Motion Sensing Behaviour in Weakly Electric Fish

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ABSTRACT

Weakly electric fish use of a self-generated electric field to probe their environment, this behaviour is known as electrolocation. This study investigated two aspects of electrolocation in two species of knifefish (*Apteronotus leptorhynchus* and *Eigenmannia virescens)*. First, we characterized the ability to track moving objects and found that tracking performance did not differ among speeds tested in either species. Second, we characterized a motion-related cue for distance perception, similar to visual parallax, for which rapidly moving objects would be perceived as closer than slowly moving objects. During tracking experiments, the fish remained centered between the moving objects. We hypothesized that the fish use electrosensory parallax to perform this centering behaviour. Thus, we predicted that if one object moved slightly slower than the other, the fish would perceive the slower-moving object as farther away, and would move towards the slower object to remain “centered.” Indeed, our results supported our hypothesis with *E. virescens* moving towards the slower object to an extent that increased with the relative decrease in speed.

RESUMÉ

Les poissons électriques utilisent un champ électrique pour sonder leur environnement, un comportement connu sous le nom d'électrolocation. Cette étude examine deux aspects de l'électrolocation sur deux espèces de poisson- électrique (*Apteronotus leptorhynchus* et *Eigenmannia virescens*). Premièrement, nous avons caractérisé leur habileté à localiser des objets en mouvement et avons découvert que la performance de localisation ne variait pas entre les vitesses étudiés. Deuxièmement, nous avons caractérisé un signal relié à la motion pour la perception de la distance, tel une parallaxe visuelle, pour laquelle des objets en mouvement rapide seraient perçus comme étant plus près que ceux en mouvement lent. Durant les expériences de localisation, les poissons restaient centrés entre deux objets en mouvement. Notre hypothèse était que les poissons utilisaient une parallaxe électro sensorielle pour se centrer. Ainsi, nous avons prédis que si un des objets en mouvement bougeait légèrement plus lentement que l'autre, le poisson le distinguerait comme étant plus loin et se dirigerait vers l'objet bougeant plus lentement pour rester ''centré''. En effet, nos résultats ont soutenu notre hypothèse que les poissons se déplaçaient vers l'objet en mouvement plus lent dans une mesure qui augmentait relativement à la réduction de la vitesse de l'objet.

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EOD - electric organ discharge

EODf - electric organ discharge frequency

N – sample size

SEM – standard error of the mean

CHAPTER ONE: INTRODUCTION

1.1 DEPTH PERCEPTION AND MOTION PARALLAX:

An animal must be able to determine whether an object in its vicinity is a predator or prey. Furthermore, it must also be able to localize the object. This task requires a number of sensory cues, including those involved in depth perception. Animals can use either passive or active sensing to acquire important depth cues. Passive sensing relies on energy already present in the environment to acquire sensory information. An example of passive sensing is a human using light from the environment for vision (Cullen, 2004). On the other hand, active sensing utilizes an animal's self-generated energy to probe the environment (Nelson and MacIver, 2006). Insect antennae, echolocation in bats and dolphins, and electrolocation in weakly electric fish are some well known biological examples of active sensing (Nelson and MacIver, 2006). Using self-generated energy allows the animal to control the intensity, direction, timing, and spectral characteristics of the signal (Nelson and MacIver, 2006). Being able to regulate direction and timing allows the animal to focus the signal towards a specific area of interest and at precise times. This focused signal will provide more relevant information with the expenditure of less energy than would be required to emit a broad wide-spread signal (Nelson and MacIver, 2006). Signal intensity and spectral characteristics may be augmented to enhance the amount of sensory information obtained or diminished to avoid conspicuousness from predators and competitors (Nelson and MacIver, 2006). Limitations of active sensing are reflected in propagation, attenuation, speed, energy requirements, and conspicuousness of the signal (Nelson and MacIver, 2006).

Consequently, active sensing serves to generate a variety of motion-related sensory cues. In the visual system, information for distance estimation is contained in convergent and divergent eye movements, and the associated disparity cues (van der Willigen et al., 2002, Watt et al., 2003). Convergent eye movements occur when focusing on close objects and consist of the eyes rotating towards each other. Divergent movements involve the eyes rotating away from each other to focus on objects that are far away (Watt et al., 2003). Binocular vision lends itself to disparity cues where the animal receives inputs from two points of view (the left and right eye). By comparing the two views the animal is able estimate the distance of an object (van der Willigen et al., 2002, Watt et al., 2003). Optical flow can provide visual depth cues from the motion of objects, boundaries and planes in a landscape (Dyhr and Higgins, 2010). A third common depth cue in vision arises from motion parallax which is generated by the relative motion of an object against a background (Kral, 2003; Ono and Wade, 2005).

Visual motion parallax is the movement of objects of differing distances across the surface of the retina at varying velocities when an animal is in motion or when the object being observed is in motion (Rogers and Graham, 1979, Kral, 1998, van der Willigen et al., 2002). Objects closer to the eye have a greater visual angle and thus pass by the retina more rapidly, while the opposite is true for objects farther away from the eye. This type of sensory recognition can have functional benefits to an animal, allowing it to correctly perceive the distance of an object and thus tailor its pursuit of the object correctly. Kral (1998) performed a study showing how *Tenodera sinensis* larvae (praying mantis) use motion parallax cues to determine depth by having them land on a target object 5 to 20 cm away from a perch. As the animal moved its head side-to-side acquiring depth cues, expecting the target to be stationary, the target was artificially shifted either in the direction or against the direction of head movement. When the experimenters disrupted the motion parallax cues by moving the target in the same direction as the head movement, the mantis larvae perceived the target to be further away and over-shot the target; when the target was moved in the opposite direction, the mantis anticipated a closer target and under-estimated the distance of the target (Kral, 1998).

As previously mentioned humans also utilise visual motion parallax to decipher depth. Rogers and Graham (1979, 1982) performed a number of experiments testing motion parallax in humans using random-dot patterns of 3-D images. These 3-D images appear flat when viewed directly, but as the observer moves his or her head from side to side the 3-D nature can be perceived. They concluded that when head movement was restricted, subjects perceived a flat vertical surface. Conversely, when head movement was unrestricted, subjects correctly identified 3-D shapes (Rogers and Graham, 1979). A subsequent study by Rogers and Graham (1982) used a 2-D random dot pattern displayed on an oscilloscope and found that dots moving in the same direction as the head movement seemed further away and dots moving in the opposite direction appeared to be closer to the observer, analogous to the perception in the praying mantis.

Visual motion parallax is conserved across the animal kingdom; the praying mantis (Kral, 1998), the honeybee and the bumble bee (Kirchner and Srinivasan, 1989, Serres et al., 2008, Dyhr and Higgins, 2010), the American barn owl (van der Willigen et al., 2002), as well as humans (Rogers et al., 1979) all have been shown to possess this visual cue. Much experimentation and knowledge exists in the field of visual motion parallax. However, it remains unclear whether motion parallax cues are used in other senses.

1.2 WEAKLY ELECTRIC FISH

Weakly electric fish experience the world in a manner that is difficult for humans to fathom. Imagine navigating your environment relying not on vision, a sense that many animals rely on for daily functioning and survival, but on the sensory signals related to self-generated electric discharges. These fish must accurately identify predators, prey, refuge, and conspecifics, using an electric sense (Rose, 2004; Moller, 1995).

Animal muscle movement requires the generation of electricity, which can be more easily detected in water. A large variety of aquatic species, from the lamprey to the duck-billed platypus, have the ability to sense this bioelectricity through electroreception (Rose, 2004). A more specialized group of electrogenic fish are able to generate electricity through an electric organ. An electric organ discharge (EOD) can be quite strong (volts) and used to immobilize prey, as in the electric eel, or an EOD can be weak (millivolts) and employed to electrolocate and communicate, as in weakly electric fish (Rose, 2004).

There are two distinct families of weakly electric fish: the Mormyriforms of Africa and the Gymnotiforms of South America. Within these families there exist both wave- and pulse-type EODs. Pulse-type weakly electric fish emit intermittent electrical signals released between longer bouts of silence. Wave-type weakly electric fish, such as *Apteronotus leptorhynchus* and *Eigenmannia virescens*, emit an uninterrupted, sinusoidal-like, wave with a regular frequency. Mormyriforms are predominantly pulse-type, and Gymnotiforms are primarily wave-type weakly electric fish (Kramer, 1999; Nelson, 2006).

Weakly electric fish, such as *A. leptorhynchus* and *E. virescens*, are nocturnal and inhabit dark murky water and therefore cannot rely on sight alone to navigate their environment. Furthermore, these fish fail to develop acute eyesight and experience “blurry” vision (Zupanc, 2004). These fish possess an electric sense to detect prey/predators, to discern their surroundings, and to communicate. Weakly electric fish generate an electric field that surrounds their body using an electric organ in their tail that is comprised of either muscle-derived (myogenic) or neural-derived (neurogenic) cells called electrocytes (Zupanc, 2004). In the electric organ, electrocytes are arranged both in series and in parallel to perform synchronized depolarization producing an EOD with amplitudes in the tens of millivolts (Zupanc, 2004). Objects in the environment produce distortions of the electric field (Lissmann and Machin, 1958). Electroreceptors located on the skin of the fish all over the body sense these distortions that provide an “electric image” of the environment (Heiligenberg and Bastian, 1984).

Furthermore, Gymnotiforms are commonly referred to as knifefish, after the “cutting” motion exhibited while they swim. It is hypothesized that this back-and-forth motion, a form of “active sensing”, allows the knifefish to acquire concise electrosensory information about the surroundings (MacIver et al., 2001; Babineau et al., 2006; Nelson and MacIver, 2006). MacIver et al (2001) hypothesized that *Apteronotus albifrons* was able to ameliorate the type of sensory information acquired by strategically positioning and moving its peripheral receptors located on the skin, through whole body movement while tracking prey. This study observed additional movement performed by *A. albifrons* during prey tracking: backward swimming and tail bending. The head region of a weakly electric fish is known to act as an electrosensory fovea and possesses a high density of electroreceptors (Carr et al., 1982). As such, backward swimming allowed the weakly electric fish to scan prey across the electroreceptors of the head, thereby presumably encoding the electric image more effectively (MacIver et al., 2001). Tail movement also affected the electric image, as it moves the electric organ located in the tail. Observers of weakly electric fish prey-capture behaviour noted that *A. albifrons* performed fast, small-amplitude tail bends when investigating objects. They believe that this behaviour assists with electrolocation, giving the fish more control over the electric image (MacIver et al., 2001).

1.3 TRACKING

With respect to weakly electric fish, tracking refers to movement by the fish that is in phase with an object or animal. In nature, weakly electric fish use tracking behaviour to locate prey, conspecifics, and shelter. Experimental studies have been able to mimic this natural shelter tracking behaviour and have concluded that weakly electric fish can track laterally-moving objects (Bastian, 1987a), longitudinally-moving shuttles surrounding the fish with windows (Rose and Canfield, 1993, Cowan and Fortune, 2007, Roth et al, 2010), and more complex prey stimuli (MacIver et al, 2001). When tracking two objects, with one on either side of the fish, weakly electric fish tend to perform centering behaviour, whereby they position themselves in the center of the space between two objects to be tracked. As an example, experiments have demonstrated that *Eigenmannia virescens* will track and stay within a refuge moving back and forth longitudinally. This study allowed the fish to use visual cues as well as electroreception to perform the tracking task (Cowan and Fortune, 2007). Additional longitudinal tracking responses of *Sternopygus* demonstrated that weakly electric fish are best at tracking moving shuttles when both vision and electrolocation are employed, while mechanoreception is not used. Whereas, frosted lenses covering their eyes and low light conditions caused a mild decrease in tracking ability. While complete darkness resulted in a further decrease in tracking performance, coupled with motion unrelated to the shuttle movement (Rose and Canfield, 1993). This, and an additional study in *E. virescens*, revealed that these fish track objects most reliably using vision and electrolocation, but are also able to track, albeit less well, in the absence of vision (Rose and Canfield, 1993; Stamper et al., 2012).

Certain parameters may affect the capacity for a weakly electric fish to track. These include conductivity, and electrical interference. *Apteronotus albifrons*, the black ghost knifefish, tracked prey better at low conductivities (35 µS / cm) than at high conductivities (600 µS / cm). High conductivities decrease the range of electrolocation; therefore the finding that weakly electric fish perform better tracking behaviour at low conductivities revealed that these fish rely heavily on electrolocation to detect and capture its prey (MacIver et al., 2001). A different study tested lateral tracking behaviour (without vision) in *Apteronotus leptorhynchus,’*, by training the fish to hover between and track two vertical aluminium rods moving side to side. During these trials, weakly electric fish were also subjected to electrical interference. This analysis concluded that broad-band noise and sinusoidal signals slightly higher and slightly lower in frequency than the fish’s own EOD decreased the capability of *A. leptorhynchus* to electrolocate (Bastian, 1987a).

Previous studies have included both lateral and longitudinal tracking in various species of weakly electric fish. In lateral tracking, the tracking objects move side-to-side along the flank of the fish. Under these conditions, the fish moves side-to-side to stay between the moving objects, so it must estimate the object location accordingly. Lateral tracking presents a ‘looming’ stimulus which moves laterally towards the observer (Clarke et al., 2013). This type of tracking can be compared to visual looming where an object is moving towards an observer from far to close. In this type of movement specific receptors on the retina are activated, and as the object gets closer it occupies more of the retina, thereby activating more receptors surrounding the originally activated receptors. As such these objects are perceived as approaching, instead of stationary or receding (Schiff et al., 1962, Schiff, 1965).

By contrast, in longitudinal tracking the fish follows a tracking shuttle moving longitudinally alongside the fish (Cowan and Fortune, 2007; Stamper et al., 2012). Longitudinal tracking involves a ‘sweeping’ stimulus which moves longitudinally, and can be compared to visual sweeping where an object moves across the visual field at a fixed distance. In this type of movement, the stimulus is sweeping from the left of your visual field to the right. Initially the receptors detecting left movement of your retina will be activated, and then as the object passes by your visual field, the center, and then the right receptors will become activated. Therefore different receptors are activated at different times (not all at once) as the stimulus moves across the receptive surface (Dyhr and Higgins, 2010).

Species differences among weakly electric fish may also contribute to diverse tracking capabilities. Field and laboratory studies have been conducted to examine the effect of group size on electrosensory interference in three wave-type weakly electric fish (Tan et al., 2005, Stamper et al., 2010). This work showed a link between increasing group size and increasing electrosensory interference which may have an effect on tracking. Therefore, those fish that occupy a larger social group, as *E. virescens* does, experience more interaction between their electric field and those of conspecifics (Tan et al., 2005). *A. leptorhynchus* are usually found in pairs and therefore encounter electric field interactions, but much less severely than *E. virescens* (Stamper et al., 2010). Lastly, *Sternopygus macrurus*, a most commonly solitary weakly electric fish, come in contact with very low or no electrosensory interference (Stamper et al., 2010). As such, the current study aimed to discover whether species differences, predominantly social organization within groups, leads to dissimilar tracking performances in *E. virescens* and *A. leptorhynchus*.

1.4 ELECTRIC IMAGE

As previously mentioned, weakly electric fish use a self-generated electric field that surrounds their body and is sensed by electroreceptors in their skin. The electric profile of a weakly electric fish is shown in Figure 1-1, with the EOD emanating from the electric organ in the tail. The weakly electric fish acts as a dipole, having a positive and a negative end. As the electric organ discharge oscillates, the positive (head) and negative (tail) ends alternate (Assad et al., 1999; Babineau et al., 2006; Caputi and Budelli, 2006).

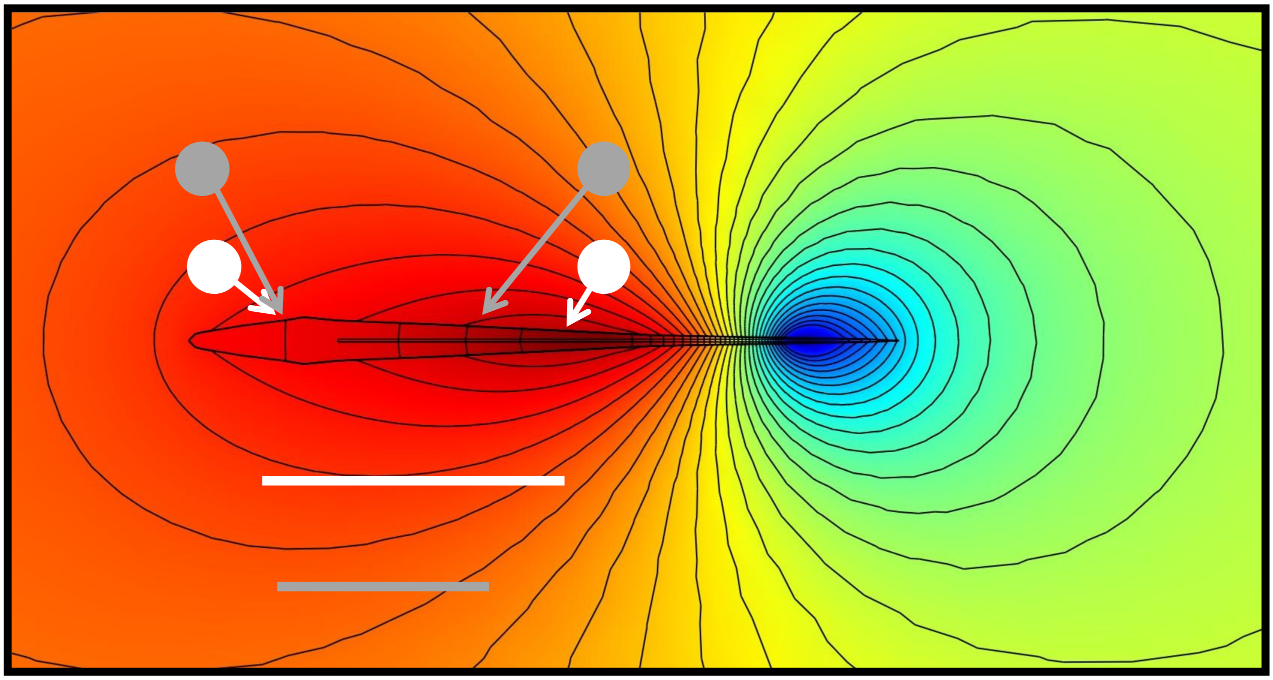

Figure 1-1: Modelling image demonstrating the electric field of the weakly electric fish, *A. leptorhynchus*. Circles indicate object location for both far (grey) and close (white) objects, whereas arrows denote where the electric image peak location projects on the fish's skin. The distance traveled by the object is the space between the two grey circles for far, and between the two white circles for close objects. The white and grey bars represent the distance traveled by the electric image for both close and far objects respectively. Isopotential lines are represented in black.

Among other functions, weakly electric fish use their electrical sense for electrolocation. When the fish senses a distortion of the electric field, it receives an electric image on the skin indicating the position of the object. An object perceived by the fish will project approximately perpendicular to the isopotential lines of the electric field. The curvature of these contours changes with distance away from the fish body axis, as can be seen in Figure 1-1. Thus, a close object will produce an electric image on the skin where the image peak location (where the image projects on the body), is very close to or at the object location. As the object gets further away the difference between the image peak location and the object location increases. As the fish swims by an object, the displacement of the image peak location along the body is larger for closer objects, therefore the image moves faster across the fish's receptors. The displacement of the image peak location is smaller for further objects; thus the image moves more slowly across the skin's receptive surface (Babineau et al., 2006; Lewis et al., 2007).

Further work in this area examined how the electric image changed as objects were presented at various lateral distances and at various rostral-caudal positions to the weakly electric fish (Babineau et al., 2006). Insight provided by previous modelling and experimental studies suggests that weakly electric fish determine how close an object is by comparing the amplitude and slope of the image peak. A close object will produce an electric image with a large amplitude and a narrow peak; whereas an object which is further away will produce a smaller amplitude coupled with a wider peak (Hoshimiya et al., 1980; Rasnow, 1996; Rother et al., 2003; Chen et al., 2005; Babineau et al., 2006). Also examined was how the electric image changes as the object is moved alongside the fish (rostral-caudal). It was observed that as the rostral-caudal position varies, so does the offset between the object location and the electric image peak location (Babineau et al., 2006). This offset, combined with the knowledge that electric images project perpendicular to object location lead to the idea of an electrosensory motion parallax in the weakly electric fish (Lewis et al, 2007). Motion parallax is an active sensing cue produced by the movement of static objects of differing distances across the receptive surface at varying velocities when an animal is in motion. Alternatively, motion parallax also includes instances where the objects are in motion and the observer is stationary (Rogers and Graham, 1979, Kral, 1998, van der Willigen et al., 2002).

The knife-like swimming of weakly electric fish suggests that motion parallax cues may be available to the electrosensory system. However, it is so far unclear whether these fish use such cues for tracking and capturing prey. In the following paragraphs, I will outline the behavioural experiments aimed at testing whether electric fish use electrosensory cues related to motion parallax.

1.5 HYPOTHESIS AND PREDICTIONS

The primary objectives of this study of motion sensing behaviour were:

- to determine whether species differences exists between *A. leptorhynchus* and *E. virescens*, in tracking behaviour, including speed preference and the effect of different objects, and

- to determine whether an electrosensory motion parallax exists in weakly electric fish and whether these fish use motion parallax cues to determine the distance of an object.

The first objective of this study was to determine whether species differences exist between *A. leptorhynchus* and *E. virescens*, in motion sensing behaviour, by testing both tracking and the use of motion parallax. Species differences may arise as a consequence of varying social organization within groups of weakly electric fish (Tan et al., 2005; Stamper et al., 2010).

The second objective was to investigate the concept of electrosensory parallax. The hypothesis of this study is that an object moving more quickly past an electric fish will be perceived as closer, and a slowly moving object will be perceived as farther away. Ideally, during perfect tracking behaviour, there will be no relative motion between the fish and the object being tracked. However, during brief periods of “slip”, there is potential for motion parallax cues, and that the fish are using these cues to determine the distance of the tracked object. Therefore if ‘slips’ are experimentally manipulated, then the weakly electric fish’s distance estimation should be positively affected. The current study utilizes tracking to test the idea of a motion parallax in the electric sense of weakly electric fish.

To test whether motion parallax cues are being used, I vary speeds of two tracking stimuli that will pass by either side of a weakly electric fish. If motion parallax is being used, when the speed of the two separate objects is different (one fast and the other slow), the fish should perceive these objects to be at different distances. I therefore predict that the position of the fish between the two tracking stimuli will systematically change from centered, to a lateral shift away from the faster stimulus and towards the slower stimulus, because it will be performing centering behaviour, perceiving one rod to be closer and the other further away, respectively. I further predict that the magnitude of shift will be correlated to the magnitude of speed difference.

CHAPTER TWO: METHODS

2.1 EXPERIMENTAL FISH

Wild-caught mature, male and female *Apteronotus leptorhynchus* and *Eigenmannia virescens* (8-15 cm in length) were bought from commercial vendors (Straits Aqualife, Below Water, Big Al's Aquarium Services). Fish were housed in small groups at the University of Ottawa Aquatic Care Facility in aerated, flow-through tanks with a 12L: 12D light cycle, a water temperature range of 26.5-29.5 °C, and a water conductivity range of 150-300 µS. Fish were frozen fed blood worms to satiation three times a week.

Preceding a trial, fish were moved to a behavioural tank with the following dimensions: length 61.4 cm, width 31.8 cm, and height 31 cm. Water temperature was 27-29 °C with a conductivity range of 185-230 µS, and the water level was 10 cm deep. Each fish was only used once; there was no repeat testing on any fish.

All protocols were approved by the University of Ottawa Animal Care Committee (BL-229).

2.2 EXPERIMENT 1: TRACKING PERFORMANCE WITH RODS

For tracking performance trials with rods, the fish were enclosed in a modified plastic cast tube measuring 30 cm in length and 4.5 cm in diameter. The tube had two large mesh-covered openings on both sides, as well as mesh covering on both ends of the tube. The mesh-covered areas allowed for electrolocation through the sides, and water flow through the ends, while keeping the fish confined when experiments took place.

In the behavioural aquarium, stainless steel rods (diameter = 1 cm, length = 22.5 cm) separated by a distance of 5 cm, were set up to pass by either side of the modified tube. These fish generally avoid metallic objects, but when tested with plastic the objects were completely ignored. In fact, after habituating to the metallic rods, the fish actively investigated the rods and tracked them more effectively than any other material tested. The rods were attached to two stepper motors located on the top of the aquarium. The two stepper motors were controlled by custom software (written by M. Murphy and J Lewis using NI LabVIEW.

Overnight, preceding the behavioural trial, the knifefish were confined to the inside of the modified tube. During the experiment both rods were moved back-and-forth in parallel with identical speeds of 1, 2, or 4 cm/s presented in random order. The trial length was 60 s stimuli followed by 30 s breaks between trials. Each fish performed 6 trials, thereby experiencing each speed tested twice.

2.3 EXPERIMENT 2: TESTING THE PARALLAX HYPOTHESIS WITH RODS

In Experiment 2, control trials were the same as in Experiment 1 (speed = 2 cm/s). In addition, parallax conditions were performed in which one of the rods was moved at 50 % of the control speed. Other than this speed difference, the methodology was similar to that in Experiment 1. The trial was broken up into control and parallax conditions. Each fish was exposed to three control conditions separated by two parallax conditions (one with the right side, and one with the left side at a reduced speed). The sides at which these speeds were presented were randomly alternated for each fish, in order to eliminate bias to any particular side. The speeds of 1 cm/s and 2 cm/s used in Experiment 1 were similar to those used in previous longitudinal tracking studies. Previous studies used ranges of 0.0025 to 4 cm/s with an optimal tracking performance within 0.0025 and 2 cm/s (Cowan and Fortune, 2007, Roth et al., 2010, Stamper et al., 2012) and 0.6 to 1.2 cm/s (Rose and Canfield, 1993). Again, the trial length was 60 s stimuli (control and parallax) followed by 30 s breaks in between trials.

2.4 EXPERIMENT 3: TESTING THE PARALLAX HYPOTHESIS WITH A SHUTTLE

In these sets of trials, the methodology was modified in an attempt to increase the time spent tracking, and the amount of time the fish remained between two objects. For this purpose, we constructed a tracking shuttle. Different varieties of shuttles made of a plexi-glass type material and with similar dimensions as ours have been used in previous tracking studies with great success (Cowan and Fortune, 2007; Roth et al., 2010). Our shuttle was adapted from that used in Roth et al. (2010) and consisted of two lexan (plexi-glass) panels which were 15 cm in length and separated by 4.5 cm; with a slit 0.6 cm in width and 8 cm in length in the middle of them running vertically, with a height that extends the water column of 10 cm. Located at the bottom and 4 cm from the bottom of the two sheets were two horizontal 2 cm wide and 15 cm long pieces of plexi-glass (Figure 2.1). This additional structure acted as a roof and floor, causing the shuttle to act as more of a refuge than a containment vessel. Prior to manufacturing, modeling work was completed using the electric field model from Babineau et al. 2006. Various shuttle designs were tested and we found that a vertical slit produces an electric image similar to that of a metal rod (J. Lewis, unpublished observations). Additionally, the slit width of 0.6 cm gave the weakly electric fish the clearest electric image (large amplitude and narrow peak). The design of our shuttle allowed the two halves of the structure to move independently of each other, allowing the sides to move at different speeds.

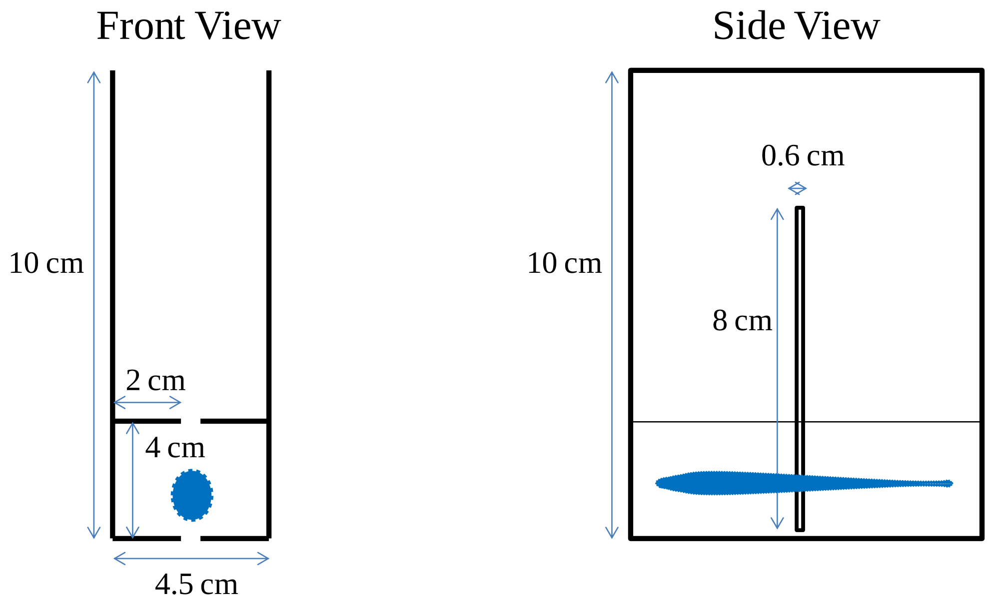

Figure 2-1: Front and side view of the experimental shuttle used. The blue circle (front view) and fish outline (side view) demonstrates the fish position within the shuttle.

Trial length was reduced to 30 s stimuli followed by 30 s breaks. Data from previous trials using rods were examined comparing time spent tracking during the first and second half of the experiment and these showed no significant difference between the beginning and end of the trials, therefore we reduced the trial length (*A. leptorhynchus*: Wilcoxon Signed Ranks Test, p =0.285,; *E. virescens*: Wilcoxon Signed Ranks Test, p = 0.388).

For this set of trials only *Eigenmannia virescens* were used, as *Apteronotus leptorhynchus* did not track or center as reliably in our previous studies. Fish were acclimated for 30 minutes to an hour preceding a trial. Once the fish took up a resting position located centrally between the two shuttle walls the trials would commence. The shuttle was attached to the two stepper motors (those used in the previous methods), and the fish was “trained” to stay between them as they moved longitudinally. Movement of metal rods directed towards the fish when it attempted to exit the shuttle provided an aversive stimulus (negative re-enforcement) causing the fish to remain in the shuttle. These quick negative re-enforcements were only needed a few times. If a fish exited the shuttle during a trial, it was not used for further analysis. During a trial, the shuttle would move in the same fashion as the rods, back and forth alongside the fish.

Just as before, these trials were divided into control and parallax conditions. Within these trials, two reduced speeds were studied, 70% and 90% reductions of the tracking speed. Within each trial, the fish was exposed to the five tracking conditions separated by four parallax conditions (two with the right side, and two with the left side at reduced speeds). The sides at which these speeds were presented were randomly alternated for each fish, to eliminate learning or bias towards one side. The reduced speeds of 70% and 90% of the tracking speeds were chosen as intermediates between the 100% and 50% speeds in the previous experiment.

2.5 ANALYTICAL MEASUREMENTS

EOD:

Electric organ discharge (EOD) frequencies were acquired for both fish identification and trend analyses. Hand-soldered electrodes (teflon-coated silver-wire electrodes, diameter of 0.38 mm, insulated excluding the ends from WPI Sarasota Florida, USA) were used to record the EOD prior to each trial. With all underwater electrical devices turned off, a positive electrode was placed at one end of the fish and a negative electrode at the other end, with the ground electrode also placed in the water. The electric signal was amplified using a WPI DAM 50 Differential Amplifier to 10x amplification with low and high frequency cut offs of 10 Hz and 3 kHz respectively. A custom made LabVIEW program (A. Tang, J. Lewis) was used to record the amplified signal through a PC sound card, and displayed the EOD frequency.

VIDEO ACQUISITION:

Trials were video-taped using a Canon FS30 A Camcorder mounted above the behavioural aquarium, giving a top down view of the experimental setup. Videos were recorded at 30 frames per second, using the “night vision” setting on the camcorder. A lab-made infrared light panel illuminated the behavioural aquarium from below, allowing trials to be video-taped in the dark. This procedure was used to ensure that the weakly electric fish can only utilize electro-sensing, and no visual cues. Infrared light has been used in preceding behavioural trials performed in the dark (MacIver et al., 2001, MacIver and Nelson, 2000).

Videos were then cropped to the appropriate trial length and converted to AVI files using Windows Movie Maker 2.6. A LabView Contrast Image Tracking Program, engineered in the Lewis lab (A. Tang, J. Saab, J. Lewis), was used to identify and extract data points for both the fish and the rod positions in the trials described in Experiments 1 and 2. Manual video tracking software, Video Point 2.5, was used to identify and extract the slit and fish position (nose, pectoral fin, and tip of tail) in the trials described in Experiments 3. Manual video tracking software was also used, which sampled the video every 200 milliseconds yielding 5 frames per second.

STATISTICAL ANALYSIS:

Data were analysed using custom scripts written by J. Lewis (MATLAB 7.0.4, The MathWorks Inc.). A correlation threshold of 0.5 was used to separate periods of tracking from periods of non-tracking during each 30 s trial. This correlation was between the fish and the rod's longitudinal position (y-axis). From this point on only data above our correlation threshold (i.e. periods during which the fish was tracking) were used for further analysis to ensure that the fish was receiving parallax cues. The following parameters were measured for various analyses:

Time Spent Tracking (%): The amount of time that the fish spent between the two rods was calculated by dividing the time spent between the rods by the duration of the trial and multiplying by 100

Lateral Deviation (cm): The standard deviation of the mean lateral position (x-axis position). This measurement indicates how stable the fish was in its lateral position (Figure 2-2). A low lateral deviation corresponds to a fish that maintains a similar lateral position, whereas a high lateral deviation reveals a fish that occupies many lateral positions and has increased movement side to side between the two tracking objects.

Longitudinal Deviation (cm): The standard deviation of the mean longitudinal position (y-axis position). This parameter indicates how stable the fish was in its longitudinal position (Figure 2-2). A low longitudinal deviation was associated with a fish that maintains a similar longitudinal position, where as a high longitudinal deviation demonstrates a fish that occupies many longitudinal positions and has increased movement front and back between the two tracking objects.

Slip (cm): Related to small, quick movements in the longitudinal direction; this measure was the instantaneous mean difference between the fish and the tracking object position over one time step (200 ms). If tracking were perfect, slip would be equal to 0.

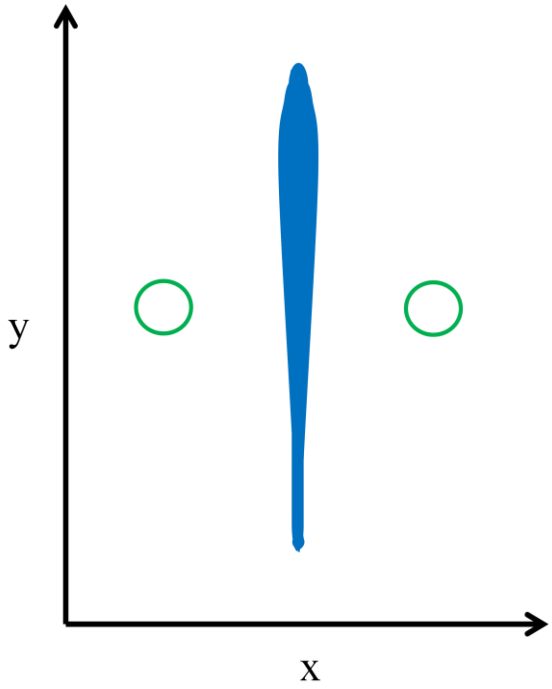

Figure 2-2: Diagram showing the weakly electric fish and its relative x- and y-axes. Longitudinal movement, front and back, represents movement in the y-axis. Lateral movement, side to side, represents movement in the x-axis. The two green circles represent the tracking stimuli, either stainless steel rods or shuttle slits.

SPSS Statistics 17.0 (SPSS Inc.) was used to analyze all data, employing two-tailed tests with a significance of $p < 0.05$. As a first step, all data were tested for normality using the Shapiro-Wilk test. Normality tests revealed that the data were non-parametric. Preliminary speed analyses used unpaired statistical tests, while parallax analyses using rods and slits as tracking stimuli employed paired statistics. All data represents means across similar conditions, unless otherwise stated.

Statistical analysis of Experiment 1: tracking with rods:

To assess tracking performance in Experiment 1 (a), we used two parameters: time spent tracking and lateral deviation. The relationships between speed and time spent tracking, and speed and lateral deviation were evaluated using a Kruskal-Wallis test. The Mann-Whitney test was used to determine whether there was a difference in time spent tracking or lateral deviation between *Eigenmannia virescens* and *Apteronotus leptorhynchus*. Spearman R correlation analyses were used to determine the effect of time spent tracking on lateral displacement.

Statistical analysis of Experiments 2 and 3: parallax conditions with rods and in a shuttle:

Spearman R correlations of various tracking parameters and time spent tracking were carried out to establish tracking trends.

To inspect the weakly electric fish's parallax behaviour, the lateral distance between the fish and the tracking rods was measured. The slope of the trajectory taken by the fish was used to assess trends in the lateral position. To determine if there was a time effect between the presentation of various conditions and the time spent tracking, Wilcoxon Signed Ranked Tests were implemented. Individual fish trajectories (raw data) and behavioural videos were also visualized and scrutinized for potential trends.

The cumulative distribution of the lateral position of *E. virescens* was also used to evaluate centering behaviour. Lateral positions of control and parallax conditions were also compared and significance was tested using Mann-Whitney tests. For the above-mentioned analysis a confidence interval of 90 % or p = 0.10 was used.

CHAPTER THREE: RESULTS

3.1 TRACKING AND CENTERING BEHAVIOUR

The results in this thesis were based on variations of previous tracking experiments in which a fish was centered between and tracks two moving aluminium rods (Bastian, 1987a and b) , Plexiglass plates (Heiligenberg, 1973) and plexi-glass shuttles with windows (Cowan and Fortune, 2007; Stamper et al., 2012). Experiments for this project were modified from these previous trials. The first set of experiments used stainless steel rods as tracking targets, whereas the second group of experiments used plexi-glass plates with a vertical slit, which were more refuge-like and produced more reliable tracking. Computational modelling studies completed in the Lewis lab have demonstrated that the slit in these plates provides an electric image that mimics that of the metallic rod (Babineau et al., 2006; Lewis et al., 2007). In the first scenario (i.e. tracking conditions), fish were positioned between two objects moving in phase at identical speeds. In the second scenario (i.e parallax conditions), the two objects moved in phase but at different speeds, one fast and one slow. Below is an example of data obtained from a tracking condition experiment with *E. virescens*.

From these traces we can see how well a fish tracks the moving objects by examining their longitudinal position (y position) with respect to the tracking object position (Figure 3-1, right panel). The data demonstrate a tracking condition where both tracking objects were moving at identical speeds. In the left panel, we can observe the fish’s lateral position (x position) in between the two tracking objects. These data represents a tracking condition where the fish performs centering behaviour between the two objects.

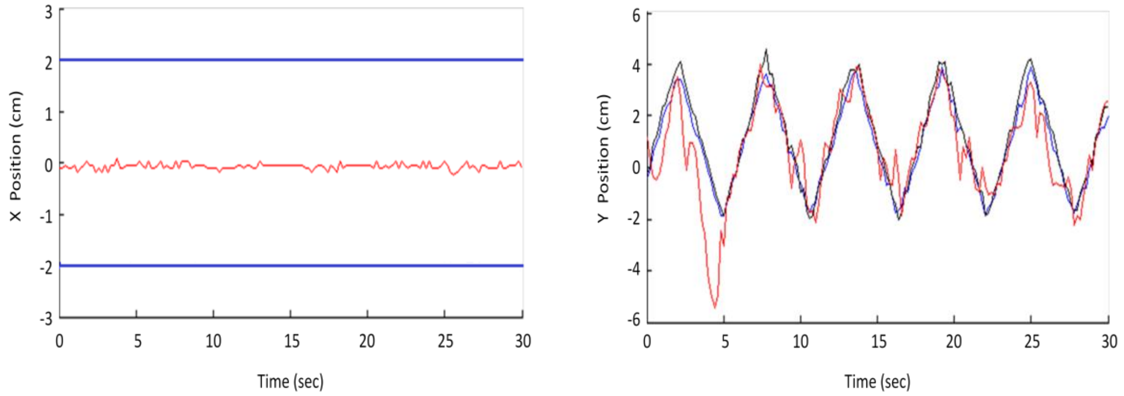

Figure 3-1: Raw data from tracking (two objects at 2 cm/s) experiments. The left panel represents the x position of the fish (red) in between the two tracking objects (blue). The right panel represents the y position of the fish (red) as it tracks the tracking objects (black and blue).

3.2 EXPERIMENT 1: TRACKING PERFORMANCE WITH RODS

Standard tracking experiments - two rods moving at the same speed - were performed to determine whether *E. virescens* and *A. leptorhynchus* track better at a particular speed. To assess performance, including the fish's ability to center itself between the rods, during these tracking experiments two parameters were used: time spent tracking and lateral deviation (see Methods).

The relationship between speed (1, 2, and 4 cm/s) and time spent tracking is presented in Figure 3-2 for both weakly electric fish species. This relationship was not statistically significant for either species (*A. leptorhynchus* Kruskal-Wallis Test, p = 0.319, n = 6; *E. virescens* Kruskal-Wallis Test, p = 0.409, n = 6). Therefore, the fish do not spend more time tracking at any of the speeds tested; these results are similar to previous studies using other tracking protocols (Cowan and Fortune, 2007, Roth et al., 2010). Furthermore, time spent tracking in *A. leptorhynchus* and *E. virescens* did not differ across all speeds combined (Mann-Whitney, p = 0.182, n = 6).

The relationship between speed (1, 2, and 4 cm/s) and lateral deviation is presented in Figure 3-3 for both weakly electric fish species. This relationship was not statistically significant for either species (*A. leptorhynchus* Kruskal-Wallis Test, p = 0.947, n = 6; *E. virescens* Kruskal-Wallis Test, p = 0.981, n = 6). This finding shows that lateral deviation does not differ over the three speeds tested and fish did not perform better centering behaviour at any of the speeds tested. Data were combined once more to examine lateral deviation between the two species of weakly electric fish and revealed a statistically significant difference (Mann-Whitney, p = 0.026, n = 6). From this difference in lateral deviation between the two fish species we can conclude that *E. virescens* show less lateral deviation (better centering behaviour), than *A. leptorhynchus*.

When we correlate the time spent tracking and the lateral deviation combined over all speeds, there was a statistically significant positive correlation for *Apteronotus leptorhynchus* (Spearman R, r = 0.354, p = 0.034) and a negative correlation for *Eigenmannia virescens* (Spearman R, r = -0.421, p = 0.011). These results suggest that time spent tracking may be correlated with lateral deviation. This result strengthens the idea that species differences exist in the tracking behaviour of weakly electric fish. *E. virescens* seemed to perform a more controlled tracking behaviour, for which an increased time spent tracking was accompanied by a decrease in lateral deviation, indicating improved centering behaviour. A less controlled tracking response was seen in *A. leptorhynchus*, where an increased time spent tracking was coupled with an increase of lateral deviation, indicating poorer centering behaviour.

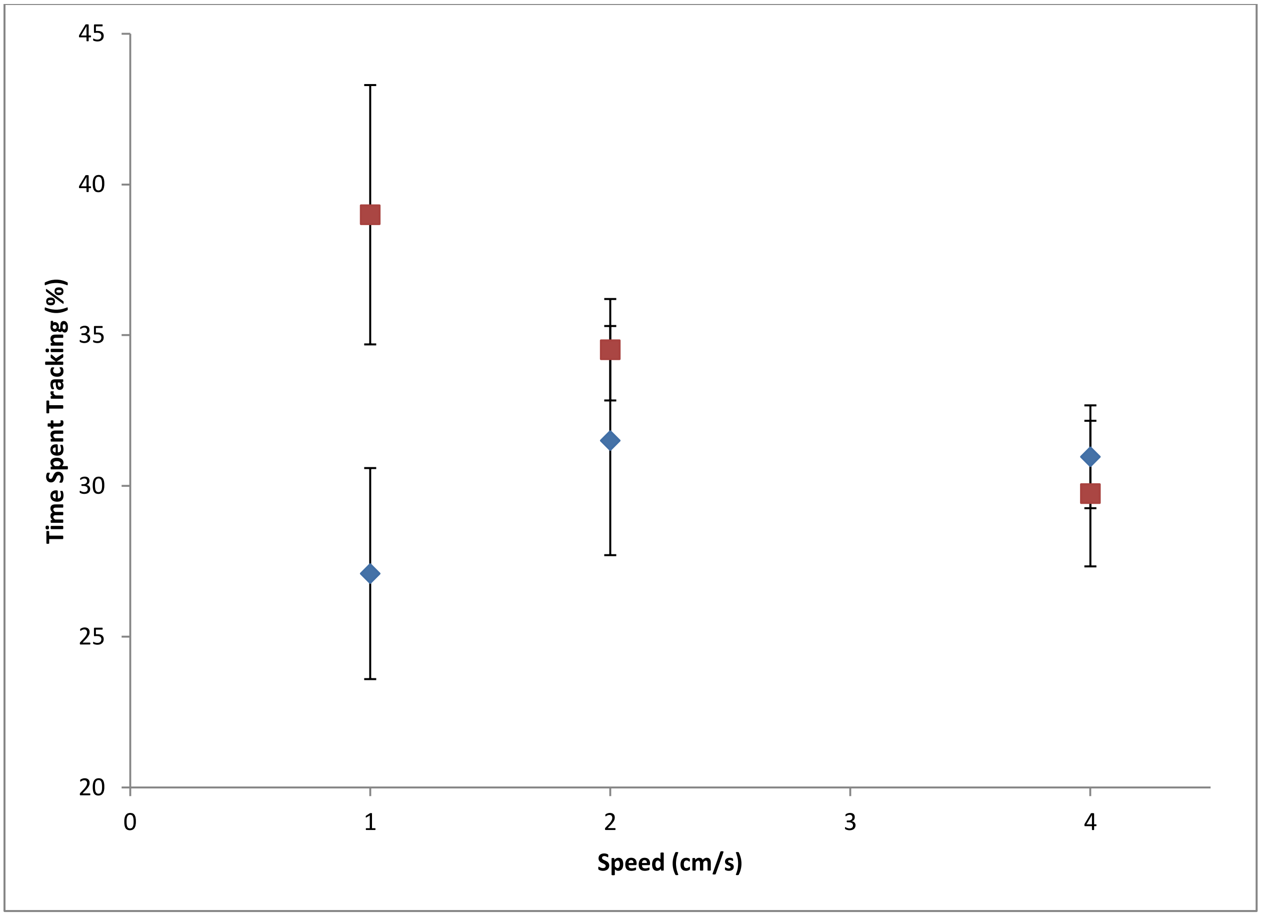

Figure 3-2: Tracking performance determined by time spent tracking in *E. virescens* (red) and *Apteronotus leptorhynchus* (blue) at three varying speeds (1, 2, and 4 cm/s). Data represent the mean of two consecutive trials**.** *E. virescens*, n = 6 and *A. leptorhynchus*, n=5. Error bars represent SEMs.

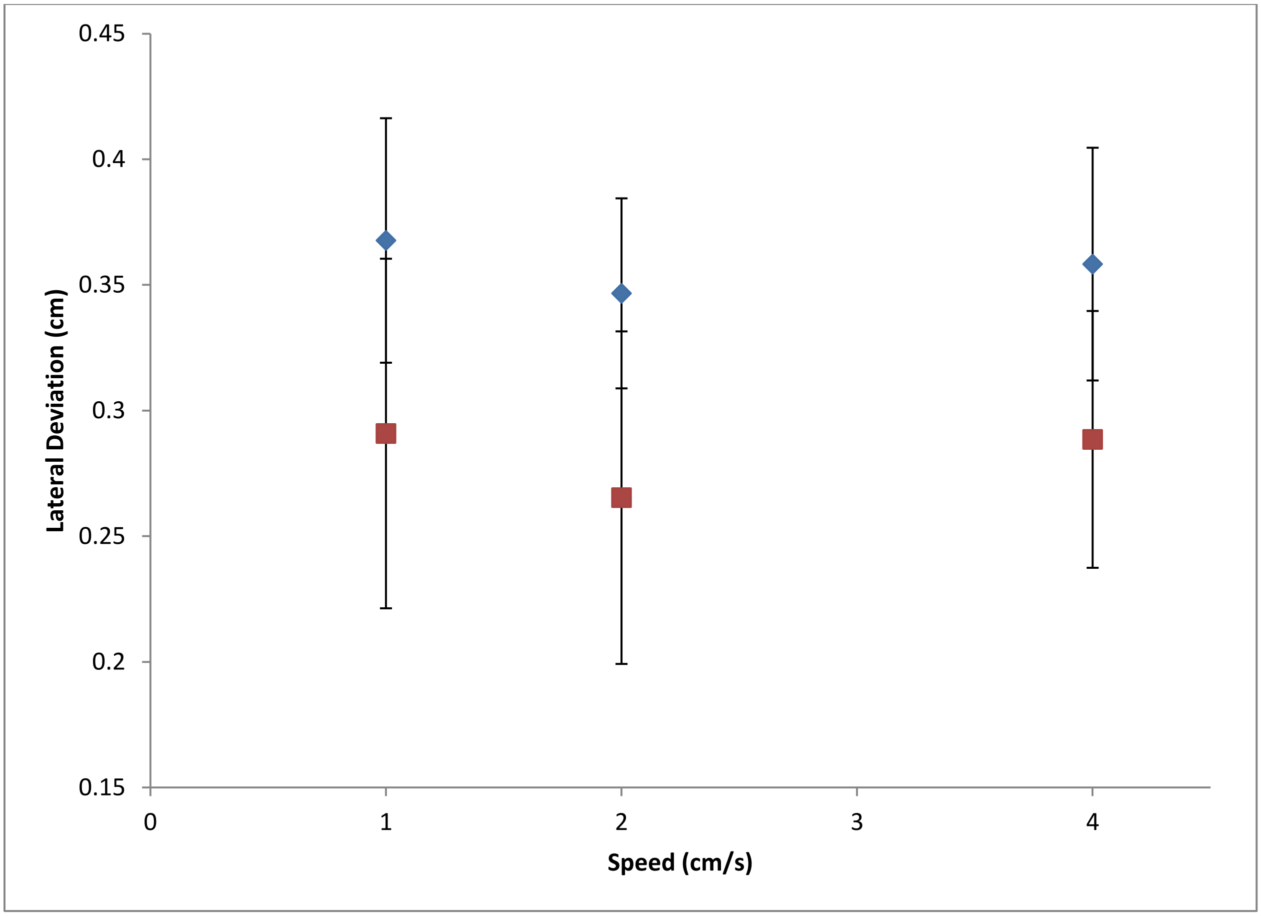

Figure 3-3: Tracking performance determined by lateral deviation in *E. virescens* (red) and *A. leptorhynchus* (blue) at three varying speeds (1, 2, and 4 cm/s). Data represent the mean of two consecutive trials. *E. virescens,* n = 6 and *A. leptorhynchus,* n=5. Error bars represent SEMs.

3.3 EXPERIMENT 2: TESTING THE PARALLAX HYPOTHESIS WITH RODS

I now consider the parallax condition in which objects can move at different speeds. As a step towards understanding this parallax condition, I first compare the tracking performance by examining several tracking parameters under both control and parallax conditions in both species (*A. leptorhynchus* and *E. virescens*).

TRACKING BEHAVIOUR WITH RODS:

To better understand tracking behaviour in both *A. leptorhynchus* (Table 3-1) and *E. virescens* (Table 3-2) several parameters were studied, as previously discussed in the methods. In these experiments rods were used as the tracking stimulus with the speed of the fast rod at 2 cm/s, and the slow rod at 1 cm/s. Results are shown for both control conditions (rods moving at identical speeds of 2 cm/s), and for parallax conditions (alternating slow and fast rods).

The relationship between time spent tracking and multiple tracking parameters is shown in Table 3-1 for *A. leptorhynchus*. During the parallax conditions, a strong positive correlation was observed between time spent tracking and longitudinal deviation (Spearman R, $r = 0.697$, $p = 0.025$). This result shows that the fish that tracked longer had more movement longitudinally front and back in the same direction of movement of the rods.

The relationship between time spent tracking and multiple tracking parameters is also shown for *E. virescens* in Table 3-2. During both control and parallax conditions a strong positive correlation was found between time spent tracking and lateral deviation (Spearman R, control; r = 0.602, p = 0.008, and parallax; r = 0.699, p = 0.011). Furthermore, during both the control and parallax conditions another strong positive correlation was discovered between time spent tracking and longitudinal deviation (Spearman R, tracking; r = 0.647, p = 0.004, and parallax; r = 0.580, p = 0.048). Considering both the parameters with positive correlations and the results for time spent tracking, we observe that fish who track for a longer period of time perform more movement in the lateral direction (side-to-side between the rods), as well as in the longitudinal direction (front and back with the rod movement).

Among these parameters, the slip did not produce any correlation to time spent tracking. In addition, there was no correlation between time spent tracking and EOD frequency, or time spent tracking and size in either control or parallax conditions.

Table 3-1: Relationship between time spent tracking and multiple tracking parameters in *A. leptorhynchus* when subjected to control and parallax conditions with rods as tracking stimuli at speeds of 2 cm/s and 1 cm/s. Significant values are marked with an asterisk. Data represent two-tailed Spearman Correlations; alpha = 0.05; n = 5.

	Control: Percent of Time spent Tracking	
	Spearman Correlation Coefficient (r)	**Significance value (p)**
EODf (Hz)	0.4	0.505
Fish Size (cm)	0.5	0.391
Lateral Deviation (cm)	-0.218	0.435
Longitudinal Deviation (cm)	0.139	0.629
Slip (cm)	0	1
	Parallax: Percent of Time spent Tracking	
	Spearman Correlation Coefficient (r)	**Significance value (p)**
EODf (Hz)	-0.5	0.391
Fish Size (cm)	0.1	0.873
Lateral Deviation (cm)	0.212	0.556
Longitudinal Deviation (cm)	**0.697***	**0.025***
Slip (cm)	0.042	0.907

Table 3-2: Relationship between time spent tracking and multiple tracking parameters in *E. virescens* when subjected to control and parallax conditions with rods as tracking stimuli at speeds of 2 cm/s and 1 cm/s. Significant values are marked with an asterisk. Data represent two-tailed Spearman Correlations; alpha = 0.05; n = 6.

	Control: Percent of Time spent Tracking	
	Spearman Correlation Coefficient (r)	**Significance value (p)**
EODf (Hz)	-0.543	0.266
Fish Size (cm)	0.6	0.208
Lateral Deviation (cm)	**0.602***	**0.008***
Longitudinal Deviation (cm)	**0.647***	**0.004***
Slip (cm)	-0.203	0.418
	Parallax: Percent of Time spent Tracking	
	Spearman Correlation Coefficient (r)	**Significance value (p)**
EODf (Hz)	-0.429	0.397
Fish Size (cm)	0.429	0.0397
Lateral Deviation (cm)	**0.699***	**0.011***
Longitudinal Deviation (cm)	**0.58***	**0.048***
Slip (cm)	-0.343	0.276

PARALLAX BEHAVIOUR WITH RODS:

We now consider these data in the context of the parallax hypothesis. The cumulative probability distribution gives the probability of observations less than the value x (Burr, 1942). In the current study the cumulative distribution function represents the probability that the fish will occupy a position less than position x between our two tracking stimuli. The cumulative probability distributions of the lateral position of *A. leptorhynchus* relative to the control and slow moving rod are shown in Figure 3-4 (upper panel). In these trials, the slow rod travelled at 1 cm/s (50% speed) and the fast rod moved at 2 cm/s (100% speed). The speed was adjusted such that when the rod on one side was fast, the other was slow, and vice versa, so that the fish experienced both speeds on both sides. Control conditions demonstrate centering behaviour (grey lines), whereas parallax conditions show more variable behaviour (blue lines). In the following figure (Figure 3-4 lower panel) we can see *A. leptorhynchus'* lateral position during control conditions (left) compared to parallax conditions (right). The line connecting the two positions represents the lateral shift an individual fish made during the parallax condition (shifts toward the slow rod are indicated in red). Shifts were calculated by subtracting the control and parallax positions, giving us the average lateral distance shifted in response to motion parallax stimuli. Data from repeated control conditions were combined for each individual fish. Data from parallax conditions were first transformed from left/right to slow/ fast and then combined for each individual fish. From this figure we can see that three of the five fish shift towards the slow rod. After statistical analysis, we can conclude that the shifts performed from control to parallax conditions by *A. leptorhynchus*, were not significant (Wilcoxon Signed Ranks Test, p = 0.180).

Figure 3-5 (upper panel) represents identical trials to those listed above in Figure 3-4, showing the results for *E. virescens*. Here both the control and parallax conditions show a tendency for the fish to move towards the fast rod. The subsequent figure (Figure 3-5 lower panel) presents *E. virescens*' lateral position during control conditions (left) compared to parallax conditions (right). Once more with the line connecting the two positions representing the lateral shift the fish has made either towards the fast (black) or slow (red) rod. Of the six fish two performed a lateral shift towards the slow rod, and four shifted towards the fast rod. Similarly to the *A. leptorhynchus* results, the shifts performed from control to parallax conditions by *E. virescens*, were not significant (Wilcoxon Signed Ranks Test, p = 0.109).

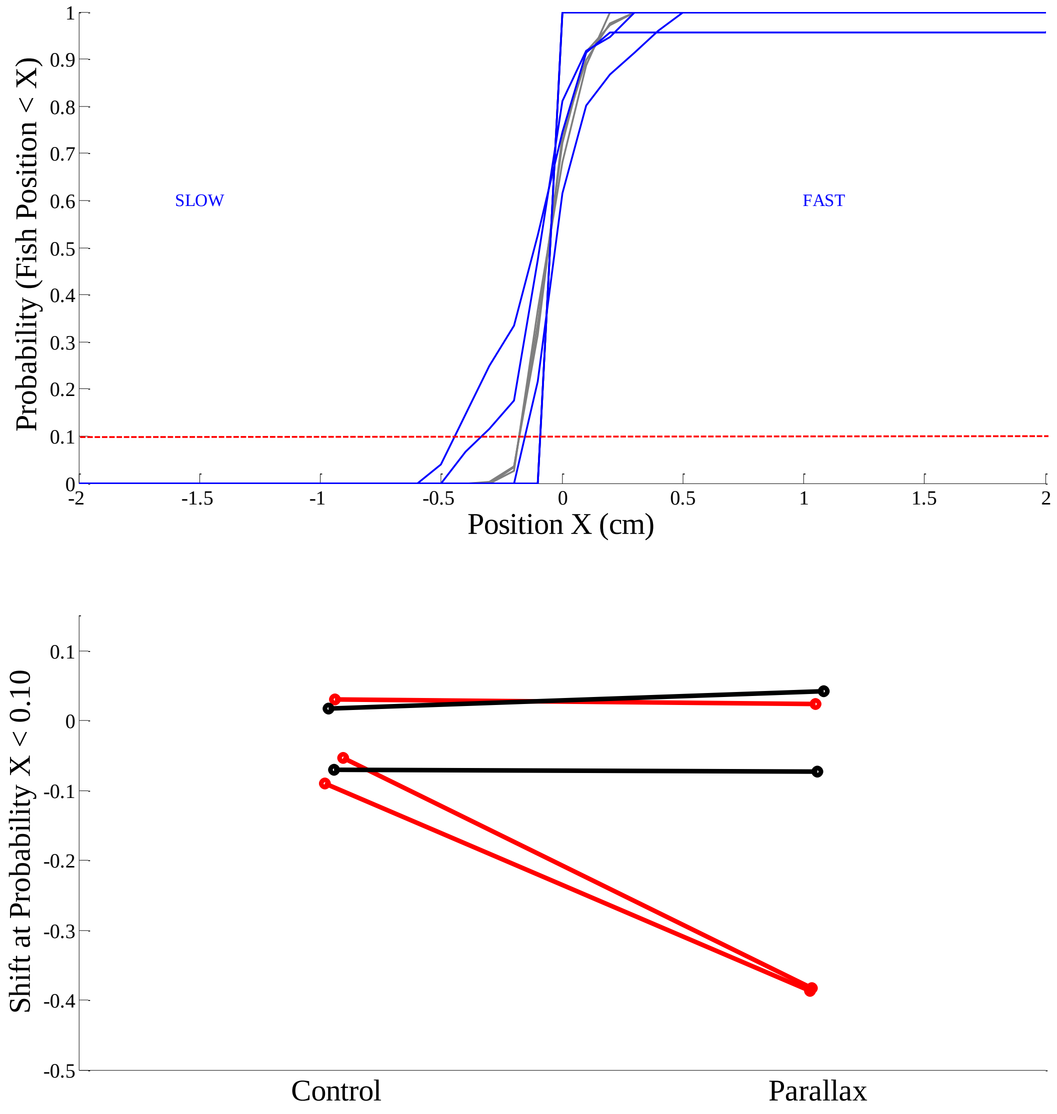

Figure 3-4: Upper Panel: Cumulative distribution of *A. leptorhynchus*'*s* lateral position. Control conditions (grey) represent both rods moving at identical speeds of 2 cm/s. Parallax (blue) represents a fast rod travelling at 2 cm/s, and a slow rod at 1 cm/s. The left of the graph is towards the slow rod while the right is towards the fast rod. The red dashed line represents the probability of fish position < 0.10 illustrated in the lower panel. Lower Panel: The magnitude of the lateral shift performed by *A. leptorhynchus* at probability of fish position < 0.10 from the control (left) to the parallax (right) condition. The bottom of the graph is towards the slow rod (red lines) while the top is towards the fast rod (black lines). N=5.

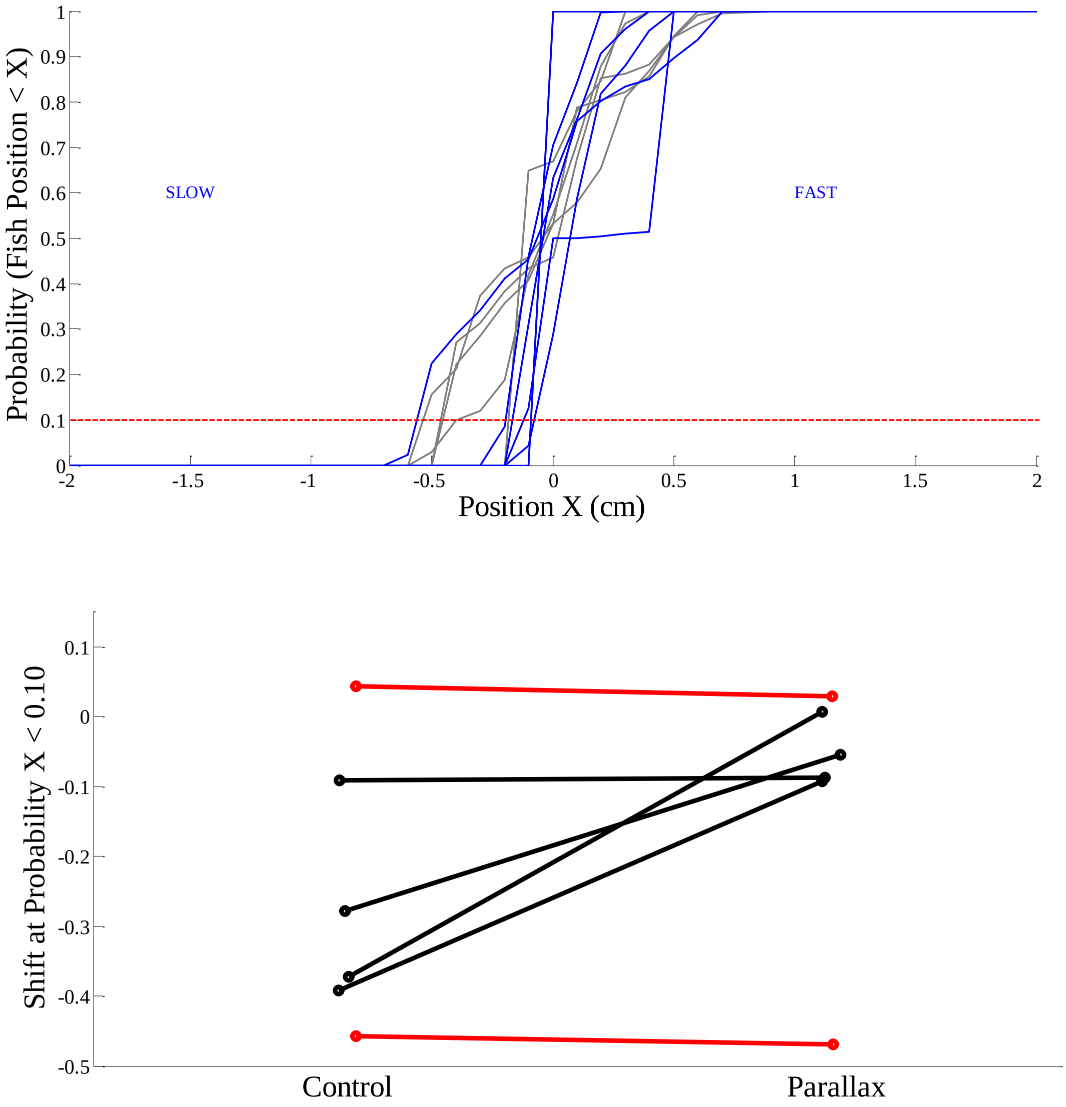

Figure 3-5: Upper panel: Cumulative distribution of *E. virescens*'*s* lateral position. Control conditions (grey) represent both rods moving at identical speeds of 2 cm/s. Parallax (blue) represents a fast rod travelling at 2 cm/s, and a slow rod at 1 cm/s. The left of the graph is towards the slow rod while the right is towards the fast rod. The red dashed line represents the probability of fish position < 0.10 illustrated in the lower panel. Lower Panel: The magnitude of the lateral shift performed by *E. virescens* at probability of fish position < 0.10 from the control (left) to the parallax (right) condition. The bottom of the graph is towards the slow rod (red lines) while the top is towards the fast rod (black lines). N=6.

These motion parallax experiments using rods as a stimulus show inconclusive results for both *A. leptorhynchus* and *E. virescens*. Statistical analysis suggests that neither of the weakly electric fish tested behave consistently when presented with motion parallax cues. These findings do not support the hypothesis that an object moving more quickly past an electric fish will be perceived as closer, and a slowly moving object will be perceived as farther away. Information acquired from these control and parallax conditions with rods was used to design a new methodology for the following trials.

Though this set of experiments provided inconclusive results, it aided in the development of methodology. The use of two metal rods for lateral tracking had been used before successfully in the literature (Bastian 1987 a and b) and initial trials showed that the fish performed more head and tail bending, probing, and scanning behaviours with the metallic rods present, than they did without the rods present. The main issue in our experiments however appeared to be our inability to keep the fish contained between the two metal rods. Thus a modified tube was used to prevent the fish from leaving the space between the tracking stimuli. However this modified tube may have impaired their ability to properly sense the rods. This impairment prompted modifications to the shuttle which will be explained in the next section.

3.4 EXPERIMENT 3: TESTING THE PARALLAX HYPOTHESIS WITH A SHUTTLE

TRACKING BEHAVIOUR IN A SHUTTLE:

Once more, several tracking parameters were examined to better appreciate *E. virescens* tracking behaviour. In the following trials using a shuttle with slits as tracking stimulus, the speed of the fast shuttle side was still 2 cm/s, while the speed of the slow side was either 1.4 cm/s (70% speed: Table 3-3) or 1.8 cm/s (90% speed: Table 3-4). The relative speeds were changed to 70 and 90 % for practical reasons, as 70 and 90 % were intermediates between the control speed of 100 % and the 50 % reduced speed from previous trials. Spearman correlations are shown for control conditions, where the shuttle sides were moving at identical speeds (2 cm/s), and for parallax conditions where the fish was presented with slow and fast sides.

The relationship between time spent tracking and multiple tracking parameters is shown in Table 3-3 for *E. virescens* at speeds of 2 cm/s and 1.4 cm/s (70% speed). During control conditions, a negative correlation was found between time spent tracking and longitudinal deviation (Spearman R, $r = -0.311$, $p = 0.028$). This correlation signifies that fish that spent more time tracking performed less movement front to back, in the same direction of the shuttle.

The relationship between time spent tracking and multiple tracking parameters is also shown for *E. virescens* in Table 3-4 at speeds of 2 cm/s and 1.8 cm/s (90% speed). During both control and parallax conditions a strong negative correlation was found between time spent tracking and lateral deviation (Spearman R, tracking; $r = -0.598$, $p < 0.001$, and parallax; $r = -0.661$, $p < 0.001$). During both the control and parallax conditions another strong negative correlation was discovered between time spent tracking and longitudinal deviation (Spearman R, tracking; $r = -0.529$, $p < 0.001$, and parallax; $r = -0.627$, $p < 0.001$). Both of these parameters combined having negative correlations with time spent tracking signifies that fish who track more perform less movement in the lateral direction (side-to-side between the shuttle slits), as well as in the longitudinal direction (front and back with the shuttle movement).

During parallax conditions an additional correlation was discovered (Table 3-4). A positive correlation between time spent tracking and slip (Spearman R, $r = 0.376$, $p = 0.012$). The relationship between slip and time spent tracking reveals that fish who track for longer periods of time perform more slip behaviour, which can be described as small movements back and forth used to probe an object.

Among these parameters, there was no correlation between time spent tracking and EOD frequency or size in *E. virescens* during either the control or parallax conditions at either speed.

Table 3-3: Relationship between time spent tracking and multiple tracking parameters in *E. virescens* when subjected to control and parallax conditions with two sides of the shuttle as tracking stimuli at speeds of 2 cm/s and 1.4 cm/s. Significant values are marked with an asterisk. Data represent two-tailed Spearman Correlations; alpha = 0.05; n = 10.

	Control: Percent of Time spent Tracking	
	Spearman Correlation Coefficient (r)	**Significance value (p)**
EODf (Hz)	0.399	0.253
Fish Size (cm)	-0.374	0.287
Lateral Deviation (cm)	-0.209	0.146
Longitudinal Deviation (cm)	**-0.311***	**0.028***
Slip (cm)	0.091	0.53
	Parallax: Percent of Time spent Tracking	
	Spearman Correlation Coefficient (r)	**Significance value (p)**
EODf (Hz)	0.28	0.434
Fish Size (cm)	-0.544	0.104
Lateral Deviation (cm)	-0.31	0.051
Longitudinal Deviation (cm)	-0.181	0.263
Slip (cm)	-0.02	0.901

Table 3-4: Relationship between time spent tracking and multiple tracking parameters in *E. virescens* when subjected to control and parallax conditions with two sides of the shuttle as tracking stimuli at speeds of 2 cm/s and 1.8 cm/s. Significant values are marked with an asterisk. Data represent two-tailed Spearman Correlations; alpha = 0.05; n = 11.

	Control: Percent of Time spent Tracking	
	Spearman Correlation Coefficient (r)	**Significance value (p)**
EODf (Hz)	-0.091	0.79
Fish Size (cm)	0.526	0.096
Lateral Deviation (cm)	**-0.598***	**0***
Longitudinal Deviation (cm)	**-0.529***	**0***
Slip (cm)	0.197	0.149
	Parallax: Percent of Time spent Tracking	
	Spearman Correlation Coefficient (r)	**Significance value (p)**
EODf (Hz)	-0.045	0.894
Fish Size (cm)	0.416	0.203
Lateral Deviation (cm)	**-0.661***	**0***
Longitudinal Deviation (cm)	**-0.627***	**0***
Slip (cm)	**0.376***	**0.012***

PARALLAX BEHAVIOUR IN A SHUTTLE:

Next the parallax hypothesis was tested using the following trials. *E. virescens* was subjected to fast slits at 2cm/s (100% speed) and slow slits at 1.4 cm/s (70% speed) and 1.8 cm/s (90% speed). To determine whether there was a difference between the two presentations of right or left slow slits (early vs late presentation) Wilcoxon Signed Ranks Tests were used. The results of these tests indicated that there was no statistical difference between the two right (Wilcoxon Signed Ranks Test, $p = 0.203$) or left (Wilcoxon Signed Ranks Test, $p = 0.799$) slow slit conditions at 70% speed. Similarly, the results of these same tests for 90% speed were that again there was no statistical difference between the two right (Wilcoxon Signed Ranks Test, $p = 0.374$) or left (Wilcoxon Signed Ranks Test, $p = 0.075$) slow slit conditions. These results showed that the order of the presentation of the slow or fast slits did not have an effect on the fish for either set of velocities. Therefore, further analyses combined the two right slow and the two left slow conditions to form one data set for the parallax conditions.

The cumulative distribution of the lateral position of *E. virescens* is shown in Figure 3-6 (upper panel). Control conditions demonstrate centering behaviour (grey lines centered at $x = 0$), while parallax conditions show a strong tendency for the fish to perform lateral shifts towards the slow rods ($x < 0$, blue lines). In the following figure (Figure 3-6 lower panel), we can see *E. virescens'* lateral position during control conditions (left) compared to parallax conditions (right) for the 70 % reduced speed. The line connecting the two positions represents the lateral shift an individual fish made either towards the fast (black) or slow (red) rod. Figure 3-6 (lower panel) goes on to confirm the tendency of the fish to move towards the slow rod in this set of trials. Of the ten fish tested, eight shift towards the slow rod, while only two did not. When the position is compared between control and parallax conditions, we observe a significant difference between the two (Wilcoxon Signed Ranks Test, p = 0.027).

Figure 3-7 depicts the cumulative distribution of the fish's lateral position for the 90% condition. *E. virescens* continues to perform centering behaviour during control conditions, but less rigidly than during parallax conditions of 70% reduced speed. The fish also contribute to the trend by laterally shifting towards the slow rod. Figure 3-7 (lower panel) reveals that ten of eleven fish shifted towards the slow slit, and only one of eleven shifted towards the fast slit. A statistical difference of shift between control and parallax conditions was also detected in the 90% conditions (Wilcoxon Signed Ranks Test, p = 0.017). Thus, overall, using slits as a stimulus, *E. virescens* showed behaviour consistent with the use of motion parallax cues.

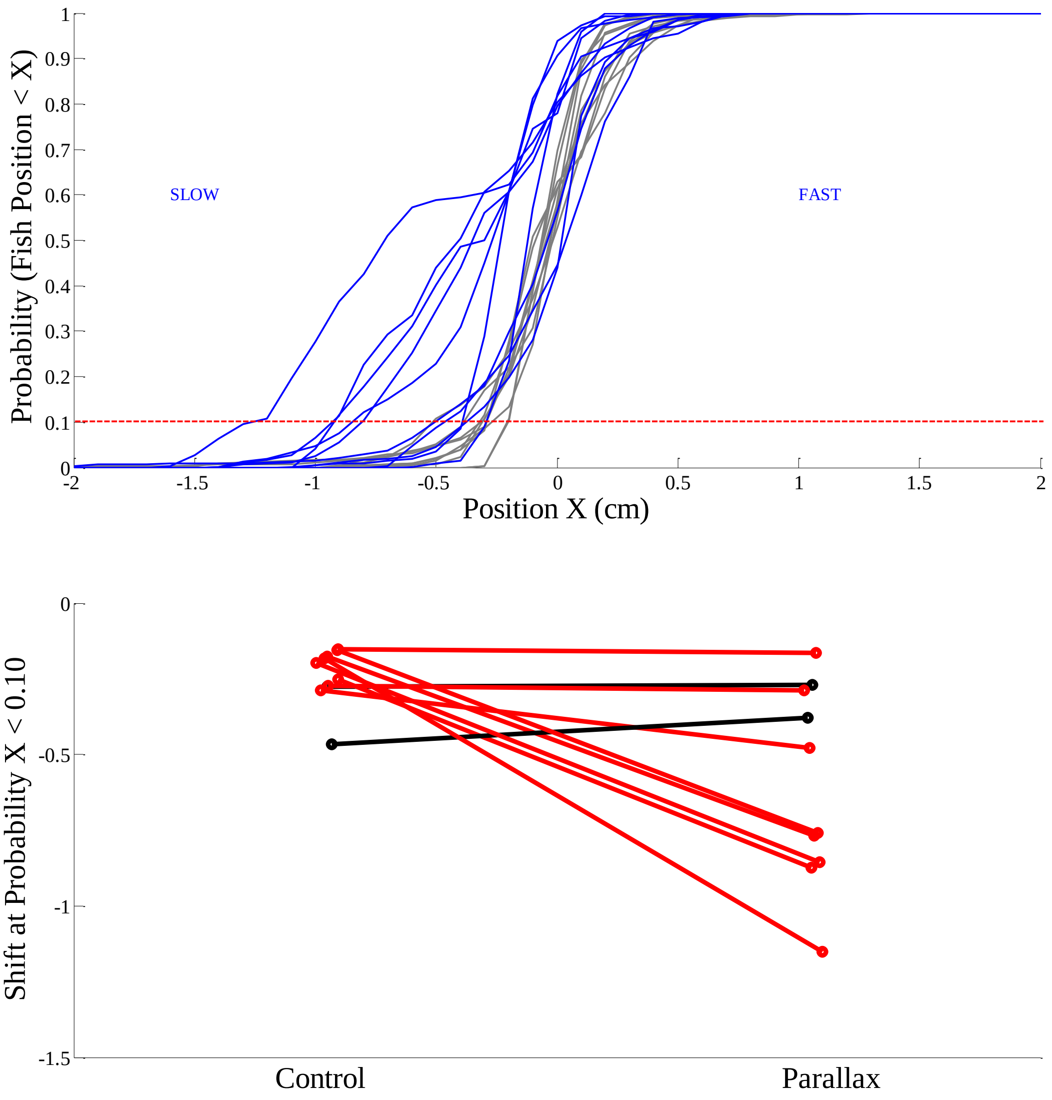

Figure 3-6: Upper panel: Cumulative distribution of *E. virescens*'*s* lateral position. Control conditions (grey) represent both shuttle sides moving at identical speeds of 2 cm/s. Parallax (blue) represents a fast side of the shuttle travelling at 2 cm/s, and a slow side of the shuttle at 1.4 cm/s. The left of the graph is towards the slow side of the shuttle while the right is towards the fast side of the shuttle .The red dashed line represents the probability of fish position < 0.10 illustrated in the lower panel. Lower panel: The magnitude of the lateral shift performed by *E. virescens* at probability of fish position < 0.10 from the control (left) to the parallax (right) condition. The bottom of the graph is towards the slow side of the shuttle (red lines) while the top is towards the fast side of the shuttle (black lines). N=10.

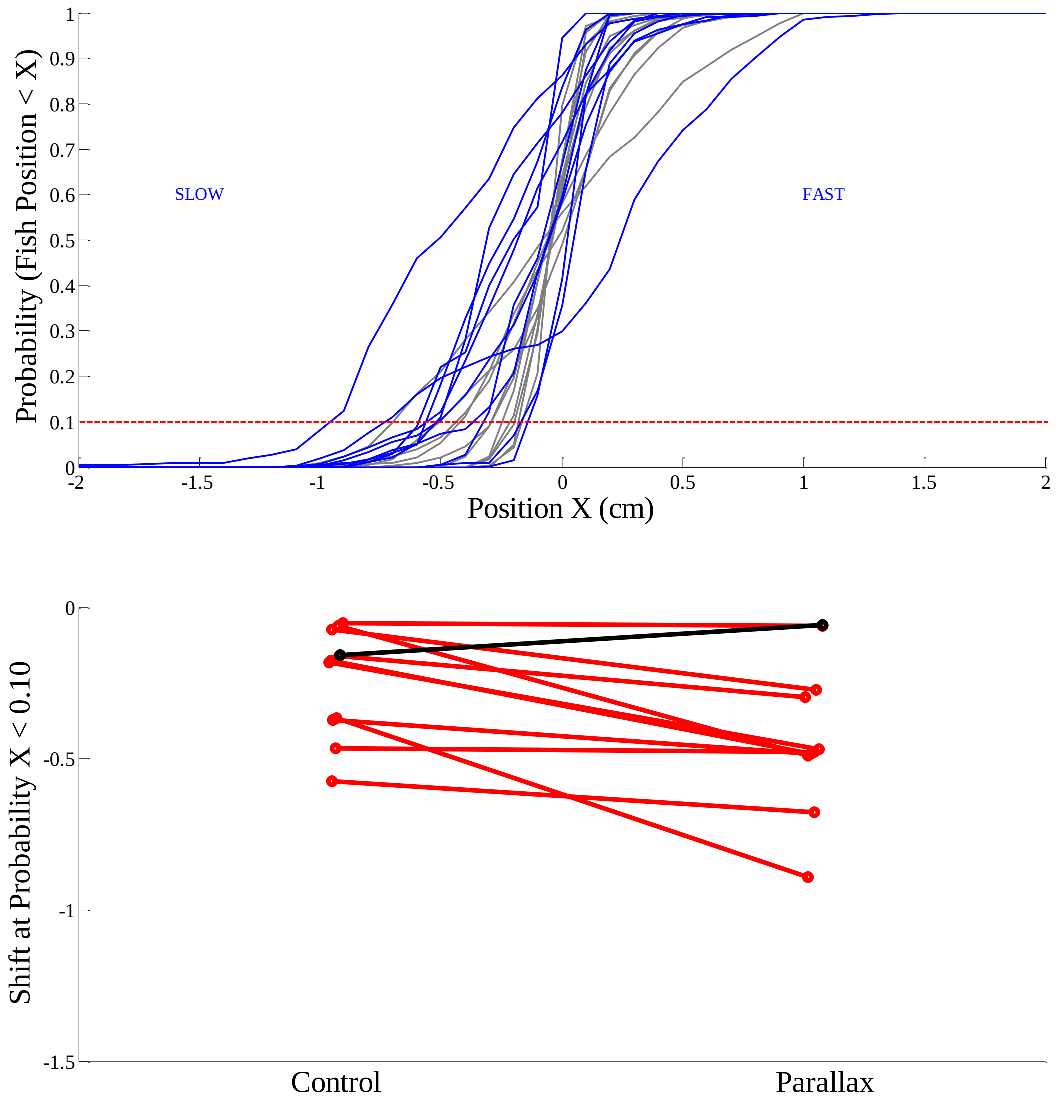

Figure 3-7: Upper panel: Cumulative distribution of *E. virescens*'s lateral position. Control conditions (grey) represent both shuttle sides moving at identical speeds of 2 cm/s. Parallax (blue) represents a fast side of the shuttle travelling at 2 cm/s, and a slow side of the shuttle at 1.8 cm/s. The left of the graph is towards the slow side of the shuttle while the right is towards the fast side of the shuttle. The red dashed line represents the probability of fish position < 0.10 illustrated in the lower panel. Lower panel: The magnitude of the lateral shift performed by *E. virescens* at probability of fish position < 0.10 from the control (left) to the parallax (right) condition. The bottom of the graph is towards the slow side of the shuttle (red lines) while the top is towards the fast side of the shuttle (black lines). N=11.

3.5 COMPARISON OF THE ROD AND SHUTTLE TRACKING METHODOLOGIES

The goal of changing methodologies from rods to shuttle slits as tracking the stimulus was to establish a better tracking performance by *E. virescens*. To determine whether the shuttle improved tracking, we compared our two tracking parameters (time spent tracking and lateral deviation) across Experiment 2, with the old method, and Experiment 3, with the new improved method for *E. virescens* only.

In the following figures we can see 1) the time spent tracking for each of the three varying speeds of parallax performed by *E. virescens* (Figure 3-8) or 2) the lateral deviation (Figure 3-9) for each of the three parallax conditions, where red bars correspond to rod tracking and blue bars to the shuttle tracking. Data shown in the following graphs were only from the control portions of the previously explained experiments; therefore lateral shifts due to motion parallax cues were not incorporated in this analysis.

Times spent tracking were lower in the experiment using the rods, and higher in those using the shuttle (Figure 3-8). This analysis reveals that the weakly electric fish were more prone to track a shuttle with slits on either side than rods. A Kruskal-Wallis Test confirms that there was a significant difference between the three experimental conditions considered (Kruskal-Wallis Test, $p = 0.002$, $n = 27$) for time spent tracking. Further analysis reveals a significant difference among the old method and the new method at both 70 % reduced speed (Mann-Whitney, $p < 0.001$, $n = 16$) and 90 % reduced speed (Mann-Whitney, $p = 0.013$, $n = 17$) in time spent tracking, whereas there was no significant difference between both experiments utilizing the new method (Mann-Whitney, $p = 0.362$, $n = 21$). Lateral deviation (Figure 3-9**)** was tested across the three sets of trials, and there was no statistical difference among experiments (Kruskal-Wallis Test, p = 0.962, n = 27). Centering behaviour was important as it gave us a comparison start point to compare our lateral deviations to in order to calculate shifts. Even with the use of our new methodology, lateral deviation performed by the fish remained relatively similar between the rod and shuttle methods. Nonetheless, an increased time spent tracking by fish in the shuttle, was consistent with previous studies (Cowan and Fortune, 2007; Roth et al., 2010; Stamper et al., 2012).

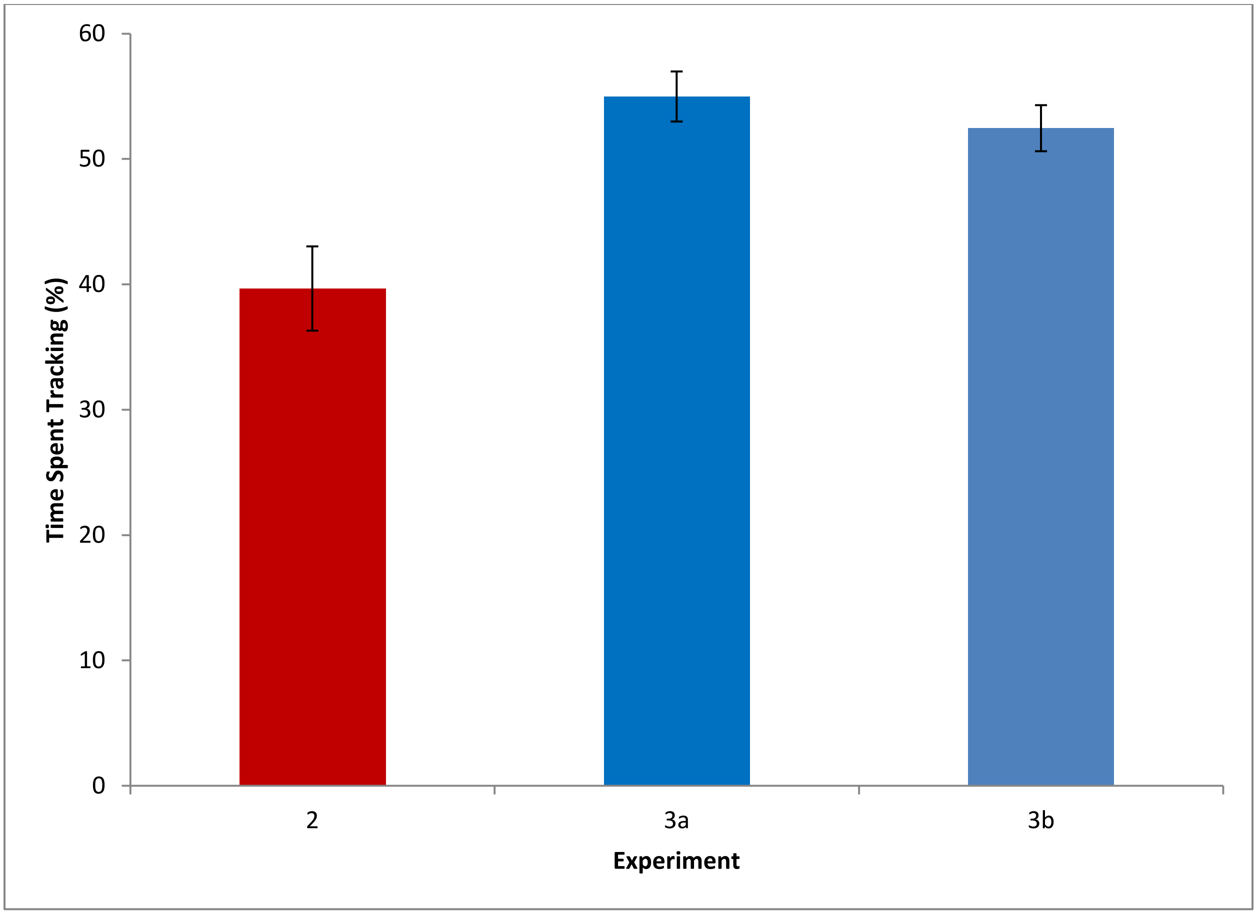

Figure 3-8: Time spent tracking over three varying experiments comparing the old (red bars) and new (blue bars) methodologies. The experiments were as follows: Experiment 2 rod method, Experiment 3a shuttle method (controls for 70 % reduced speed), and Experiment 3b shuttle method (controls 90 % reduced speed). Note that although only control portions were analyzed the speed reduction was used to identify the three experiments. Sample sizes were as follows: Experiment 2, n = 6; Experiment 3a, n = 10; and Experiment 3b, n = 11. Error bars represent SEMs.

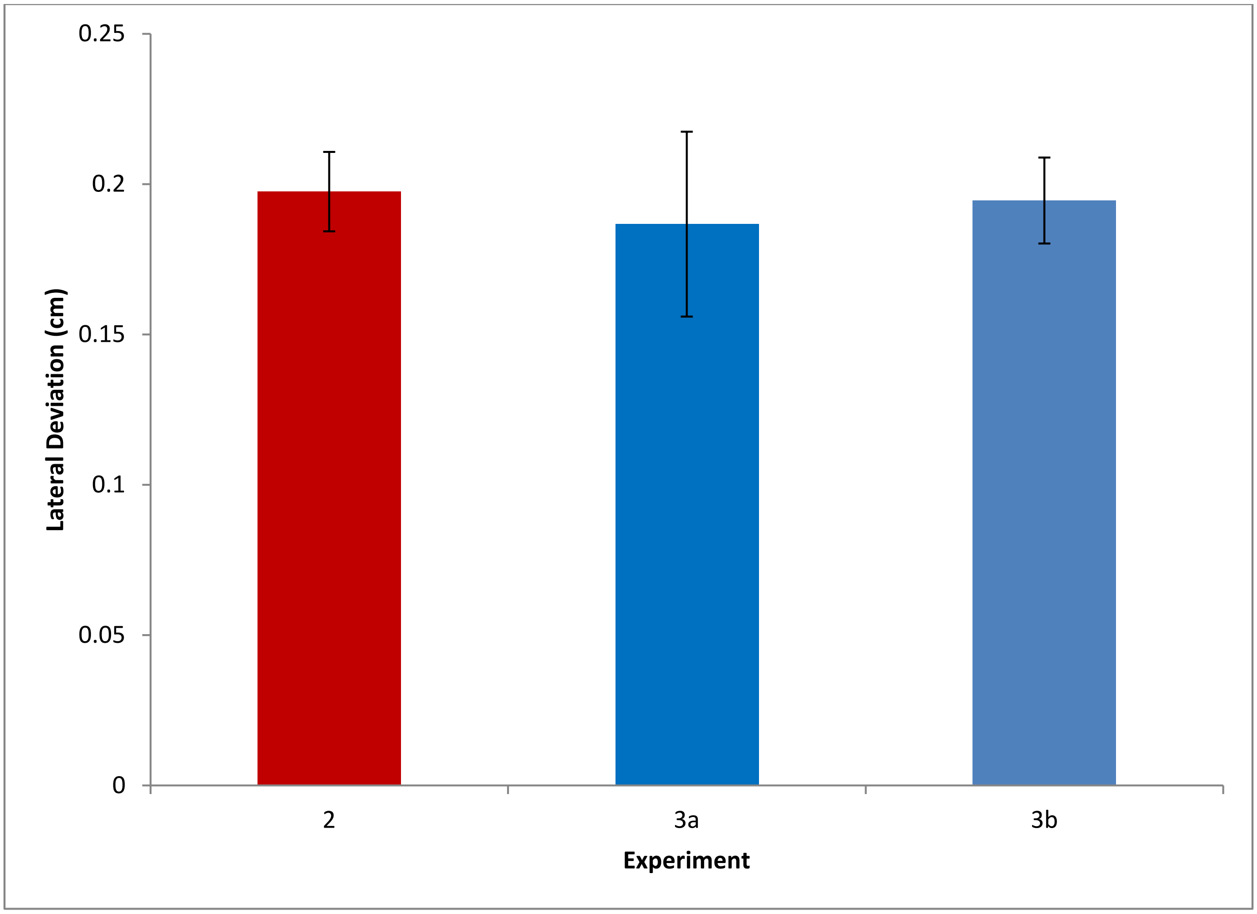

Figure 3-9: Lateral deviation over three varying experiments comparing the old (red bars) and new (blue bars) methodologies. The experiments were as follows: Experiment 2 rod method, Experiment 3a shuttle method (controls for 70 % reduced speed), and Experiment 3b shuttle method (controls 90 % reduced speed). Note that although only control portions were analyzed the speed reduction was used to identify the three experiments. Sample sizes were as follows: Experiment 2, n = 6; Experiment 3a, n = 10; and Experiment 3b, n = 11. Error bars represent SEMs.

CHAPTER FOUR: DISCUSSION

Electric fish live in a complex environment. Poor vision and murky water conditions necessitated the development of sensory systems that circumvent these visual deficiencies. The self-generated electric sense of these fish allows for precise and accurate monitoring of external stimuli, including motion sensing.

The present motion sensing study of weakly electric fish aimed:

- to determine whether species differences exist between *A. leptorhynchus* and *E. virescens*, in tracking behaviour, including speed preference and the effect of different tracking stimuli, and
- to determine whether an electrosensory motion parallax exists in weakly electric fish and whether these fish use motion parallax cues to determine the distance of an object.

As demonstrated by previous tracking studies, as well as the above mentioned data, the electrolocation ability of weakly electric fish extends not only to stationary objects, but to moving objects as well (Bastian, 1987a; Rose and Canfield, 1993, Cowan and Fortune, 2007, Roth et al, 2010; MacIver et al, 2001). Furthermore, results presented above indicate that *E. virescens* has demonstrated lateral shifts towards the slower stimulus, indicative of motion parallax behaviour. This result reveals that their electric sense can assess complex motion cues past simple presence detection and as a result, these fish modify body position and behaviour in a manner that suggests that electrosensory motion parallax cues were at play.

4.1 DISCUSSION OF EXPERIMENT 1: TRACKING PERFORMANCE WITH RODS

Preliminary tracking experiments revealed that weakly electric fish track metal rods more reliably at slower speeds (1 to 4 cm/s) and at reduced ranges of motion (6 cm) (data not shown). These findings were in agreement with those found in Cowan and Fortune (2007) and Roth et al. (2010). As such the remainder of the trials discussed will be within these ranges.

Quantifying the system:

Experiment 1 was completed to assess our system and determine under which parameters and speeds our control and parallax conditions would be tested. At tracking speeds of 1, 2, and 4 cm/s, *A. leptorhynchus* and *E. virescens* do not exhibit increased tracking performance (measured by an increase in time spent tracking and/ or an increase in centering behaviour) at any particular speed. This finding allowed further control and parallax conditions to take place and be compared at any speed within this range. The current study corroborates findings of a previous study performed by Cowan and Fortune, that weakly electric fish track equally well at these different speeds (Cowan and Fortune, 2007).

As previously mentioned, tracking is used to locate prey, conspecifics, and shelter. Each of these three types of tracking is performed at different velocities and is described as either global or local. A global signal activates an entire receptor array, whereas a local signal only activates a restricted portion of the array (Cowan and Fortune, 2007). Prey capture tracking involves a local signal at velocities of approximately 10 cm/s (MacIver et al., 2001). Social signals from conspecifics constitute a global signal and travel at velocities greater than 10 cm/s

(Cowan and Fortune, 2007). Lastly, refuge tracking is considered to be both a local and global signal, and occurs at speeds less than 10 cm/s (Cowan and Fortune, 2007). For each type of tracking, there seems to be a velocity range that is optimum. Perhaps having decided that at the speeds presented the tracking rods were not prey or conspecifics, the speed and the composition of the shuttle revealed that this was a shelter.

Neither the velocity nor the trajectory of the rods in Experiment 1 resembled prey or conspecific movement. Speeds tested in Experiment 1 could resemble those at which aquatic plants or debris sway back and forth in the water. Weakly electric fish are often found in the water near the shore, hiding in aquatic plants or debris (Tan et al., 2005; Stamper et al., 2010). The 1, 2, and 4 cm/s moving tracking rods could be within the range of these slow moving natural shelters. In their natural environment, weakly electric fish have much practice tracking shelters and vegetation that move with the water currents, which could explain why weakly electric fish are so apt and consistent when tracking our slowly moving rods. (Heiligenberg, 1973).

Species comparison:

Additionally Experiment 1 allowed the comparison of the tracking behaviour exhibited by both weakly electric fish species. Correlation results of Experiment 1 lend to the idea that *E. virescens* performs a better tracking behaviour than *A. leptorhynchus*. *E. virescens* demonstrated an increased time spent tracking accompanied by a decrease in lateral deviation showing more controlled tracking behaviour. Increased time spent tracking linked with an increase in lateral deviation, depicts a less controlled tracking behaviour in *A. leptorhynchus*.

4.2 DISCUSSION OF EXPERIMENT 2: TESTING THE PARALLAX HYPOTHESIS WITH RODS

In Experiment 2 several tracking parameters were initially examined in an attempt to identify trends present in control and parallax conditions, followed by an analysis of the effect of a motion parallax stimulus using rods. In the following sections I will examine the results obtained from control and parallax conditions for *A. leptorhynchus* and *E. virescens.* In this instance the parallax condition had a 50% difference in speed between the fast and the slow rod.

Apteronotus leptorhynchus:

A strong positive correlation was found during parallax conditions between time spent tracking and longitudinal deviation, in conjunction with the raw data traces of both the rod and fish's movement. This correlation indicates that during parallax conditions, *A. leptorhynchus* often overshot the tracking stimuli and moved excessively in the longitudinal plane. The current correlations contribute to the theory mentioned above that *A. leptorhynchus* was an inferior tracker.

Motion parallax conditions reveal high variability in *A. leptorhynchus'* tendency to move towards either the faster or slower rod. The motion sensing behaviour performed by *A. leptorhynchus* was quite inconsistent, which makes it difficult to discern whether a trend towards the slow rod exists.

Eigenmmania virescens:

Like *A. leptorhynchus, E. virescsens* also demonstrated increasing longitudinal and lateral deviation with increased time spent tracking. The increased lateral movement performed by *E. virescens* may be due to the fish's tendency to move widthwise in the tube to investigate the moving stimuli. This finding opposes the results of Experiment 1 control condition which described a negative correlation between the time spent tracking and lateral deviation. These discrepancies suggest that a weak or a lack of relationship exists in this data, and further experimentation in this area may be required to determine concrete trends.

Species comparison:

The differences in tracking behaviour between *A. leptorhynchus* and *E. virescens* can be seen in the results of both Experiments 1 and 2. Inconsistencies and a poorer tracking performance by *A. leptorhynchus* contrasted by a more centered and investigative tracking performance by *E. virescens* motivated our decision to only use *E. virescens* in further motion parallax experiments. In reviewing previous non-prey tracking studies in the literature, *E. virescens* was found more often in tracking experiments, while *A. leptorhynchus* was utilized less (Cowan and Fortune, 2007; Roth et al., 2010; Stamper et al., 2012 versus Bastian, 1987a).

Both of these weakly electric fish species occupy similar habitats. Although they can be found throughout the water column, *A. leptorhynchus* and *E. virescens* are most commonly found at the edges of lakes and streams, hiding in root systems, leaf litter, aquatic vegetation, and fallen trees (Stamper et al., 2010, Tan et al., 2005). These habitats and the tendency of these fish to hide under debris and inside crevices, requires the weakly electric fish to possess an acute ability to perceive distance (von der Emde et al., 1998) and movement such as small prey (MacIver et al., 2001; Babineau et al., 2007) using their electric sense in lieu of vision. Perhaps this environment has contributed to the refinement of the weakly electric fishes` motion sensing behaviour over evolutionary time.

Seeing these species differences warrants a closer look at the social organization of these two weakly electric fish species. Group size and density may have contributed to these tracking differences. The group size determines how many electric signals are being emitted by nearby weakly electric fish, while density determines the proximity of these signals. *E. virescens* have been observed in the wild to preferentially occupy groups of 17 to 3 individuals (Tan et al., 2005). By contrast, *A. leptorhynchus* prefer groups of 2 individuals, although they may travel alone or in slightly larger groups (Stamper et al., 2010). Being part of a larger and more closely distributed group would subject the fish to a greater number of electric signals at greater signal strengths. With increased signal abundance and strength the weakly electric fish would experience greater sensory interference, and therefore would need to rely on other strategies to modify incoming sensory information to differentiate between social signals and environmental cues (Stamper et al., 2010). These additional electrosensory signals present in weakly electric fish groups have been demonstrated to cause short-term synaptic depression in *E. virescens* (Rose and Fortune, 1999). Furthermore, Ramcharitar et al. propose that this short-term synaptic depression caused by sensory interference improves motion detection, such as those used in tracking tasks, in weakly electric fish (Ramcharitar et al., 2004). Although more work needs to be completed in this area, this difference could help to explain the finding in the current study that *E. virescens* performs better tracking behaviour than *A. leptorhynchus*.

Additionally, the presence of a group may increase *E. virescens* tracking ability as they must be aware of their conspecific's locations and move fluidly as a group during schooling behaviour. Conversely, *A. leptorhynchus* may rely less on tracking behaviour due to their small or solitary group dynamic, therefore reducing importance of constantly knowing the location of conspecifics. In fact, when *A. leptorhynchus'* groups were analyzed most pairs were male and female. This observation should be noted as males produce a higher EOD frequency, than females, thereby decreasing sensory interference between their signals (Stamper et al., 2010).

Even though it appeared that there was a trend in shifts towards the fast rod, the shift between control and parallax conditions performed by *E. virescens* were not statistically significant. One reason why the weakly electric fish might have chosen to move towards the faster rod was because it was a novel and more interesting stimulus (due to its increased speed), a reason having nothing to do with motion parallax cues.

The behaviour demonstrated in these motion parallax experiments using rods as a stimulus shows inconsistent results for both weakly electric fish tested. Data from Experiment 2 using rods as a tracking stimulus was unable to identify whether the weakly electric fish tested behaved as though motion parallax cues were being detected. Thus, Experiment 2 failed to support the hypothesis that an object moving quickly past an electric fish will be perceived as closer, while an object moving slowly will be perceived as further away.

There are various explanations as to why *A.* leptorhynchus and *E. virescens* tracking performances were sub-optimal in the above mentioned tracking and parallax experiments.

Perhaps being enclosed in the modified tube in Experiments 1 and 2 affected the weakly electric fish's ability to detect the rods. Subsequently the enclosure could have caused a distraction, thereby decreasing the fish's attention given to the moving objects and thus its behaviour. Being trapped within the modified tube could have caused the fish to search for an exit rather than use motion related cues to track the rods. A previous study restrained weakly electric fish (*Brachyhypopomus brevirostrus*) in a PCV tube covered with nylon mesh, which was very similar to the modified tube used here. This report showed little change in EOD pulse rates during investigation of novel stimuli; however restraint could still have an effect on behaviour (Hitschfeld et al., 2009).

In retrospect, it is possible that the speed difference between the two rods in the parallax stimuli of 50 % speed was too great to give the fish proper motion parallax cues to perform reliable shifts. Although the rods were always in phase, the large speed difference between the two rods could have made the rods seem as if they were independent stimuli, and the fish could have been paying attention to just one rod at a time.

Overall, Experiment 2 showed many inconsistencies, which lead to a change in methodology from rods to a shuttle as the tracking stimulus for Experiment 3 to ensure a more reliable tracking performance and to justly test our motion parallax hypothesis. The shuttle provides a better tracking stimulus because the fish prefer to stay within it. It presents a refuge to these fish who naturally want to hide. As aforementioned, in their natural environment weakly electric fish seek refuge in aquatic root systems, vegetation, leaf litter and other debris (Stamper et al., 2010, Tan et al., 2005). The movement of the shuttle may resemble the back and forth movement and speed of aquatic plants (Heiligenberg, 1973).

4.3 DISCUSSION OF EXPERIMENT 3: TESTING THE PARALLAX HYPOTHESIS WITH A SHUTTLE

Experiment 3 follows the same format as Experiment 2 and begins with the examination of several tracking parameters, to more thoroughly understand *E. virescens'* tracking behaviour using a shuttle. This group of experiments also analyzed the cumulative distribution of the lateral fish position and the magnitude of lateral shift to determine whether electrosensory motion parallax cues were being exploited.

Learning from Experiment 2, this set of trials used parallax speeds that were closer to the control speeds (70% and 90% speed), than the previous parallax speed used (50% speed). Working under the assumption that speed reduction is proportional to the lateral shift lead to the realization that a 50% speed difference between slow and fast tracking stimuli would lead to a significantly large lateral shift. If this large lateral shift were to occur, the increased distance between the fish and the tracking object may impede the fish's electrosensing ability, as the weakly electric fish's signal intensity decreases with increasing distance (Nelson and MacIver, 2006). The parallax hypothesis predicts that the reduced speed of 70 % would induce a greater lateral shift from the centered position, than a reduced speed of 90 %. Therefore, the more the speed is reduced, the larger the predicted lateral shift.

Species differences highlighted in Experiments 1 and 2 motivated our choice of only using *E. virescens* to further test motion parallax in a shuttle. As a result of changes, Experiment 3 produced more reliable and centered tracking with decreased variability. This improved tracking behaviour allowed the motion parallax hypothesis to be tested more effectively. In these experiments, the fish tracked slits on either side of a moving shuttle. As these fish naturally take up residence in plastic tubes and other shelters, they may be more receptive to tracking the shuttle slits in a moving refuge. Using this method, the weakly electric fish were surrounded by the tracking stimulus, which could have also made it easier to obtain motion parallax cues. Results from Experiment 3 (new protocol) produced an increased time spent tracking by *E. virescens.* This finding strengthens the argument that weakly electric fish track better utilizing the new shuttle tracking method, and validates the changes made to our methods.

Contrary to results of Experiment 2, during control conditions with the shuttle as a tracking stimulus, fish that spent more time tracking performed less longitudinal movement at the 70 % reduced speed. Traces of the fish and shuttle movement revealed that when tracking in a shuttle, *E. virescens* was capable of matching the speed of the tracking shuttle and moving in the longitudinal direction in phase with it.

Results of Experiment 3 at 90 % reduced speed revealed that fish that track more perform less unnecessary movement in either lateral or longitudinal directions. *E. virescens* tracks the moving shuttle reliably without additional movement from sided-to-side or back-and-forth as seen in Experiment 2. These fish do not seem to be concerned with trying to escape or prodding at the sides of their enclosure nearly as much as those enclosed in the modified tubes with a rod tracking stimulus. The fish may be more apt to tracking the shuttle than the rod because their surroundings were moving all around them, making it easier to obtain motion tracking and motion parallax cues.

Another trend that is worthy of noting was an increase in ‘slip’ behaviour with increased time tracking at the 90% speed. This finding reveals that those weakly electric fish that were able to track for prolonged periods of time were performing increased ‘slip’, which is a type of scanning motion described as small movements back and forth used to probe an object. This finding supports the hypothesis that the weakly electric fish may be using ‘slip’, an active sensing behaviour, for acquiring electrosensory motion parallax cues that allow them to assess the distance of the tracked objects, as previously described (MacIver et al., 2001; Babineau et al., 2006; Nelson and MacIver, 2006). What makes this trend even more robust was that this increase in ‘slip’ was found during a parallax condition when there exists a slight difference in speed between the fast and slow side of the shuttle, thereby forcing the fish to rely heavily on motion parallax cues to decipher which objects were closer and which were farther away. This finding suggests that these fish were in fact paying attention to our stimuli and were modifying their behaviour to better their motion sensing ability.

Encouraging results of cumulative probability and direction of lateral shifts distribution in *E. virescens* were observed for both 70 % and 90 % reduced speeds which showed a lateral position that was shifted closer to the slow rod during parallax conditions. Recall that we hypothesize that the slower moving object was farther away, thereby in shifting towards the slow side of the shuttle we believe that *E. virescens* was continuing to perform centering behaviour based on the motion parallax cues that the fish was receiving. Therefore this lateral shift supports the hypothesis that an electrosensory motion parallax exists in the electric sense of weakly electric fish.

Interestingly, when compared to the 70 % reduced speed, centering behaviour of the control at 90 % speed was less rigid. Therefore, *E. virescens* maintains a more central control position within the shuttle at the 70% reduced speed, versus the 90 %. Additionally, the shift from the control to the parallax position performed by *E virescens* was greater at the 70 %

reduced speed, than the 90 % reduced speed. This result was as predicted in that the more the speed was reduced, the larger the predicted lateral shift. Recall that a close object produces an electric image on the receptive surface at a location that is at or very close to the image peak location. A far object generates an electric image on the skin that is farther away from the image peak location. When the weakly electric fish scan an object, the displacement of the image peak location along the body is larger for closer objects, therefore the image moves faster across the fish’s receptors. For farther objects the displacement of the image peak location is smaller for further objects, thus the image moves more slowly across the skins receptive surface (Babineau et al., 2006; Lewis et al, 2007). For that reason the 70 % speed reduction causes the image to move across the fish’s receptive surface more slowly than the 90 % reduced speed, thereby indicating an object that is further away thereby eliciting a larger lateral shift towards the slow shuttle side. In summation, Experiment 3 demonstrated better tracking and a consistent shift to the slow side in a speed-dependent way. The consistency shown in these trials gives confidence that a motion parallax effect is present.

Overall, the results of Experiment 3 suggest that the electrosensory parallax provides an additional distance cue that can be used for electrolocation and navigation. Therefore motion is critical for electrosensory processing, and these results suggest that motion also plays a role in the acquisition of electrosensory parallax cues. From previous studies, we learned that visual motion parallax is a motion related cue (Kral, 1998; Kirchner and Srinivasan, 1989; Franceschini et al., 2008; Dyhr and Higgins, 2010; van der Willigen et al., 2002; Rogers et al., 1979). The conclusions of this current study contribute to the proposed idea that in the unique sensory system of the weakly electric fish, an electrosensory motion parallax exists. This report constitutes the first to provide evidence that the electric field supplies a foundation for motion parallax in *E. virescens* and that during tracking and centering weakly electric fish perceive an object moving slower as farther away, and faster as closer.

4.4 EOD FREQUENCY AND SIZE

There was no correlation between EOD frequencies or size with time spent tracking at any of the speeds tested in Experiments 2 and 3, in either *A. leptorhynchus* or *E. virescens*. The lack of these correlations goes on to suggest that there were no tracking or parallax behavioural differences between males and females, as EOD frequency and size can both be gender indicators in weakly electric fish. *A leptorhynchus* has an EOD frequency of 600-1000Hz, with males emitting EODs > 800 Hz, and females emitting EODs < 800 Hz (Zakon et al., 2002). Furthermore, *E. virescens* emit an EOD frequency between 225 and 700 Hz, where once more males emit higher frequencies than females (Tan et al., 2005). Size is also correlated with gender, generally larger weakly electric fish are male, while smaller fish are female (Curtis and Stoddard, 2003).

No significant correlation was found between EOD frequencies and time spent tracking. However, the higher EODs emitted by *A. leptorhynchus* were accompanied by more lateral deviation, and a poorer tracking performance, while the lower EODs produced by *E. virescens* showed less lateral deviation and a more controlled tracking behaviour. These EOD differences associated with tracking performance could be due to species differences. In order to prove that there is a relationship between EOD frequency and tracking performance a more thorough within species comparison is needed. Future work could test this theory by examining the difference in tracking ability between high and low EOD frequency *E. virescens*.

An additional consideration when investigating EODs during tracking experiments is the affect of amplitude modulations. Amplitude modulations are caused by the movement of either the fish or the object being sensed, as well as due to interactions between EODs of different frequencies. Rods and slits can therefore produce amplitude modulations of the electric image when sweeping past a fish. The fish can also cause amplitude modulations by moving past the rods and slits. In perfect tracking, completely in phase with no error/ slips, there are no amplitude modulations in time (i.e. the electric image is stationary on the body surface). However slip of the shuttle or rods along the body surface (tracking error), whole-body oscillations, and tail bending produce amplitude changes in time. Each type of amplitude modulation occurs in a different frequency range: tracking error equals the stimulus frequency (current study = 0.17-0.33Hz), whole-body oscillations (0.1-1 Hz), and tail bending (1.5-2.35 Hz) (Stamper et al., 2012). Although amplitude modulations do occur during tracking behaviour, they occur at very low frequencies and are oversampled even by the EOD frequencies of the fish.

4.5 INCONSISTENCIES

Over the course of this study, the many fish tested did not always behave uniformly. A source of inconsistent behaviour could be related to how we acquired these fish. Due to the inconsistency of the weakly electric fish supply, we often had to use several different suppliers to obtain fish as needed. Different suppliers most likely handled fish differently. Handling stress has been shown to alter EOD frequency and amplitude in *E. virescens* (Hitschfeld et al., 2009). Since handling is known to change EOD frequency, it may translate to changes in behaviour as well. To combat the effect of handling stress, fish were acclimated for a period of 2 weeks in laboratory conditions prior to experimentation. An additional factor of the fish coming from various suppliers could have been that our fish may have come from different geographical regions, and different populations. A study of the three-spined stickleback (*Gasterosteus aculeatus*) examined a group of correlated behaviours, known as behavioural syndromes, across 12 different fish populations. The behaviours tested across trials were: aggression, activity, and exploration of food or environment. This study found that behavioural syndromes vary across fish populations. Those sticklebacks living in large piscivorous ponds possessed a behavioural syndrome where aggression, activity and exploration were all correlated. However, fish from small ponds in the absence of predators did not show these correlated behaviours (Dingemanse et al., 2007). The data for three-spined stickleback reveals that habitat and ecological pressures can produce variation across different populations of fish. The existence of behavioural syndromes is also known in weakly electric fish. Isabelle Shank from the Lewis lab found that a behavioural syndrome exists between exploration of novel objects and feeding in male *A. leptorhynchus* in a laboratory setting. Conversely, this study did not find a syndrome between anti-predator, conspecific intrusion or novel environment behaviours, demonstrating that variance (perhaps due to natural population differences) does exist across weakly electric fish, and specifically those in the Lewis lab (Shank, 2013).

The weakly electric fish were acquired all year round; as such varying batches of fish may have been going through different seasonal cycles at the point of capture and delivery to our facility. The effect of breeding or spawning season, as well as other important parts of their yearly cycle could affect how the weakly electric fish perform in the lab. In the lab we also received fish of various ages, thus different maturities may have caused a difference in behaviour among weakly electric fish.

Additionally, our lab conditions (although designed to mimic natural environments) do vary from the habitat occupied by these fish. Glass aquaria, foreign plastic surroundings, not to mention odd moving man-made objects are not encountered in the natural environments and could have an influence on various behaviours not related to the motion sensing.

4.6 FUTURE STUDY

Results suggest that motion parallax cues were being utilized by the weakly electric fish. Nevertheless, other factors could be contributing to the shift towards the slow rod, such as mechanical stimuli through water movement. The current study employed several measures to decrease mechanical stimuli. First, in Experiment 2 a modified tube covered in mesh was used to confine the fish to the area between the two rods. This mesh which covered the tube also served to dampen mechanical stimuli produced by the rods which created water movement. Second, the tracking stimuli were moved through the water longitudinally, as such the thin ends of the shuttle in Experiment 3 were able to cut through the water causing minimal drag. Thicker rods used in Experiment 2 would have produced more mechanical stimuli, than the thin shuttle walls of Experiment 3. The increased water movement in Experiment 2 may have contributed to the observed variability in results. Furthermore, earlier tracking studies using plexi-glass blades situated both in front and on both sides of the fish, as well as a shuttle demonstrated that weakly electric fish do not rely on mechanosensory information to track (Heiligenberg, 1973; Rose and Canfield, 1993). Future study is needed to tease apart which sense is acting and how mechanical stimuli is perceived. For instance a similar study without the movement of water, where a fish is exposed to electrical stimuli which mimics a moving object (one fast and one slow) could aid in determining which sense is at play.

Future work in the Lewis lab will resemble the current study where a motion parallax stimulus is presented alongside a tracking weakly electric fish. However the motion parallax stimulus will be generated electrically using a modified shuttle lined with electrodes, which can send out tracking and parallax stimuli that mimics the electric appearance of rods or shuttles. This work can be used to aid in the creation of weakly electric fish models that can be used to further uncover the mechanisms of an electrosensory motion parallax.

Further investigation could be directed towards understanding how electrosensory parallax information is acquired by the nervous system. Also important would be to determine how this sensory information is transformed and transmitted to the motor system. It would be interesting to determine whether similar neuronal pathways used for an electrosensory motion parallax are also used for electrolocation and tracking. Alternatively research could focus on possible similarities between electrical and visual motion parallax in regards to their sensory and motor systems.

One could also conduct a study of motion parallax with vision as well as the electric sense to establish whether the fish perform the same (vision does not affect motion parallax), better (vision increases motion parallax performance), or worse (vision conflicts with electro-sensory cues thereby decreasing motion parallax performance) using two sensory mechanisms. These experiments could then be performed with vision only, eliminating the use of the electric sense. Previous studies in the absence of vision have demonstrated that weakly electric fish were still able to detect and follow motion cues (Rose and Canfield, 1993), which suggests that electrolocation is necessary for the motion sensing associated with electrosensory motion parallax. Additionally tracking studies performed with both the use of sight and electrolocation show more reliable tracking performance than tracking studies with electrolocation alone (Stamper et al., 2012; Rose and Canfield, 1993).Thus, the elimination of vision may lead to a decrease of the weakly electric fish's ability to perceive motion parallax cues and respond accordingly.

Further experiments could be conducted with varying conditions such as conductivity, electrical noise similar to various tracking studies (MacIver et al., 2001; Bastian, 1987a). These studies can help determine whether motion parallax cues can still be utilized with altered conditions and to determine limits of their detection.

An additional study to outline the limits of parallax detection would involve decreasing the speed difference between the two shuttle walls to determine the threshold of motion parallax detection. Alternatively, studies increasing the speed difference between the two shuttle walls would help ascertain at which point weakly electric fish stop receiving motion parallax cues and begin interpreting the movements as some other cue. A large difference between the two shuttle walls could be perceived as two independently moving stimuli, or even as one moving and one stationary object.

4.7 SUMMARY

Preliminary tracking experiments were performed to assess whether the two species of weakly electric fish tracked better at 1, 2, or 4 cm/s revealed that neither of our fish, *A. leptorhynchus* or *E. virescens,* performed a better tracking behaviour (described as high time spent tracking and low lateral deviation) at any of the aforementioned speeds tested. Therefore our results coincide with those found in the literature, and with speeds associated with tracking shelter or aquatic vegetation (Heiligenberg, 1973; Cowan and Fortune, 2007)

The difference in tracking abilities between *A. leptorhynchus* and *E. virescens* may be due to species differences in group size, group density and schooling tendencies, as *A. leptorhynchus* is most commonly found in pairs, while *E. virescens* in groups of 3 to 17 (Stamper et al., 2010; Tan et al., 2005).

The majority of previous motion parallax studies has focused on the visual sensory systems (Kral, 1998; Kirchner and Srinivasan, 1989, Serres et al., 2008, Dyhr and Higgins, 2010; van der Willigen et al., 2002; Rogers et al., 1979). In spite of all this research on motion parallax, the present work constitutes the first demonstration of a motion parallax in an electrosensory system. Examination of lateral shifts performed when *E. virescens* was presented with a motion parallax stimulus supported our hypothesis that an object moving more quickly past an electric fish will be perceived as closer, and a slowly moving object will be perceived as farther away. In conclusion, *E. virescens,* uses electrosensory motion parallax cues for distance perception. Furthermore these fish perform lateral shifts towards the slow shuttle wall in a speed-dependent fashion. The finding that these fish not only shift towards the slow side of the shuttle, but that they also do so in a speed-dependent way strengthens the conclusion that electrosensory motion parallax cues were being employed by *E. virescens*.

Just as the visual system gave us a clue that motion parallax may also be occurring in other systems, this study will lend insight into motion sensing behaviour of other biological systems and senses. This work will contribute to the motion sensing and more specifically the motion parallax knowledge base, and will help focus further behavioural and neural coding research.

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