the posture of the eyebar, helps to simplify orienting and to reduce the degrees of freedom an octopus is generally able to move and sense with, because of its loose or non-rigid body structure.[60] The optokinetic nystagmus and the integration of vestibular data into posture control are just two examples of how humans and octopuses use their senses to augment how they will immediately continue to intake sensory data. For example, if the statocyst (or utricle and saccule) sense the direction of gravity change, that data can be used to reorient how visual data being received should be interpreted, compared to how it was before the direction of gravity changed in relation to the position of the vestibular organs. While it’s possible some organisms are more adaptable in regards to these sorts of adjustments, consistently adjusting to different head positions or a visually spinning environment can make the performance of otherwise normal behaviors more complicated and require more coordination. Many organisms attempt to avoid having to factor in these sorts of sensory corrections, evidenced by the stabilization found in humans, birds, and lizards, among many other animals[59–61]. I would expect that while octopuses attempt to control the amount of change of their bearing-heading difference, thus keeping it rather low, there is some natural variation.

If octopuses were disoriented by spinning, I would expect the average change in bearing-heading difference to be greater. Across full videos and in crawling movements, no significant effect of trial type was found, however. In full videos, octopuses averaged 0.25° per frame of average change in bearing-heading difference in the control trials. (Table 4; Fig. 11) This amounts to 1.51°/second of average increase in bearing-heading difference. In crawling movements, this average was slightly lower for controls at 0.028° per frame or about 0.17°/second (Table 5; Fig. 18) These are relatively small numbers, and represent biologically small movements or changes. These small changes suggest a relatively tight control on how the octopus “faces” compared to its direction of movement.

When statistically analyzing the average change in bearing-heading difference, the absolute values were used, as what is relevant for this factor is the magnitude of change, rather than necessarily the direction. While, as I noted, the averages using non-absolute values was relatively low in controls, they still ranged up to 10° per frame in full videos and up to 50° per frame in crawling movements. These values are rather high and suggest that octopuses’ average change in bearing-heading difference could be up to 300° per second. This is obviously not realistic. In order to determine what may have caused this average, I visually inspected some of the videos in which the magnitude of average change in bearing-heading difference was particularly high. In the case of the second trial for Octopus #5, who had an unusually large change of -42.46° per frame, the octopus was in fact moving in circles. Specifically, the octopus was raised up on its arms and its eyes being at the top of its body in this position were swaying in a consistent circle, while the rest of its body was mostly stationary. This highlights a key issue with this metric, which is that changes in bearing are not weighed in importance by when the octopus is actively moving. Therefore the natural variations of label placement can introduce variations in the calculation of the center point from which movement direction is calculated from. Therefore, a stationary octopus would still be attributed a change in bearing-heading difference. Generally, this shouldn't affect results too drastically, as again most of this variation would be random and therefore average out to zero. Its only effect would be dragging down the average. A way of modifying this measurement in the future to possibly control the error of stationary changes in heading would be to create a threshold for the distance travelled each frame. This would essentially bypass the inclusion of stationary octopuses. This method of filtering the data might also be useful when calculating averages for bearing change and bearing-heading difference in the full video data set.

These other specific cases of large magnitude changes in bearing-heading difference, create an issue where the octopus's movement is accurately being tracked, but the type of movement is different enough in character that it makes the results difficult to reasonably interpret alongside movements that involve more linear motions (those that seem to be moving the octopus through space rather than rotating or wobbling around a point). While this sort of wobbling would rather cartoonishly seem like something that might be the result of disorientation, this wobbliness was observed several times outside of the trials, and was likely the instability of octopuses attempting to raise themselves on their arms.

Directed Movement Classification

"Crawling" was defined as a catch all for movements that were not jetting. It is possible the highly varied styles of movement within this category may have introduced too much noise into some of the metrics. Though jetting movements were not able to be analyzed based on my data, these movements may present much more conclusive or more easily interpreted results due to their more defined movement aspects. Jetting movements' paths, as I mentioned earlier, are largely decided at the beginning of the movement by the initial thrust. These factors make jetting movements a likely more directly applicable movement type for studying the effects of spinning specifically on their accuracy when leaping for the crab or on their length or duration of the jetting movements. While octopuses significantly chose to perform crawling movements over jetting movements in spinning trials, the proportion of jetting movements was still relatively high and would likely be sufficient to analyze in a project with greater amounts of spinning trials.

Conclusion

In general, this project attempted to lay the groundwork for future projects that want to examine orientation and disorientation. Ultimately, the biggest complication with the experiment was malfunctions of the spinning tank, which cut short my ability to perform spinning trials throughout over half of the experiment. Similarly, the inability to analyze jetting movements likely greatly limits the view I was able to get of how the spinning trials affected octopuses' orientation.

The metrics used to measure possible disorientation could receive some modification after careful thought as to how each of these metrics is affected when averaging or subsetting, as well as how the methodology is affecting the observed values. For example, not only does the catch success rate in trials not provide evidence of disorientation alone, it might be substantially altered by the length of time given to catch the crab. In my project, this may have led to an elevated catch success rate. Similarly, the relative frequency of occurrence of crawling vs jetting movements may be affected by how one classifies and separates these movement types. Jetting movements by nature may contain less variability and natural noise than crawling movements. Similarly, jetting movements' direction being largely controlled by an initial launch may make these movements more clearly interpretable as examples of changes in directness and speed. It may be worthwhile to further separate crawling actions into more specific classifications, however with the variability of octopuses' movements this may be infeasible. The significant decrease in jetting movements in spinning trials provides evidence that something about the spinning trials affected how octopuses chose to catch crabs. Whether this was fear, stress, or disorientation will need more clarification. It may not be reasonable to assume that it would have only been one of these factors rather than some combination.

The idea that octopuses may have had time to recover from initial disorientation before making directed movements towards the crab, means that that evidence of disorientation (were it occurring) would have been suppressed when analyzing directed movements. This dynamic may make it hard, even if differences are found in the full video data set, to attribute them directly to disorientation, as there is no clear intent of motions made outside of directed movements. Finding ways to attribute factors like time before a directed movement or time spent unmoving may be needed to build a picture of how octopuses are behaving before they attempt to catch the crab. Specifically, this reorientation would likely increase the amount of time before a directed movement was made, therefore lengthening the duration of the full video. In this project no change in the duration of full videos was observed; which, in combination with no significant change in average size-corrected speed, suggests that this sort of reorientation was likely not occurring.

Ultimately, octopuses were surprisingly unaffected by the spinning trials. After being spun for a full minute at 60° per second, octopuses showed no differences in their catch rate of crabs, duration, average size-corrected speed, average bearing change, or average change in bearing-heading difference in either the full video or crawling movements. This suggests that by definition they were not disoriented in their movement. However, octopuses performed jetting movements significantly less after spinning suggesting that there was some limitation of their movement. Directness of octopuses' movement towards the crab, which would have likely been most visible in crawling movements (compared to jetting movements), was also not significantly affected. All together, the results from these less "voluntary" aspects of motion, which I would have expected to be signs that an octopus was less able to coordinate its movements because of disorientation, provide little clear evidence of disorientation.

The consistency of the average bearing-heading difference at around 50° is exciting, and suggests that *O. rubescens* might have preferential eye use when moving. The decrease in average bearing-heading difference after being spun in the full video data set might suggest that the octopuses were actually controlling the coordination of their bearing and heading more during crawling movements where no effect was found between trial types. This may represent a valuable line of future research.

Lastly, the interaction between the date of trial and trial type on bearing change in crawling movements gives some evidence that octopuses were acclimating or adapting to being spun. as seen in decrease over time in the bearing changes in spinning trials (Fig. 12). This differential effect of date on spinning trials versus controls was not observed for any other metric. If adaptation was occurring, this should be a consideration for future projects, as it would implicitly affect the character of one's data.

While the results of this project are far from conclusive, it has laid a foundation for future research into disorientation and the vestibular system of octopuses and other cephalopods. With more trials, successful neural network training, and improvements to the reliability of some of the calculated movement metrics, this methodology can provide a wealth of data not only on disorientation, but on the nature of octopus movement itself.

Future Directions

This project contains a lot of data not only on how octopuses were affected by spinning, but also on what did and did not work about this novel methodology. Future projects should attempt to refine the methods and their relationship to the metrics being observed. For example, how long to allow for the octopus to catch a crab and how to subset the data of a full video to be able to examine information about directed movements and the behaviors outside of them.

Based on the post-hoc power analyses completed on metrics where no significant difference was found, it may also be possible to rule out some metrics from future implementations of this methodology. Bearing-heading difference may be one such metric that while still necessary to determine the change in bearing-heading difference may not be biologically relevant to disorientation. According to the power analyses, the full video data set would have required up to 26° difference in average bearing change to reach a statistical power level of ~0.8. (Table 6) In crawling movements, this mean difference would have had to be up to almost 95°. (Table 7) Biologically, this change is very unlikely and would require octopuses to consistently increase or decrease their average bearing-heading difference in one direction. If disorientation does affect bearing-heading difference, it likely would not affect it consistently and severely and so may not be able to be detected with simple averages. One alternative would be to examine the distribution of bearing-heading differences, to get a better picture of whether octopuses might increase or decrease a range that they keep their bearing-heading difference within.

Standardizing a way to place crabs and the octopuses' distances to the crab would help to limit any skewing of the data that might occur due to the distance and movement modalities for a larger versus smaller octopus. One way of going about this would be to find a way to keep the octopus in the center of the tank while spinning, both ensuring a standard spinning experience and the ability for placement of the crab to be standardized from the center to the edge of the tank. Though keeping them in the center of the tank through food association was unsuccessfully tried during the pre-trial period of this project, it may still be possible using a scented/flavored but non-consumable object in the center of the tank. Though, this may affect the octopus's willingness to retrieve the crab. Another solution to standardizing distance, would be to instead find a way to keep them near a side of the tank during spinning.

This project could have also benefited from a better filming set up. Finding ways to increase the clarity and quality of the recorded videos might increase the accuracy of artificial neural networks trained on the locations being tracked in this study. One complication of direct lighting is surface glare on the water that obstructs analysis. It may be possible to use thin fabrics such as cheese cloth to diffuse light before it hits the surface of the water without substantially dimming it. Another possible avenue for increasing video quality would be to instead use red light while keeping the rest of the environment dim. This of course introduces many challenges, and would require a camera sensitive to these conditions. How this would affect the behavior of the animals would also be important to consider, as this may simulate night conditions. Being able to train a better model with the same or less manually labelled frames may allow for an accurate enough model to estimate the positions of the octopus eyes reliably enough to be used in conjunction with the crab-labelling DeepLabCut model.

Similarly, videos with higher contrast and overall better quality may also allow for some other measurements to be accurately taken. For example, with sufficient lighting and contrast background subtraction could be used in ImageJ to compare the overall “spread” of the octopus to see if octopuses change how much they spread out when spun.

As optic reflexes can be an important indicator for sensed motion in the vestibular system, finding a way to record the eyes of the octopus after a spinning trial would allow for a solid baseline as to whether the octopus was perceiving motion. This may even allow for a differentiation between sensed vestibular and visual motion based on whether the octopus is exhibiting a vestibulo-ocular reflex or an optokinetic nystagmus.

LITERATURE CITED

1. Schone, H. *Spatial Orientation: The Spatial Control of Behavior in Animals and Man*. (Princeton University Press, 2014).
2. Lalazar, H. & Vaadia, E. Neural basis of sensorimotor learning: modifying internal models. *Curr. Opin. Neurobiol.* **18**, 573–581 (2008).
3. Kawato, M. & Wolpert, D. Internal Models for Motor Control. in *Novartis Foundation Symposium 218 - Sensory Guidance of Movement* 291–307 (John Wiley & Sons, Ltd). doi:10.1002/9780470515563.ch16.
4. Anderson, S. R. *et al.* An Internal Model Architecture for Novelty Detection: Implications for Cerebellar and Collicular Roles in Sensory Processing. *PLoS ONE* **7**, e44560 (2012).
5. Häder, D.-P., Braun, M. & Hemmersbach, R. Gravity Sensing, Graviorientation and Microgravity. in *Gravitational Biology I: Gravity Sensing and Graviorientation in Microorganisms and Plants* (eds. Braun, M., Böhmer, M., Häder, D.-P., Hemmersbach, R. & Palme, K.) 1–11 (Springer International Publishing, Cham, 2018). doi:10.1007/978-3-319-93894-3_1.
6. Jamon, M. The development of vestibular system and related functions in mammals: impact of gravity. *Front. Integr. Neurosci.* **8**, (2014).
7. Straka, H., Zwergal, A. & Cullen, K. E. Vestibular animal models: contributions to understanding physiology and disease. *J. Neurol.* **263**, 10–23 (2016).
8. Rosenhall, U. Some morphological principles of the vestibular maculae in birds. *Arch. Für Klin. Exp. Ohren- Nasen- Kehlkopfheilkd.* **197**, 154–182 (1970).
9. Galante-Oliveira, S., Marçal, R., Ribas, F., Machado, J. & Barroso, C. Studies on the morphology and growth of statoliths in Caenogastropoda. *J. Molluscan Stud.* **79**, 340–345 (2013).
10. Sötje, I. *et al.* Comparison of the statolith structures of Chironex fleckeri (Cnidaria,

Cubozoa) and Periphylla periphylla (Cnidaria, Scyphozoa): a phylogenetic approach. *Mar. Biol.* **158**, 1149–1161 (2011).

11. Wittmann, K. J., Schlacher, T. A. & Ariani, A. P. Structure of recent and fossil mysid statoliths (Crustacea, Mysidacea). *J. Morphol.* **215**, 31–49 (1993).
12. Ibsch, M., Vöhringer, P., Anken, R. H. & Rahmann, H. Electronmicroscopic investigations on the role of vesicle-like bodies in inner ear maculae for fish otolith growth. *Adv. Space Res.* **25**, 2031–2034 (2000).
13. Young, J. Z. The statocysts of *Octopus vulgaris*. *Proc. R. Soc. Lond. B Biol. Sci.* **152**, 3–29 (1997).
14. Schöne, H. & Budelmann, B.-U. Function of the Gravity Receptor of *Octopus vulgaris*. *Nature* **226**, 864–865 (1970).
15. Dilly, P. N. The structure of some cephalopod statoliths. *Cell Tissue Res.* **175**, 147–163 (1976).
16. Collewijn, H. Oculomotor reactions in the cuttlefish Sepia officianalis.
17. Beisel, K. Development and evolution of the vestibular sensory apparatus of the mammalian ear. *NIH* 225–241 (2005).
18. Budelmann, B. U. Cephalopod sense organs, nerves and the brain: Adaptations for high performace and life style. in *Physiology of Cephalopod Mulluscs* (ed. Pörtner, Hans O.; O’Dor, Ron K; Macmillan, D. L.) 13–33 (Gordon and Breach Publishers, 1994).
19. Day, B. L. & Fitzpatrick, R. C. The vestibular system. *Curr. Biol.* **15**, R583–R586 (2005).
20. Ashcroft, D. W. & Hallpike, C. S. On the Function of the Saccule. *J. Laryngol. Otol.* **49**, 450–460 (1934).
21. Budelmann, B. U., Sachse, M., Staudigl, M. & Young, J. Z. The angular acceleration receptor system of the statocyst of *Octopus vulgaris*: morphometry, ultrastructure,

and neuronal and synaptic organization. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **315**, 305–343 (1997).

22. Wu, X., Yu, S., Shen, S. & Liu, W. Exploring the biomechanical responses of human cupula by numerical analysis of temperature experiments. *Sci. Rep.* **11**, 8208 (2021).
23. Budelmann, B. U. Cephalopod sense organs, nerves and the brain: Adaptations for high performance and life style. *Mar. Freshw. Behav. Physiol.* **25**, 13–33 (1995).
24. Chin, S. Visual vertigo: Vertigo of oculomotor origin. *Med. Hypotheses* **116**, 84–95 (2018).
25. Bronstein, A. M. Vision and vertigo. *J. Neurol.* **251**, 381–387 (2004).
26. Straka, H. & Dieringer, N. Basic organization principles of the VOR: lessons from frogs. *Prog. Neurobiol.* **73**, 259–309 (2004).
27. Robinson, D. A. The behavior of the optokinetic system☆. in *Progress in Brain Research* (eds. Anastasio, T. et al.) vol. 267 215–230 (Elsevier, 2022).
28. Collewijn, H. Oculomotor reactions in the cuttlefish, *Sepia officinalis*. *J. Exp. Biol.* **52**, 369–384 (1970).
29. Budelmann, B. U. & Young, J. Z. The statocyst-oculomotor system of octopus vulgaris: extraocular eye muscles, eye muscle nerves, statocyst nerves and the oculomotor centre in the central nervous system. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **306**, 159–189 (1997).
30. Macefield, V. G. The roles of mechanoreceptors in muscle and skin in human proprioception. *Curr. Opin. Physiol.* **21**, 48–56 (2021).
31. Peterka, R. J. Chapter 2 - Sensory integration for human balance control. in *Handbook of Clinical Neurology* (eds. Day, B. L. & Lord, S. R.) vol. 159 27–42 (Elsevier, 2018).
32. Wells, M. J. Proprioception and Visual Discrimination of Orientation in Octopus. *J. Exp. Biol.* **37**, 489–499 (1960).

33. Bender, J. A. & Frye, M. A. Invertebrate solutions for sensing gravity. *Curr. Biol.* **19**, R186–R190 (2009).
34. Brandt, T., Dietrich, M. & Strupp, M. *Vertigo and Dizziness*. (Springer).
35. Boycott, B.B. The Functioning of the Statocysts of <i> Octopus vulgaris <i>. 78–87 (1960) doi:10.1098.
36. Reiley, A. S., Vickory, F. M., Funderburg, S. E., Cesario, R. A. & Clendaniel, R. A. How to diagnose cervicogenic dizziness. *Arch. Physiother.* **7**, 12 (2017).
37. Magnusson, M. & Malmström, E.-M. Chapter 26 - The conundrum of cervicogenic dizziness. in *Handbook of Clinical Neurology* (eds. Furman, J. M. & Lempert, T.) vol. 137 365–369 (Elsevier, 2016).
38. Lee, S.-H. & Kim, J. S. Benign Paroxysmal Positional Vertigo. *J. Clin. Neurol.* **6**, 51–63 (2010).
39. Macias, J. D., Lambert, K. M., Massingale, S., Ellensohn, A. & Ann Fritz, J. Variables Affecting Treatment in Benign Paroxysmal Positional Vertigo. *The Laryngoscope* **110**, 1921–1924 (2000).
40. Bhattacharyya, N. *et al.* Clinical Practice Guideline: Benign Paroxysmal Positional Vertigo. *Otolaryngol. Neck Surg.* **139**, 47–81 (2008).
41. Sole, M. Does exposure to noise from human activities compromise sensory information from cephalopod statocysts?
42. Solé, M. *et al.* Ultrastructural Damage of Loligo vulgaris and Illex coindetii statocysts after Low Frequency Sound Exposure. *PLOS ONE* **8**, e78825 (2013).
43. Navarro, M. O., Bockmon, E. E., Frieder, C. A., Gonzalez, J. P. & Levin, L. A. Environmental pH, O2 and Capsular Effects on the Geochemical Composition of Statoliths of Embryonic Squid Doryteuthis opalescens. *Water* **6**, 2233–2254 (2014).
44. Maneja, R., Piatkowski, U. & Melzner, F. Effects of ocean acidification on statolith calcification and prey capture in early life cuttlefish, Sepia officinalis. *J. Shellfish Res.*

30, 1011 (2011).

45. Colmers, W. F. *et al.* “Spinner” cephalopods: defects of statocyst suprastructures in an invertebrate analogue of the vestibular apparatus. *Cell Tissue Res.* **236**, 505–515 (1984).
46. Hanlon, R. T. Strontium is required for statolith development and thus normal swimming behavior of hatchling cephalopods. J. exp. Biol. **141**, 187-195 (1989).
47. Kaplan, M. B., Mooney, T. A., McCorkle, D. C., Cohen, A. L. Adverse effects of ocean acidification on early development of squid (*Doryteuthis pealeii*). *PLoS ONE* **8**, e63714 (2013).
48. Ikeda, Y., Arai, N., Kidokoro, H. & Sakamoto, W. Strontium: calcium ratios in statoliths of Japanese common squid *Todarodes pacificus* (Cephalopoda: Ommastrephidae) as indicators of migratory behavior. *Mar. Ecol. Prog. Ser.* **251**, 169–179 (2003).
49. Kharlamova, A. *et al.* Cerebellar morphology and behavioural correlations of the vestibular function alterations in weightlessness. *Neurosci. Biobehav. Rev.* **126**, 314–328 (2021).
50. Goddard, M., Zheng, Y., Darlington, C. L. & Smith, P. F. Locomotor and exploratory behavior in the rat following bilateral vestibular deafferentation. *Behav. Neurosci.* **122**, 448–459 (2008).
51. Stackman, R. W. & Herbert, A. M. Rats with lesions of the vestibular system require a visual landmark for spatial navigation. *Behav. Brain Res.* **128**, 27–40 (2002).
52. Lewis, R. F. Vestibular implants studied in animal models: clinical and scientific implications. *J. Neurophysiol.* **116**, 2777–2788 (2016).
53. Mathis, A. *et al.* DeepLabCut: markerless pose estimation of user-defined body parts with deep learning. *Nat. Neurosci.* **21**, 1281–1289 (2018).
54. Liu, Y. The Confusing Metrics of AP and mAP for Object Detection. *Medium*

https://yanfengliux.medium.com/the-confusing-metrics-of-ap-and-map-for-object-detection-3113ba0386ef (2020).

55. MacIver, M. A., Patankar, N. A. & Shirgaonkar, A. A. Energy-Information Trade-Offs between Movement and Sensing. *PLOS Comput. Biol.* **6**, e1000769 (2010).
56. Frasnelli, E., Ponte, G., Vallortigara, G. & Fiorito, G. Visual Lateralization in the Cephalopod Mollusk Octopus vulgaris. *Symmetry* **11**, 1121 (2019).
57. Schnell, A. K., Hanlon, R. T., Benkada, A. & Jozet-Alves, C. Lateralization of Eye Use in Cuttlefish: Opposite Direction for Anti-Predatory and Predatory Behaviors. *Front. Physiol.* **7**, (2016).
58. Sakurai, Y. & Ikeda, Y. Effect of visual lateralization on the spatial position of individuals within a school of oval squid (Sepioteuthis lessoniana). *J. Comp. Physiol. A* **210**, 381–398 (2024).
59. Goyens, J. & Aerts, P. Head stabilisation in fast running lizards. *Zoology* **127**, 114–120 (2018).
60. Pete, A. E., Kress, D., Dimitrov, M. A. & Lentink, D. The role of passive avian head stabilization in flapping flight. *J. R. Soc. Interface* **12**, 20150508 (2015).
61. Pozzo, T., Berthoz, A. & Lefort, L. Head stabilization during various locomotor tasks in humans. *Exp. Brain Res.* **82**, 97–106 (1990).
62. Pinheiro J, Bates D, R Core Team (2023). _nlme: Linear and Nonlinear Mixed Effects Models_. R package version 3.1-164, https://CRAN.R-project.org/package=nlme.
63. Pinheiro JC, Bates DM (2000). _Mixed-Effects Models in S and S-PLUS_. Springer, New York. doi:10.1007/b98882 https://doi.org/10.1007/b98882.
64. Bradski, G. (2000). The OpenCV Library. Dr. Dobb's Journal of Software Tools.
65. Umesh, P. (2012). Image Processing in Python. CSI Communications, 23.

Tables and Figures

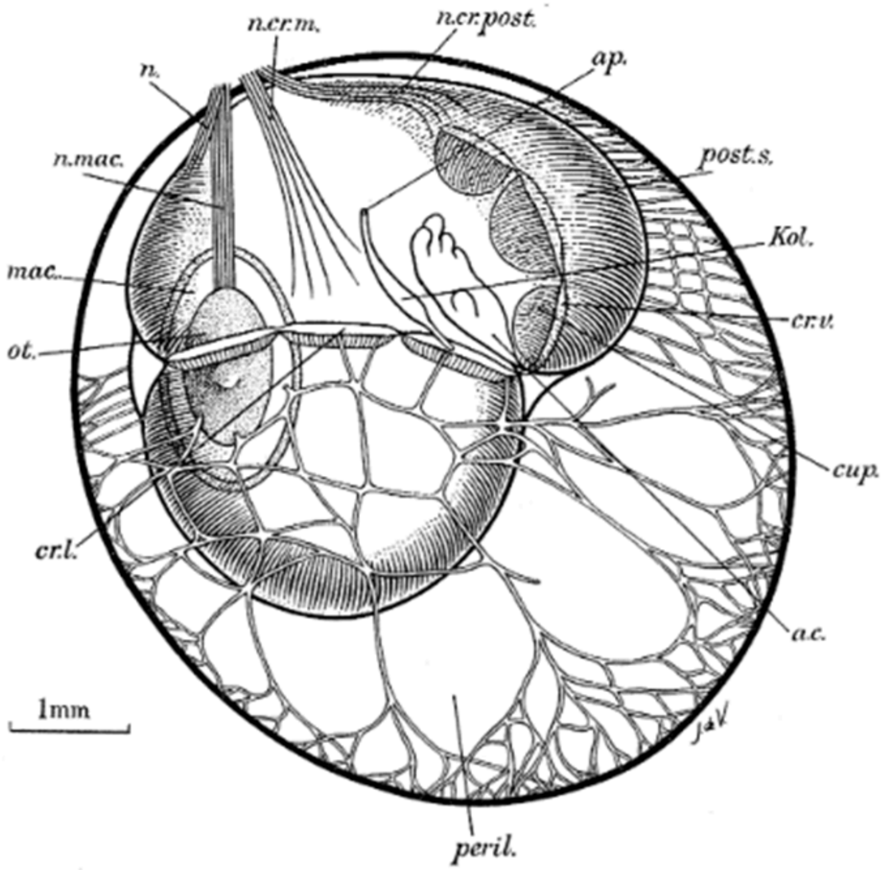

Figure 1: A diagram from Young 1959 illustrating the statocyst of Octopus vulgaris sitting inside the cartilaginous sac. “ot.” and “mac.” markers point to the macula and statolith gravity sensing system. “cup.” marker denotes one of the cupula found in the posterior chamber.

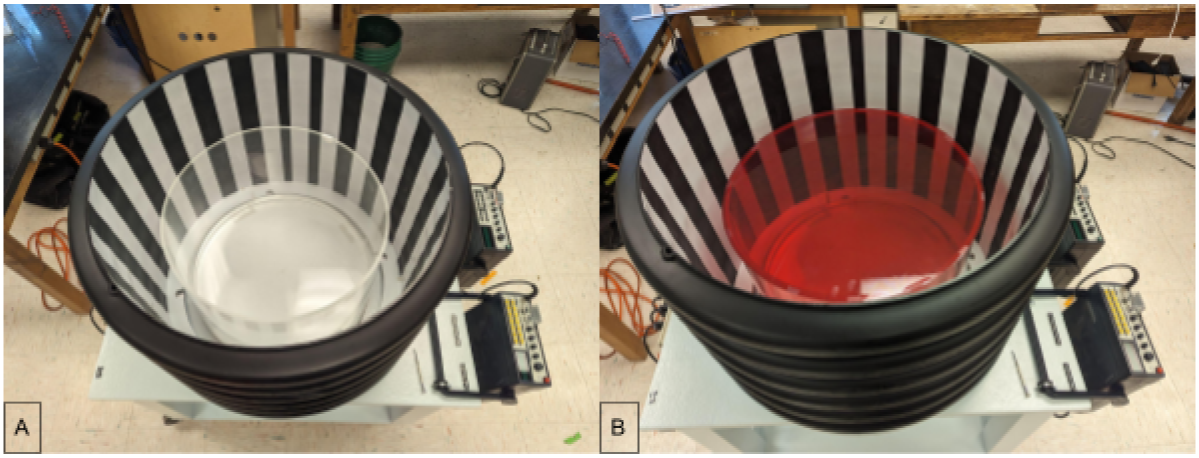

Figure 2: Spinning Tank. Outer drum consists of alternating black and white stripes facing in towards an inner drum. The inner drum is a hollow, acrylic cylinder with a removable red top. The inner drum acts as the tank or aquarium for the octopus and was the only drum spun during this project's data collection.

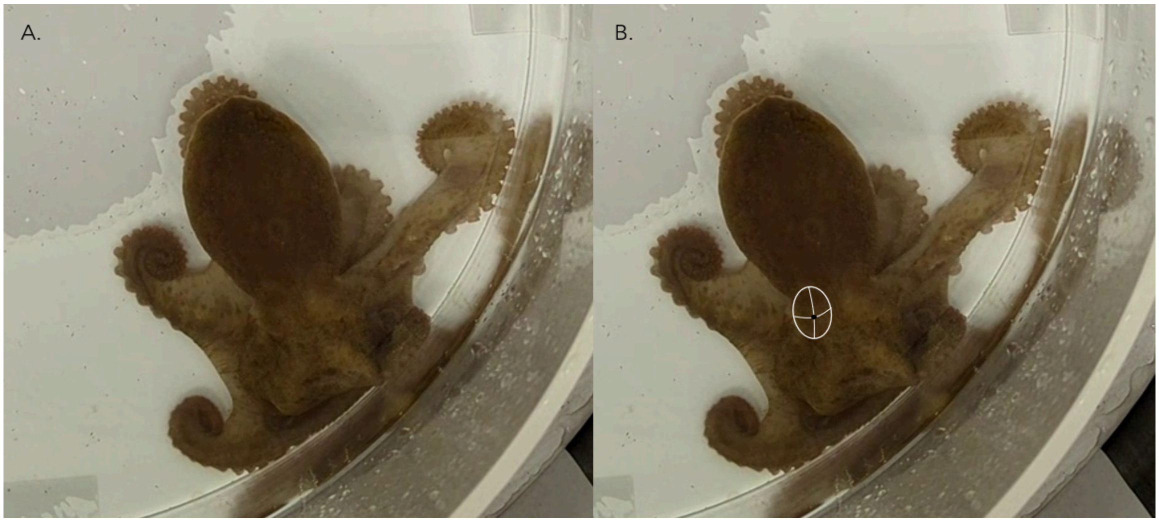

Figure 3: A) A frame from a video of an octopus in the spinning tank. B) The same image overlaid with white lines illustrating the three dimensional curvature of the eye, and a black dot at their center showing a theoretical label placement for the right eye. Cues such as light contouring, "eye-bar" displays, and dark eye ring displays helped to determine the placement of the label.

Figure 4: Examples of the appearance of octopuses during jetting motions. Generally the arms trail opposite the direction of motion.

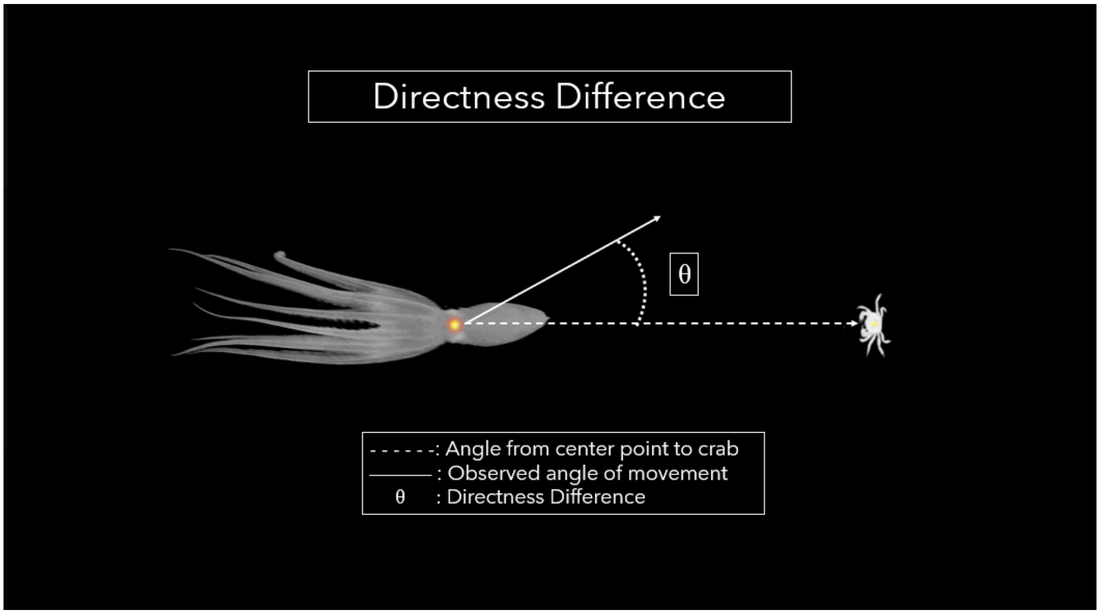

Figure 5: A diagram illustrating the two vectors from which directness was measured. Both originate from the center point between the left and right eye of the octopus. One vector points to the labelled center of the crab. The other points in the direction of the octopus' center point in the following frame.

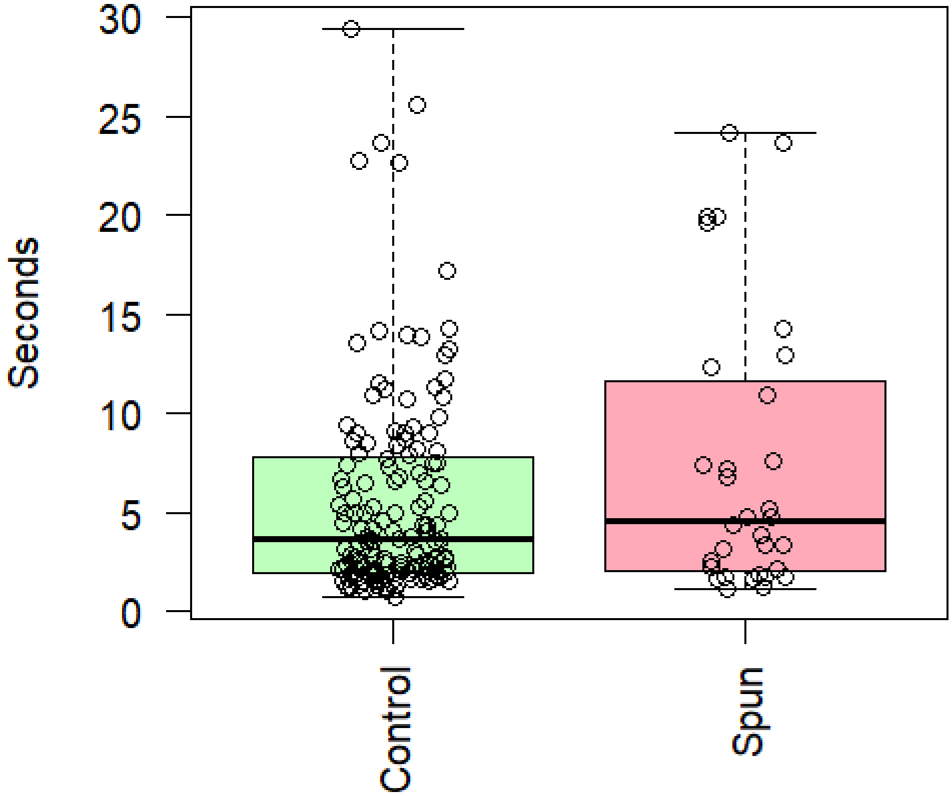

Figure 6: Duration of full videos from control and spinning trials. Linear Mixed Effects Model Results: Chi-square: 3.265; df: 1; p-value: 0.07077.

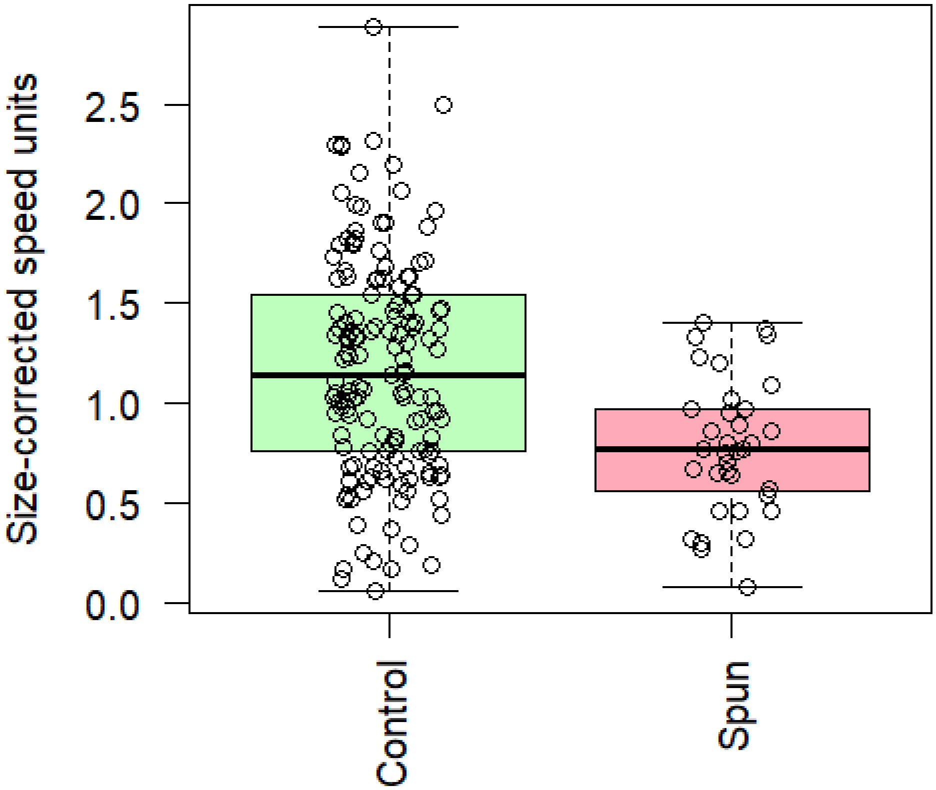

Figure 7: Size-corrected speed of full videos from control and spinning trials. Linear Mixed Effects Model Results: Chi-square: 2.2061; df: 1; p-value: 0.1374629.

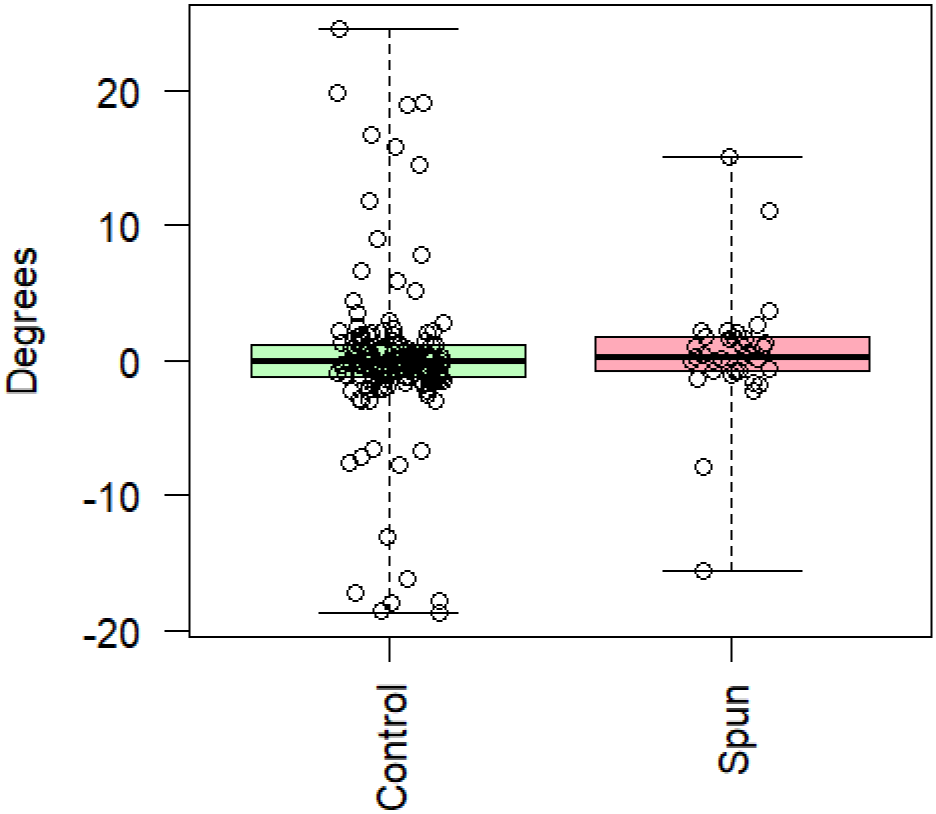

Figure 8: Average bearing change of full videos from control and spinning trials. Linear Mixed Effects Model Results: Chi-square: 0.0720; df: 1; p-value: 0.7884.

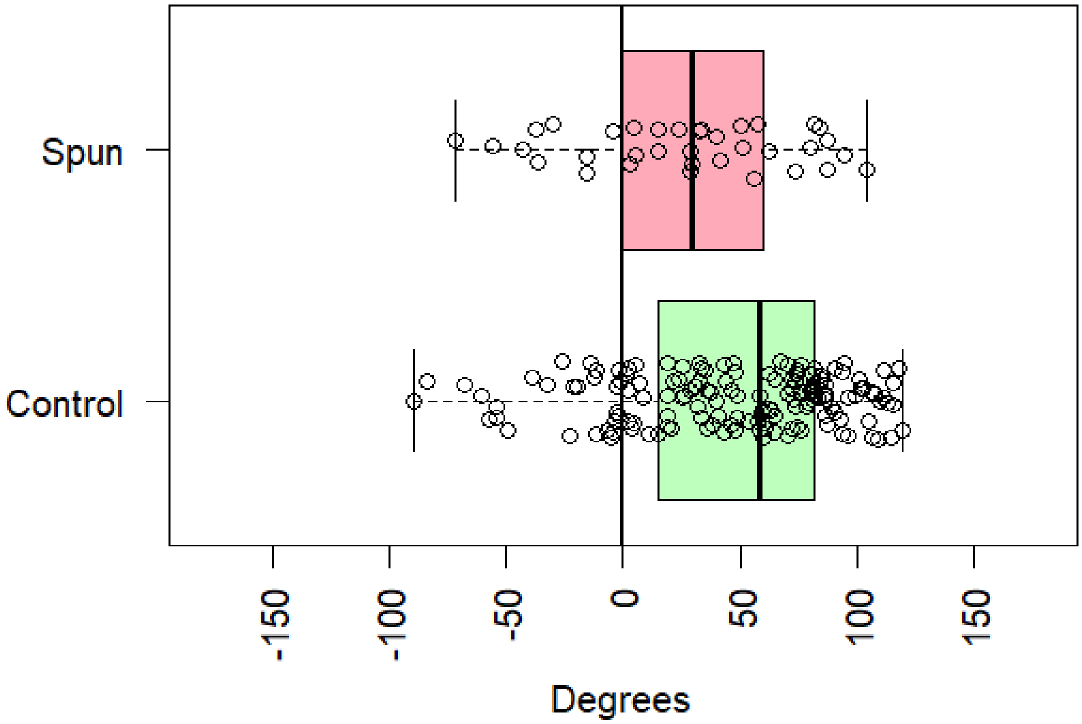

Figure 9: Average bearing-heading difference of full videos from control and spinning trials. Linear Mixed Effects Model Results: Chi-square: 5.9723; df: 1; p-value: 0.01453.

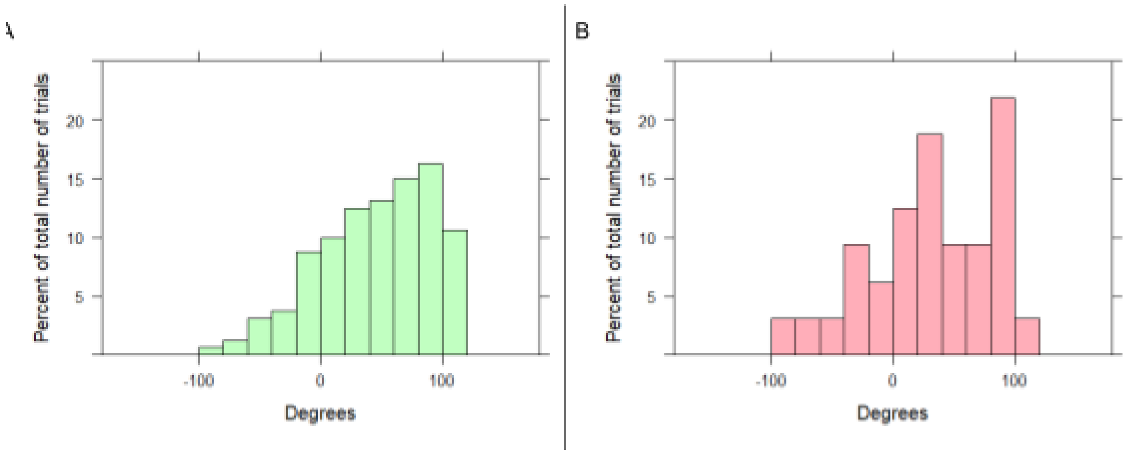

Figure 10: Average bearing-heading difference of full videos from control (A) and spinning (B) trials displayed in histograms.
Linear Mixed Effects Model Results: Chi-square: 5.9723; df: 1; p-value: 0.01453.

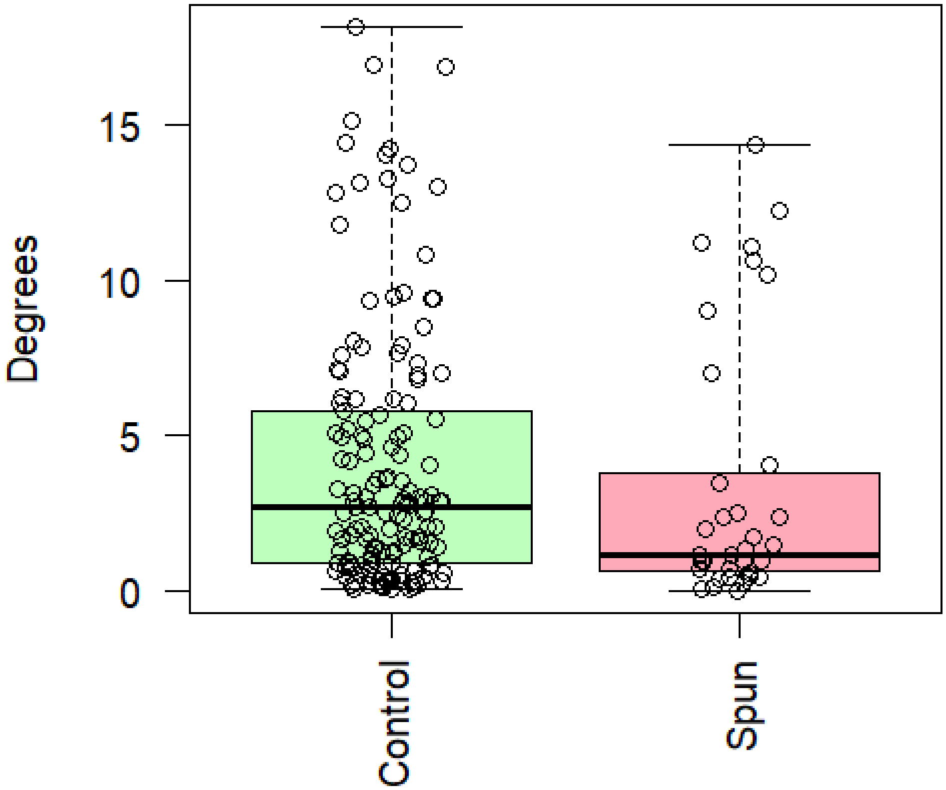

Figure 11: Average change in bearing-heading difference of full videos from control and spinning trials.

Linear Mixed Effects Model Results: Chi-square: 1.3979; df: 1; p-value: 0.2371.

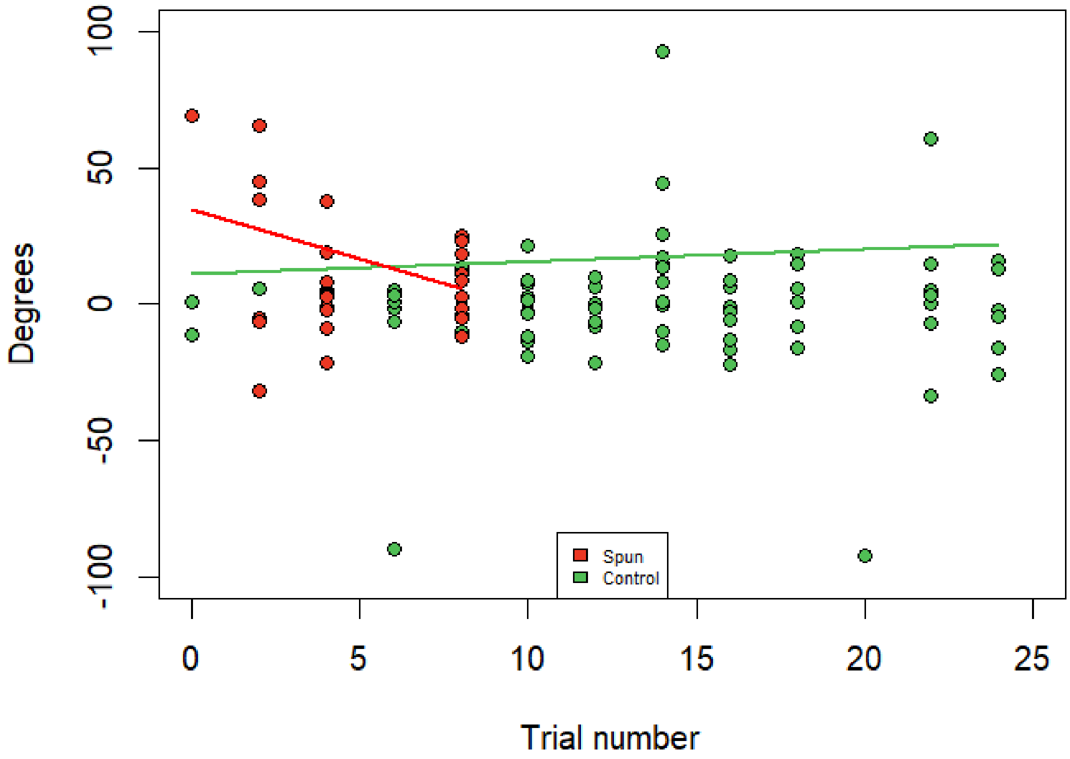

Figure 12: Average bearing change for crawling movements from control and spinning trials. The linear regressions predicted by the linear mixed effects model fit to the control trials' data is plotted by the green line. The linear regression predicted by the linear mixed effects model fit to the spinning trials' data is plotted by the red line.
Linear Mixed Effects Model Results: Chi-Square: 4.0949; df = 1; p-value: 0.0430137.

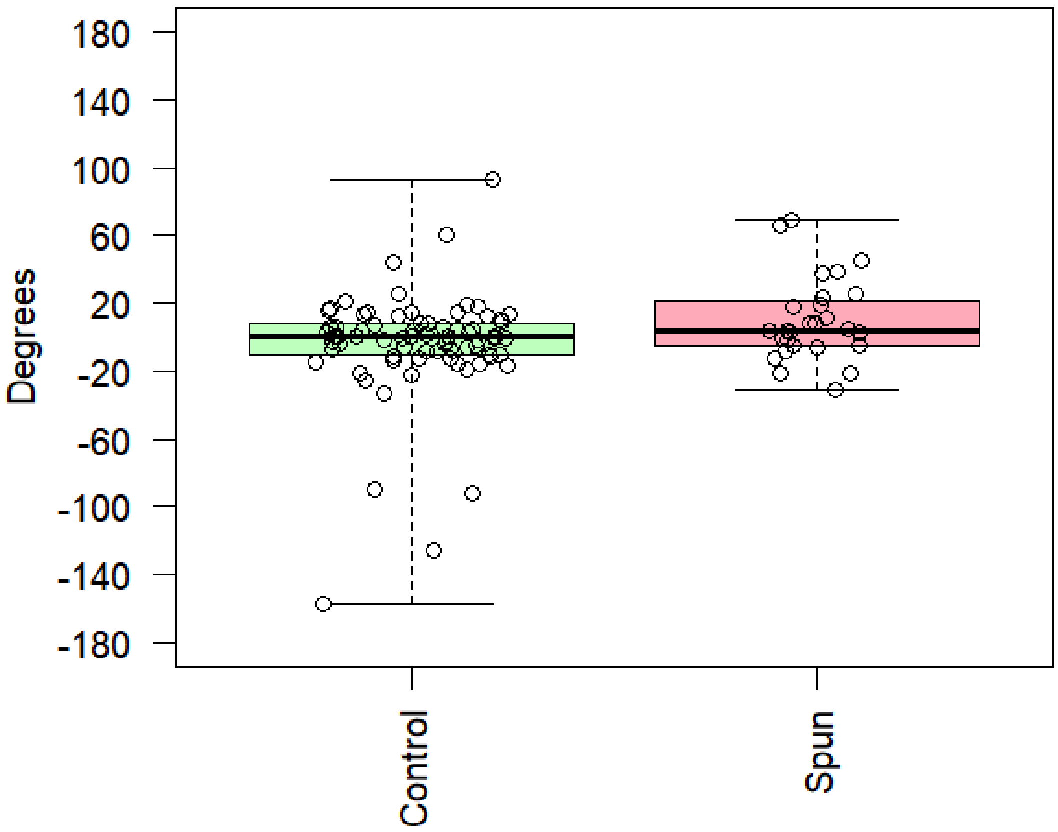

Figure 13: Average bearing change in control versus spun trials for crawling movements. Linear Mixed Effects Model Results: Chi-square: 3.2622; df: 1; p-value: 0.0708953.

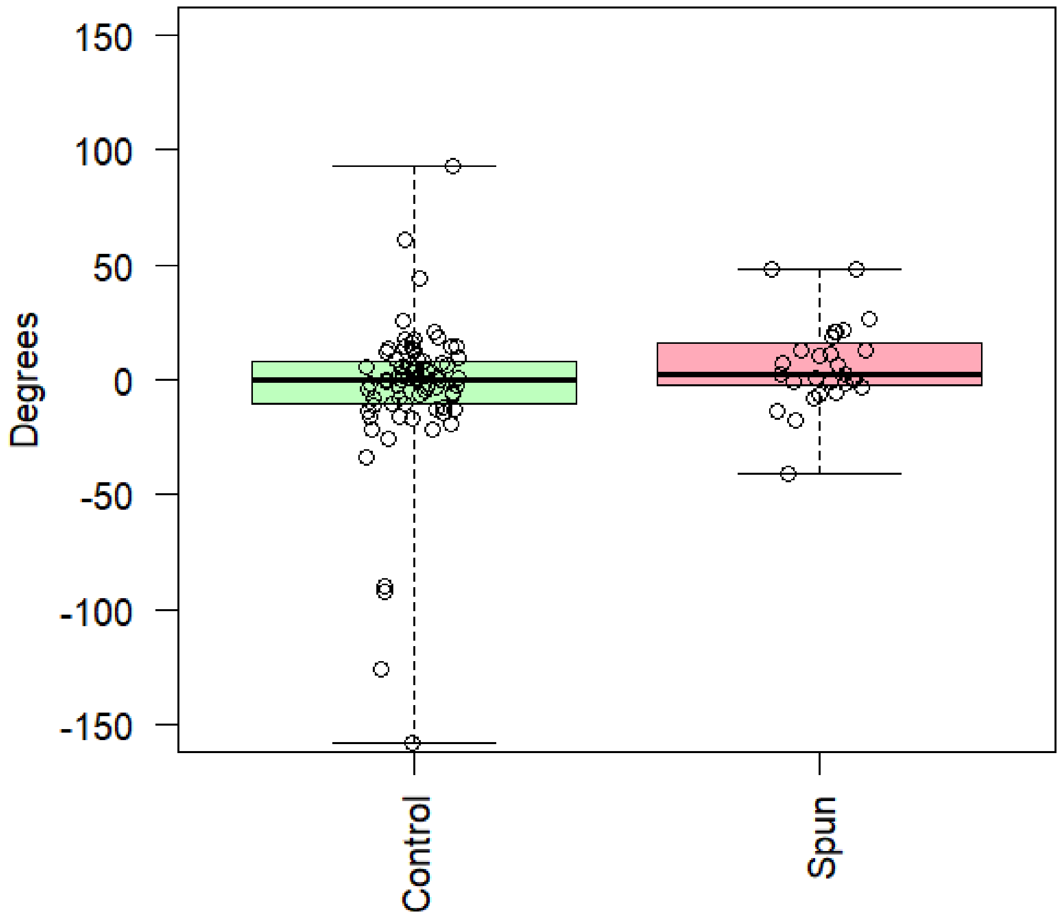

Figure 14: Average directness for crawling movements from control and spinning trials. Linear Mixed Effects Model Results: Chi-square: 0.4118; df: 1; p-value: 0.521081.

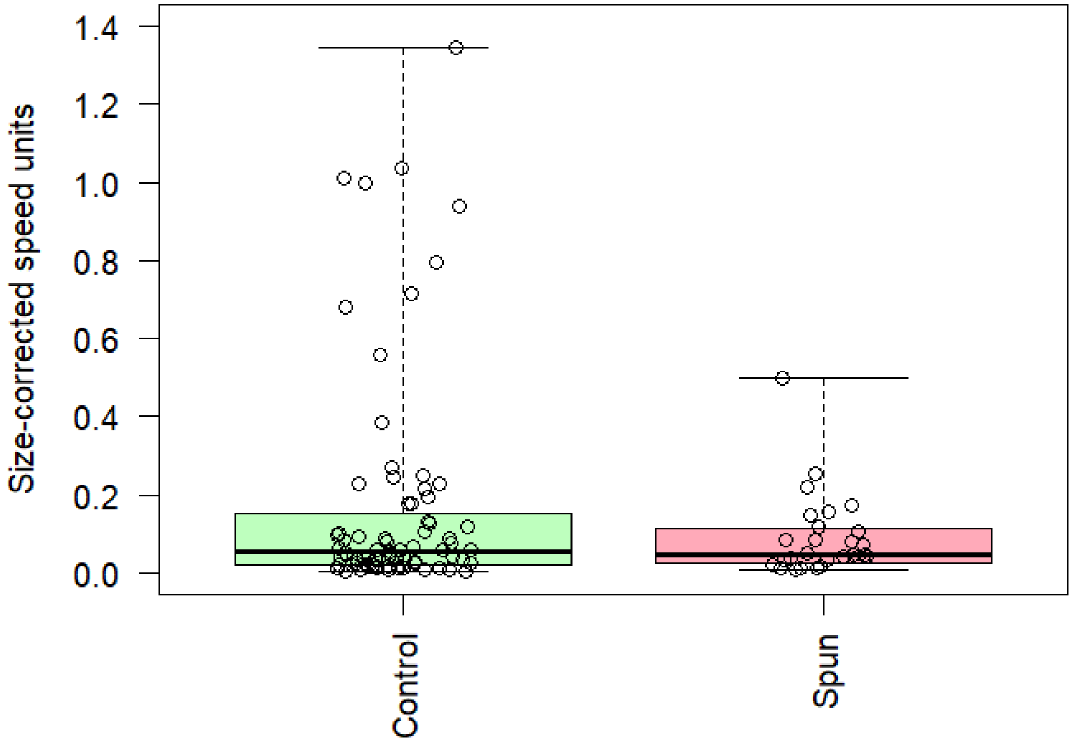

Figure 15: Average size corrected speed for crawling movements from control and spinning trials. Size-corrected speed units represent pixels per frame values divided by each octopus's respective mantle index. Linear Mixed Effects Model Results: Chi-square: 0.0782; df: 1; p-value: 0.7797.

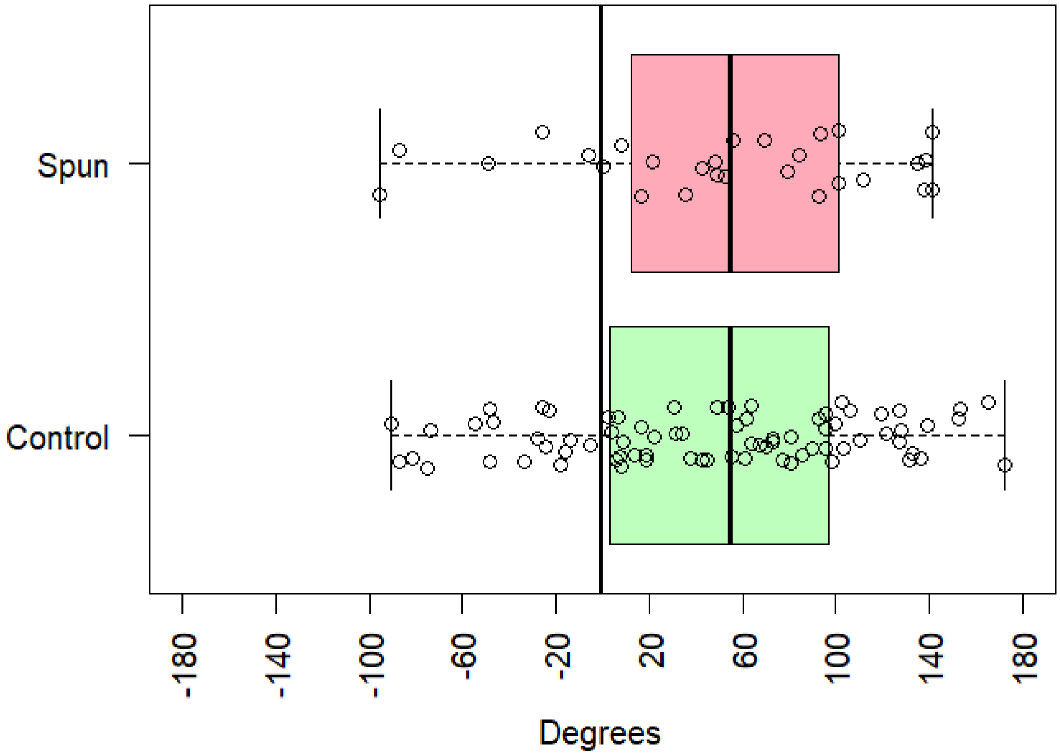

Figure 16: Average bearing-heading difference for crawling movements from control and spinning trials. Linear Mixed Effects Model Results: Chi-square: 0.9419; df: 1; p-value: 0.3318.

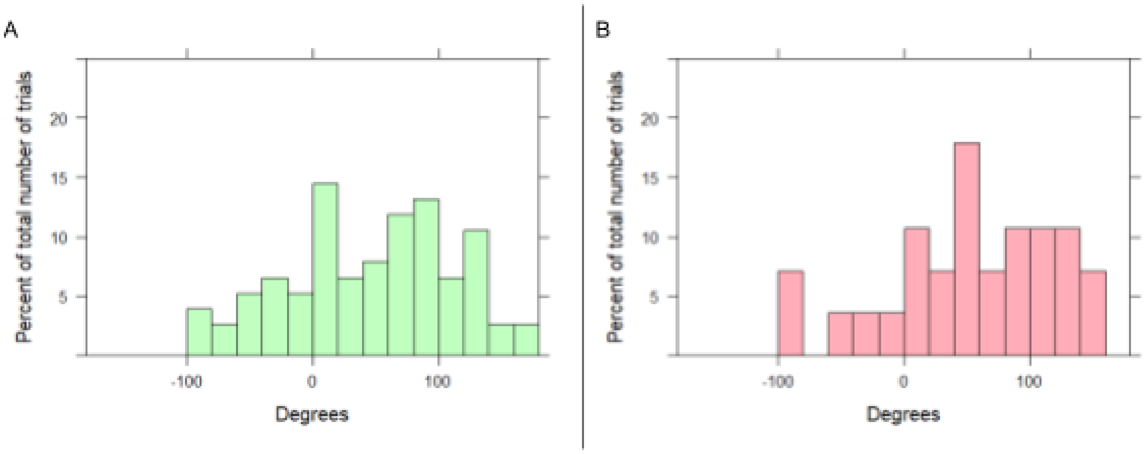

Figure 17: Average bearing-heading difference for crawling movements from control (A) and spinning (B) trials. Linear Mixed Effects Model Results: Chi-square: 0.9419; df: 1; p-value: 0.3318.

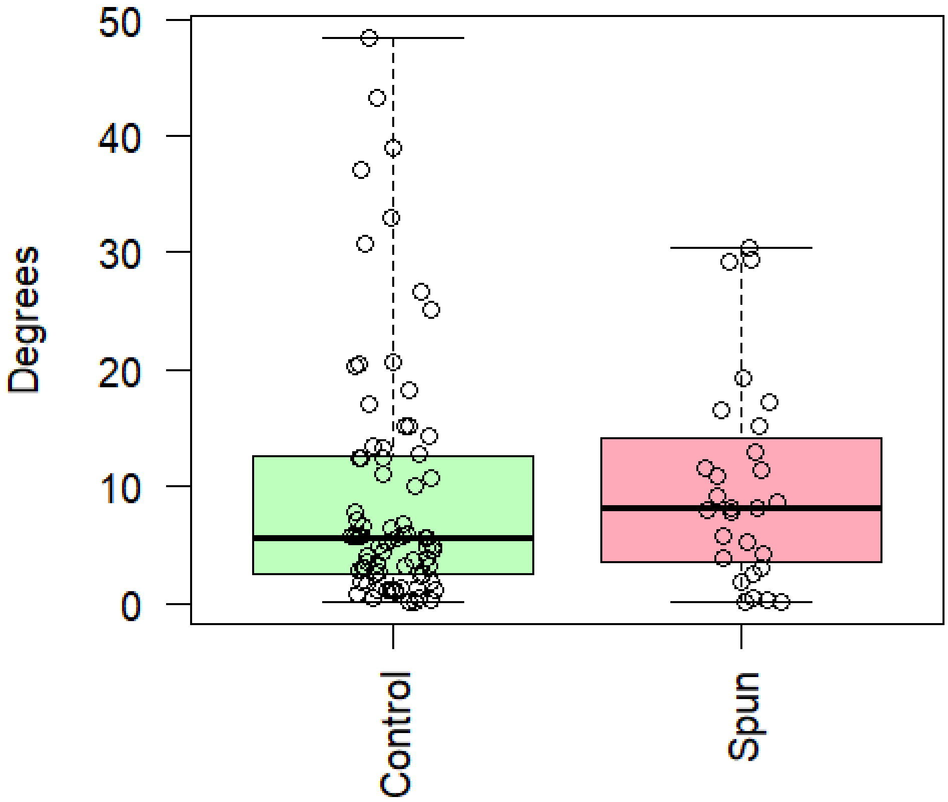

Figure 18: Average change in bearing-heading difference for crawling movements from control and spinning trials. Linear Mixed Effects Model Results: Chi-square: 0.0986; df: 1; p-value: 0.75355.

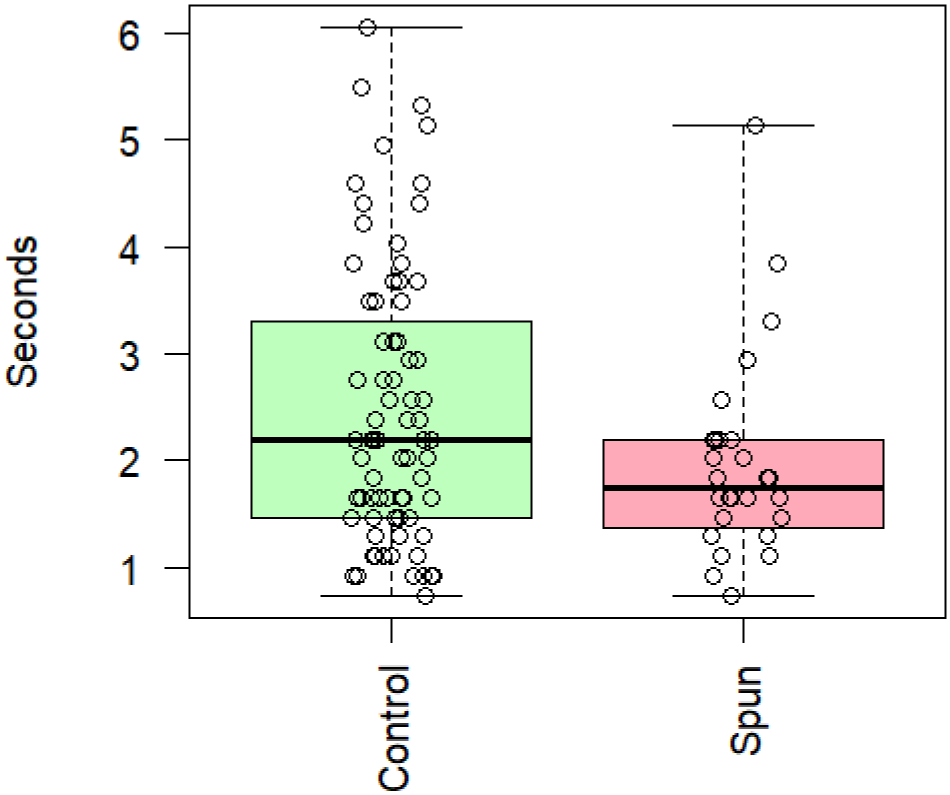

Figure 19: Durations of crawling movements from control and spinning trials. Linear Mixed Effects Model Results: Chi-square: 1.0178; df: 1; p-value: 0.313.

Table 1: Chi-squared tests for movement type and crab catching success between trial types

Variable	X-squared	df	p-value
Movement Type	15.613	1	7.770E-05
Catch Success	8.77E-30	1	1

Table 2: Distribution of crab catching success between trial types

	Failure	Success	
Control	22	143	
Treatment	6	35	
	X-squared	df	p-value
Difference in catch success	8.77E-30	1	1

Table 3: Distribution of movement type occurrences between trial types

	Crawling	Jetting	
Control	76	84	
Treatment	28	4	
	X-squared	df	p-value
Difference in frequency of movement type	15.613	1	7.770E-05

Table 4: Linear Mixed Effects Model Anova III Test Results for the Full Video Data Set

Data Set : Variable	Analysis	p - value	Spinning Mean	Control Mean
	~ Trial Type*Date | Trial Type	0.1374		
Average Size-corrected Speed (speed units)	~ Trial Type*Date | Date	0.4587	0.7784	1.1778
	~ Trial Type*Date | Trial Type:Date	0.8222		
	~ Trial Type*Date | Trial Type	0.7884		
Average Bearing Change (degrees)	~ Trial Type*Date | Date	0.5155	0.4842°	0.0576°
	~ Trial Type*Date | Trial Type:Date	0.5428		
	~ Trial Type*Date | Trial Type	**0.0382**		
Average Bearing-Heading Difference (degrees)	~ Trial Type*Date | Date	0.1865	27.2806°	45.1210°
	~ Trial Type*Date | Trial Type:Date	0.1764		
	~ Trial Type*Date | Trial Type	0.2371		
Average Change in Bearing-Heading Difference (degrees)	~ Trial Type*Date | Date	0.3091	-1.1950°	0.2522°
	~ Trial Type*Date | Trial Type:Date	0.3814		
	~ Trial Type*Date | Trial Type	0.4911		
Duration (Seconds)	~ Trial Type*Date | Date	0.3329	7.5328	5.6931
	~ Trial Type*Date | Trial Type:Date	0.5721		

Table 5: Linear Mixed Effects Model Anova III Test Results for the Path Analysis Data Set

Variable	Analysis	p - value	Spinning Mean	Control Mean
	~ Trial Type*Date | Trial Type	0.5210		
Directness Difference (degrees)	~ Trial Type*Date |Date	0.6303	6.1956°	1.3237°
	~ Trial Type*Date | Trial Type:Date	0.5235		
	~ Trial Type*Date | Trial Type	0.7797		
Average Size-corrected Speed (speed units)	~ Trial Type*Date |Date	0.9877	0.7784	1.1778
	~ Trial Type*Date | Trial Type:Date	0.8661		
	~ Trial Type*Date | Trial Type	0.0709		
Bearing Change (degrees per frame)	~ Trial Type*Date |Date	0.0621	0.4843°	0.0577°
	~ Trial Type*Date | Trial Type:Date	**0.0430**		
	~ Trial Type*Date | Trial Type	0.3318		
Average Bearing-Heading Difference (degrees)	~ Trial Type*Date |Date	0.2642	27.2806°	45.1211°
	~ Trial Type*Date | Trial Type:Date	0.2870		
	~ Trial Type*Date | Trial Type	0.7536		
Average Change in Bearing-Heading Difference (degrees per frame)	~ Trial Type*Date |Date	0.6682	-1.1951°	0.2522°
	~ Trial Type*Date | Trial Type:Date	0.6319		
	~ Trial Type*Date | Trial Type	0.3130		
Duration (seconds)	~ Trial Type*Date |Date	0.8260	7.5329	5.6932
	~ Trial Type*Date | Trial Type:Date	0.7950		

Table 6: Power Analysis of the Full Video Data Set

Variable	Power	Required mean difference	Observed mean difference
Average Size-corrected Speed (speed units)	0.8485	0.6390	0.4096
Average Bearing Change (degrees per frame)	0.8023	-3.3295°	-0.3329°
Average Bearing-Heading Difference (degrees)	0.8141	26.7606°	17.8404°
Average Change in Bearing-Heading Difference (degrees per frame)	0.8025	4.6314°	1.4473°
Duration (seconds)	0.8178	-3.0055	-1.8784

Table 7: Power Analysis of the Path Analysis Data Set

Variable	Power	Required mean difference	Observed mean difference
Directness (Degrees)	0.8084	-33.1294°	-4.8720°
Average Size-corrected Speed (speed units)	0.8032	0.4149	0.0830
Average Bearing Change (degrees)	0.8037	-26.8985	-13.5946
Average Bearing-Heading Difference (Degrees)	0.8027	-94.7718°	-6.9177°
Average Change in Bearing-Heading Difference (Degrees)	0.8112	16.6897°	3.2725°
Duration (Seconds)	0.8209	1.6376	0.4962

APPENDIX I

DeepLabCut Crab Model Evaluation

%Training dataset	0.95
Training epochs	180
pcutoff	0.60
Train rmse	1.11
Train rmse_pcutoff	1.11
Train mAP	98.85
Train mAR	99.76
Test rmse	4.09
Test rmse_pcutoff	3.87
Test mAP	81.00
Test mAR	85.96