A thesis presented for the degree of *Doctor of Philosophy*

Sensory-motor integration in *Gnathonemus petersii*



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DECLARATION

The thesis presented here is my own work, but nonetheless, the following people made contributions towards its content:

1. My primary supervisor, Prof Theresa Burt de Perera, provided support throughout the whole project. She also supplied feedback on the content and structure of this thesis.

2. My secondary supervisor, Prof Gerhard von der Emde, helped with experimental design and data interpretation.

3. Oliver Padget from the Oxford Navigation group helped with developing the generalised linear mixed models used in data chapters two and three.

4. Toby Skeels-Jungius proof-read this thesis.

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GENERAL ABSTRACT

Animals are inundated by stimuli as they undergo the challenges of daily life. To cope, they possess senses which allow them to obtain information from their environment. They can tune into important aspects of this information and decide on the most appropriate response, requiring coordination of the animal's sensory and motor systems. This interaction is not one directional. Animals can actively shape their perception with self-driven motion. Weakly electric fishes are an excellent group of animals to study this bidirectional interaction because they possess a unique active sensing system (their electric sense) which seems to be closely coupled with their own body movements. The aim of this thesis was to investigate sensory-motor integration in the weakly electric fish *Gnathonemus petersii (G.petersii)*. In data chapter one, we found that electric organ discharges were generated during forwards and backwards swimming, suggesting that *G.petersii* have the capacity to collect electrical information bidirectionally. Importantly, we found that electrical activity generated in the two swimming modes only differed when they were investigating objects, indicating that the movements had a role in sensory acquisition. In data chapter two, we found that egocentric motion is important for object recognition, specifically movement-induced modulations (MIMs), which seem to provide information regarding an object's shape. Lastly, we found in data chapter three that MIMs is not always a robust cue for shape recognition, especially when the objects differ subtly in shape and scanning space is compromised. Some evidence suggests that individuals might be able to use other movement related cues instead, e.g. electrical snapshots. This thesis reinforces the importance of self-generated motion in enhancing perception, especially in environments where information is difficult to extract (e.g. the murky rivers that *G.petersii* inhabit). These findings not only improve our understanding of active electrolocation, but of active sensing more generally.

GENERAL INTRODUCTION

1. The principle of sensory-motor integration and the use of active sensing strategies to study this relationship

How sensory and motor systems interact is essential for understanding an animal's behaviour. Sensory systems obtain information which help an individual to decide on what to do in a particular situation, and, in turn, their body movements can sculpt the information detected by these sensing systems (reviewed by Hofmann *et al.,* 2013b). It is important for an individual to identify and categorise signals correctly, as a mistake could have consequences on an individual's survival or reproductive success. For example, mistaking a predator for a conspecific could lead to serious injury or death if courtship behaviours are triggered rather than defensive or escape behaviours.

Active sensing and active sensing strategies are commonly used for studying sensory-motor interactions. Active sensing occurs when an animal emits energy to investigate their surroundings (Nelson and MacIver, 2006). Active sensing strategies are the processes by which sensory flow is modified by movement (Hofmann *et al.,* 2013b). There are many examples of these strategies across the animal kingdom from active eye scanning in insects (Wallace, 1959) to active touch in rodents (Hartmann, 2001). Movements can be isolated to the sensor/emitter of the signal, or be more wide ranging, consisting of whole-body movements (Hofmann *et al.,* 2013b). Echolocation has been well studied in this context- primarily in bats. Echolocation is a form of active sensing, where an animal will produce a series of high frequency acoustic calls and then listen to their echoes as they bounce off objects within the environment. The information from these echoes can then be used to locate prey and to navigate around obstacles (Surlykke *et al.,* 2009). Research has been undertaken on how bats can modify the information they get from this sensory modality, by either moving body parts associated with call production or detection. For example, *Myotis daubentonii* are thought to change the aperture of their mouths to control their 'acoustic

gaze', with a wider mouth allowing for a louder, narrower echolocation beam, meaning that they can be more directional with their calls (Surlykke *et al.,* 2009). Echolocating porpoises can also adjust their 'acoustic gaze' depending on the environmental context (Wisniewska *et al.,* 2012). Additionally, rhinolophid and hipposiderid bats will move their ears quickly which seems to allow them to encode the direction of their target (usually prey) in the form of time-frequency Doppler signatures (Yin and Müller, 2019).

The active sensing strategies we have described so far have focussed on movements of the emitter or sensor, however sometimes whole body movements are involved. Optic flow is a phenomenon observed in insects (as well as other animals). It results from egocentric movement which causes visual images to shift across the retina. Changes in how these images are projected onto the retina provide an animal with precise information about the layout of their environment, thereby allowing them to navigate and forage in complex environments effectively (Srinivasan *et al.,* 2000; Egelhaaf *et al.,* 2014; Kugler *et al.,* 2019). Another example of how egocentric motion can create sensory flow is seen in blind cave fish. They have been shown to use the water movement as they swim past an object to obtain information about its identity and location through their lateral line- known as hydrodynamic imaging (von Campenhausen *et al.,* 1981; Weissert and von Campenhausen, 1981; Windsor *et al.,* 2008). In sum, movements isolated to the sensors or emitters usually play a role in sculpting the sensory input so that it is optimised for perception. Whole-body movements tend to modify the spatiotemporal dynamics of the sensory flow, allowing extraction of certain kinds of information from the environment (Hofmann *et al.,* 2013b).

2. Using weakly electric fish to study the interactions between sensory and motor systems during active electrolocation

Weakly electric fishes are another suitable group for exploring sensory-motor integration, because they have a well characterised active electric sense and show body movements that seem to relate to this modality (e.g. Toerring and Belbenoit, 1979; Toerring and Moller, 1984; Sawtell *et al.,* 2005; von der Emde, 2006; Hofmann *et al.,* 2013b; Engelmann *et al.,* 2021). These fishes have evolved the ability to generate and detect low voltage fields in the environment which they use for localisation. This is commonly referred to as 'active electrolocation' (Lissmann, 1951, 1958; Lissmann and Machin, 1958; Heiligenberg, 1973; reviewed by von der Emde, 2006). They can also communciate with conspecifics using their electric signals, which is known as 'electrocommunication' (Moller and Bauer, 1973; Gebhart *et al.,* 2012; Worm *et al.,* 2017). There are two independent lineages of weakly electric fish: the Gymnotiformes from the Americas (specificially South America) and the Mormyriformes from Africa (Bullock *et al.,* 1983; Winemiller and Adite, 1997). Both lineages developed these abilities at a similar time to one another (Lavoué *et al.,* 2012). Within the gymnotiformes, fish generate their electric fields by producing either pulse or wave-type electric organ discharges (EODs). The vast majority of mormyrids produce pulse-type EODs however. This has led to different ways of encoding environmental information: broadly speaking wave-type species use a frequency code and pulse-type species use a temporal code (reviewed by Sawtell *et al.,* 2005).

Despite being less studied than in other animal groups, a range of active sensing strategies have been identified in both gymnotiform and mormyrid fish. Tail bending around objects is a common behaviour seen in both families, and is thought to improve perception of electric images (object-induced changes to the electric field- these will be discussed in more detail later) and provide cues for lateral distance estimation (Heiligenberg, 1975; MacIver *et al.,* 2001; Sim and Kim, 2011). All weakly electric fish have a semi-rigid body axis thought to keep the electric organ rigid and

enable the integrity of the electric field surrounding them to be maintained (Lissmann, 1958; Lannoo and Lannoo, 1993). Having a stiff body was thought to limit the reafferent noise generated during swimming, however it seems that this is not necessarily the case, and that the nervous system can account for the reafference (Bastian, 1995; MacIver *et al.,* 2001; Sawtell, 2017; Perks *et al.,* 2020). Several set behaviours ('probing motor acts') have been identified in mormyrids during novel object exploration (Toerring and Belbenoit, 1979; Toerring and Moller, 1984). One example is *'va-et-vient'* where the fish swims back and forth repeatedly along an object (Toerring and Belbenoit, 1979; Toerring and Belbenoit, 1984). Similar bidirectional movements have been observed in gymnotiformes (Lannoo and Lannoo, 1993; Nanjappa *et al.,* 2000; MacIver *et al.,* 2001; Snyder *et al.,* 2007; Ruiz-Torres *et al.,* 2013). This motion generates electrosensory flow which is thought to help perception in a number of ways (Hofmann *et al.,* 2013b). It is thought to modify sensory feedback so that it matches with what the neural system is attuned to (Stamper *et al.,* 2012) as well as increasing the sensory volume of the animal, and in doing so, maximising the chance of detecting objects in the environment, for example prey (MacIver *et al.,* 2001; Snyder *et al.,* 2007). It also appears to provide cues for continuous distance estimation, and makes distance perception by motion parallax possible (Hofmann *et al.,* 2013a, 2013b, 2017; Pedraja *et al.,* 2018). Nonetheless, the study of how and why movement modulates sensory flow in weakly electric fish is still in its development, with new insights continuing to be made (Hofmann *et al.,* 2013b; Engelmann *et al.,* 2021).

3. Gnathonemus petersii as a model and their life history

Gnathonemus petersii (G.petersii) is a good candidate species for studying the interactions between sensory and motor systems, given the wealth of information on its active electric sense and various behaviours (e.g. Toerring and Belbenoit, 1979; Toerring and Moller, 1984; reviews: von der Emde, 2006; Hofmann *et al.,* 2013b). *G.petersii* (Günther 1862) is a mormyrid species

found in turbid rivers and lakes across Central and Western Africa. These fish are most active at twilight and night when they will forage for small invertebrates hidden in substrate using an array of senses (Corbet, 1961; Moller *et al.,* 1979; von der Emde and Bleckmann, 1998; Olaosebikan *et al.,* 2020; Fig. 1). Their habitats typically experience rainy and dry seasons (Wuraola and Adetola, 2011). As a result, individuals tend to migrate between different areas over the course of the year- for example they tend to breed during the rainy season in flooded plains (Okedi, 1969 cited in Cain *et al.,* 1994; Landsman, 1991). Like other mormyrids, it is thought that they are predated upon by larger fish, such as electrosensing catfish (Hanika and Kramer, 1999, 2000). As they live in poorly lit and sediment-filled environments, they are heavily reliant on their electrosensory system, though they do have a visual system that is well adapted to these conditions (Landsberger et *al.,* 2008; Kreysing *et al.,* 2012; Pusch *et al.,* 2013a, 2013b; Francke *et al.,* 2014).

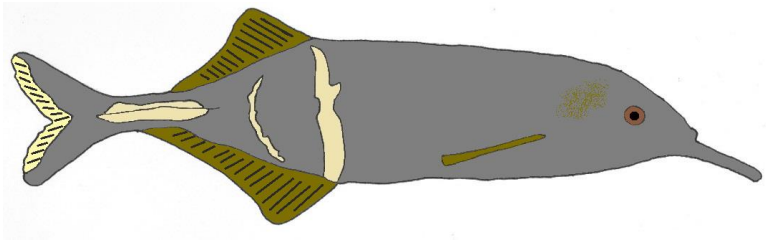


Fig. 1. Illustration of the mormyrid, *Gnathonemus petersii.* They have a striking appearance, which includes an enlarged anal fin, distinctive stripes on the lower flank, and a movable chin appendage (Schnauzenorgan).

4. G.petersii's active electric sense and its role in object localisation

G.petersii use pulse-type EODs to generate their electric fields. These short, discrete pulses are generated from an electric organ (comprising of modified muscle cells known as electrocytes) found in the caudal peduncle (Lissmann, 1958; Bennett, 1971; Moller, 1980; Markham, 2013; Fig. 2). The firing of these electrocytyes is controlled by the pacemaker nucleus in the hindbrain (Bennett, 1971; Markham, 2013). Each EOD produces a three-dimensional electric field around

the fish with a set geometry according to properties of the fish itself (Schwarz, 2000 as cited in von der Emde, 2006; Caputi and Budelli, 2006; von der Emde, 2006). It is an asymmetric dipole field, with a larger pole covering the body anterior to the electric organ, and a smaller pole at the fish's tail (von der Emde, 2006; Engelmann *et al.,* 2008). Objects in close proximity to the generated field distort it as well as local EODs. How the field is distorted depends on the properties of the object. For example, an object which is an electrical conductor will increase field line density, but also increase the amplitude and distort the waveform of individual EODs (von der Emde 2006; Figs. 2 and 3). Specialised tuberous electroreceptors on the skin of the fish (known as mormyromasts) are tuned to detect these voltage pattern changes caused by the object- so called 'electric images' (Bell *et al.,* 1989; von der Emde and Bleckmann, 1992a; Caputi and Budelli, 2006; von der Emde, 2006). The mormyromasts are distributed asymmetrically on the body. The highest density of receptors can be found on the head, particularly in the nasal region (just above the mouth) and on the Schnauzenorgan (their movable chin appendage). Few receptors are found on the sides, and none on the tail (von der Emde and Schwarz, 2002; Hollmann *et al.,* 2008; Pusch *et al.,* 2008; Fig. 2). The Schnauzenorgan and nasal region are considered 'electric foveae', not only due to the high density of receptors they both have, but because of the morphological, physiological, and behavioural adaptations associated with these regions, which seem to make them well tuned for different aspects of foraging (Bacelo *et al.,* 2008; Hollmann *et al.,* 2008; Pusch *et al.,* 2008; von der Emde *et al.,* 2008; Amey-Özel *et al.,* 2012).

The electric images produced are blurred as there is no focussing mechanism akin to that found in vertebrate visual systems (Caputi and Budelli, 2006; von der Emde, 2006; Fig. 3). Nevertheless, this sense provides excellent fine scale spatial information about objects in close proximity to the fish (von der Emde, 2006; von der Emde *et al.,* 2010; Schumacher *et al.,* 2017a). Since *G.petersii's* EODs have a fairly stereotypical (biphasic) appearance, but the time between their pulses (known as inter-discharge intervals, or IDIs) is highly variable, they encode information in a temporal format (von der Emde, 1993; Sawtell *et al.,* 2005; Engelmann *et al.,* 2008). The electrical

information is first transmitted to the electrosensory lateral line lobe in the hindbrain, and then sent to the nucleus lateralis in the midbrain for further processing where a somatotopic map of the active electric sense exists (Hollmann *et al.,* 2016). Mormyrids also have another set of epidermal, tuberous receptors known as 'knollenorgans'. These are tuned to electric signals produced by conspecifics, so are involved in social behaviours (Szabo and Fessard, 1974; Moller, 1976; Hopkins and Bass, 1981; Bell and Grant, 1989; Poulet and Hedwig, 2007; Bacelo *et al.,* 2008). Note that we will not be touching on the topic of electrocommunication in this thesis, as our interests lie in how the active sense is used for object detection and recognition instead.

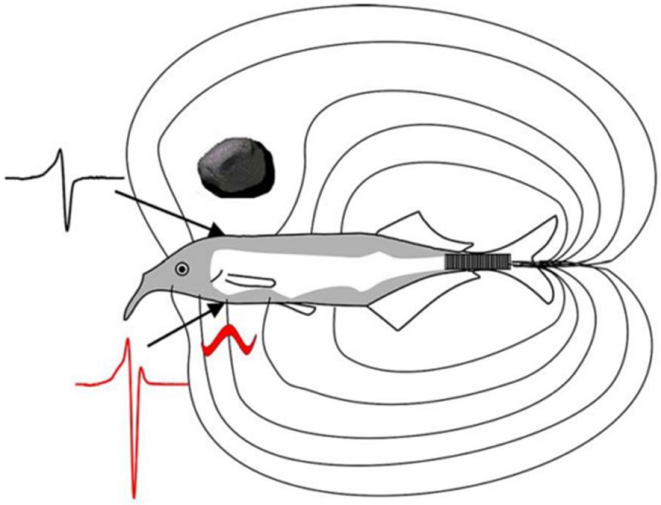


Fig. 2. Schematic of *G.petersii's* active electrosensory system. EODs are produced by the electric organ in the caudal peduncle (black bar) to form electric fields around the animal. The field and local EODs are modified by nearby objects. Modification depends on the properties of the object. The worm (shown in red) is a conductor so increases field line density, and increases the amplitude of local EODs in addition to distorting their waveforms. Conversely, the stone (shown in black) is a non-conductor so field line density decreases. At the same time, the amplitude of local EODs decreases but their waveforms remain the same. These object-induced changes are then detected by specialised receptors on the skin (shown in grey). Diagram from von der Emde (2006).

5. Other sensory systems used by G.petersii

The active electric sense is *G.petersii's* most dominant sense, particularly at short distances- less than four centimetres (von der Emde *et al.,* 2010; Schumacher *et al.,* 2016a, 2017a, 2017b). However, they will use other sensory modalities depending on the circumstances (von der Emde and Bleckmann, 1998; Moller, 2002; Rojas and Moller, 2002; Schumacher *et al.,* 2016a, 2017a, 2017b). Having multiple senses is advantageous, as they can complement one another if they provide similar types of information, and they may even work 'synergistically', meaning that perception is better with both senses than with one alone (Moller, 2002; Rojas and Moller, 2002; Schumacher *et al.,* 2017a). They can also be used 'redundantly' where one sense will replace another if it is deemed more reliable (Moller, 2002; Rojas and Moller, 2002; Schumacher *et al.,* 2017a). Together, these senses will enable an individual to behave robustly, especially during periods of variability, such as the changing of the seasons.

Like many aquatic vertebrates, *G.petersii* can detect external electric fields via ampullary receptors on their skin (a process known as passive electrolocation) which can provide cues for prey capture, and possibly has a role in navigation (Peters and Bretschneider, 1972; Bell and Russell, 1978; Kalmijn, 1982; von der Emde and Bleckmann, 1998; Collin and Whitehead, 2004; Caputi and Budelli, 2006; Baker *et al.,* 2013). As mentioned earlier, *G.petersii* have eyes that are highly adapted to their environment. Cone and rod photoreceptors are arranged in bundles within the retina. It is thought that this arrangement helps *G.petersii* to spot large, moving objects even in poor lighting (Landsberger *et al.,* 2008; Kreysing *et al.,* 2012; Pusch *et al.,* 2013a, 2013b, Francke *et al.,* 2014). These fish will switch to using visual cues over longer distances when the active sense is no longer reliable, showing a dynamic weighting of incoming cues (Schumacher *et al.,* 2016a, 2017a, 2017b). This weighting is seen in other species of weakly electric fish as well (Dangelmayer *et al.,* 2016; Sutton *et al.,* 2016). *G.petersii* also exhibit cross-modal recognition where they can obtain and store information about an object from one sense, and then access

and use this information with another (Schumacher *et al.,* 2016a). This has been studied in the context of the active electric sense and vision, but probably applies to other combinations of senses. This flexibility in sensing allows individuals to respond to things in their environment more accurately and quickly (Schumacher *et al.,* 2016a).

Touch is an important component of *G.petersii's* perception of their environment. In mormyrids, touch plays a role in object exploration (e.g. von der Emde and Schwarz, 2002) as well as in social interactions, such as courtship displays (Kirschbaum, 1987) and agonistic encounters (Crockett, 1983, 1986 cited in Moller, 1995; Terleph, 2004). As we have established, *G.petersii's* Schnauzenorgan plays a prominent role in electrosensing, acting as an electric fovea, being highly adapted to short range object detection (von der Emde and Schwarz, 2002; Bacelo *et al.,* 2008; Hollmann *et al.,* 2008; von der Emde *et al.,* 2008; Amey-Özel *et al.,* 2012). However, the Schnauzenorgan is also considered to be a source of tactile information during object exploration, as *G.petersii* have often been observed using their long chin appendages to tap objects in close proximity to them (von der Emde and Schwarz, 2002; von der Emde and Fetz, 2007, Fechler *et al.,* 2012). Recent work suggests that the Schnauzenorgan does contain structures akin to human touch receptors, so it is feasible that touch could be used in part to detect and potentially aid discrimination between objects (Amey-Özel *et al.,* 2019). Nevertheless, it seems that tactile cues are not necessary for successful object recognition (von der Emde and Fetz, 2007). Instead, tactile information obtained from the Schnauzenorgan likely plays a supporting role, complementing and/or adding to the information acquired through electrosensing, especially since there seems to be a coupling between the two sensory systems (Amey-Özel *et al.,* 2015, 2019). In some situations, touch may even act as a replacement sense when other sensory modalities are compromised (Walton and Moller, 2010). As we have mentioned before, having access to multiple senses is beneficial to *G.petersii,* as it enables them to maintain a stable and reliable impression of their environment even if conditions change around them (Schumacher *et al.,* 2017a).

G.petersii also have an array of other lesser known sensory modalities which can work alongside the more primary sensory modalities. They possess a mechanosensory lateral line that can be used to detect pressure changes in water which seems to be useful for locating moving prey (von der Emde and Bleckmann, 1998). Having said that, their lateral line is significantly reduced when compared with other teleost fish (Schumacher, 2018). This is likely due to the lateral line system being outcompeted by the electrosensory system, which occupies a similar niche but can provide more detailed information about the environment (Moller, 2002; Schumacher, 2018). As such the lateral line may play more of a supporting role, being more useful when considered alongside other sensory modalities (Schumacher, 2018).

G.petersii also have excellent hearing. This species is able to detect a broad range of sound frequencies (between 100 and 2500 Hz) with maximum sensitivity ranging from ca. 300 to 600 (Moller, 1995; Moller, 2002) or up to 1000 Hz (McCormick and Popper, 1984). As such, they have the ability to respond to a wide range of sound cues within their environment. This species is also known to produce clicking sounds from very low frequencies up to 8000 Hz (Rigley and Marshall, 1973). The function of these clicks is unknown, but they seem to be associated with their righting response and relate to agonistic encounters with conspecifics (Rigley and Marshall, 1973; Moller, 1995). As such, it is possible similar cues might be used in communication, e.g. during territorial conflicts with conspecifics or even when mating (Rigley and Marshall, 1973; Moller, 2002).

Additionally, this species has a relatively well developed olfactory system which enables them to use chemical cues during prey searching (Rooney *et al.,* 1989; von der Emde and Bleckmann, 1998). It is likely this sense is utilised when in combination with a more dominant sense (e.g. active electric sense) or when other senses become less reliable or are unavailable (von der Emde and Bleckmann, 1998).

G.petersii can also use hydrostatic pressure cues (the pressure exterted by water due to gravity) to navigate (Cain, 1995). Individuals seem to use these cues in association with the active

electrosense to build up a spatial representation of their environment, relying on hydrostatic cues more once the representation has been sufficiently developed (Cain, 1995).

Despite the array of senses they have access to, we will primarily be focussing on *G.petersii's* active electric sense in this thesis, given its dominance in object recognition tasks over short distances, and because many of the interesting body movements seen in this species seem to relate to its electrosensory system (e.g. Toerring and Belbenoit, 1979, Toerring and Moller, 1984; von der Emde *et al.,* 2010; Hofmann *et al.,* 2013b; Schumacher *et al.,* 2016a, 2016b, 2017a).

6. G.petersii's object recognition abilities with their active electric sense

6.1. Deriving information from a single electric image

As we have established, *G.petersii* primarily uses their active electric sense at short range, and since it has high spatial resolution, it is the preferred sense for object recognition (e.g. von der Emde, 2006; von der Emde *et al.,* 2010; Schumacher *et al.,* 2016a, 2016b, 2017a, 2017b). Research has shown that different image parameters from a single electric image can be used to work out a whole array of object properties. In mormyrids like *G.petersii,* a typical electric image projected onto the skin surface has a centre-surround ('Mexican hat') profile, with a large central region surrounded by a small contrasting rim area, with the effects dependent on the conductance of the object (Caputi *et al.,* 1998; Caputi and Budelli, 2006; von der Emde, 2006; Fig. 3). From the electric image, various parameters can be extracted, such as: EOD waveform modulations, image width, maximal slope, and peak amplitude (Fig. 3). Using one or more of these parameters, *G.petersii* can detect where an object is located, the object's distance, the size of the object, and the object's resistance as well as its capacitance (reviewed by von der Emde, 2006).

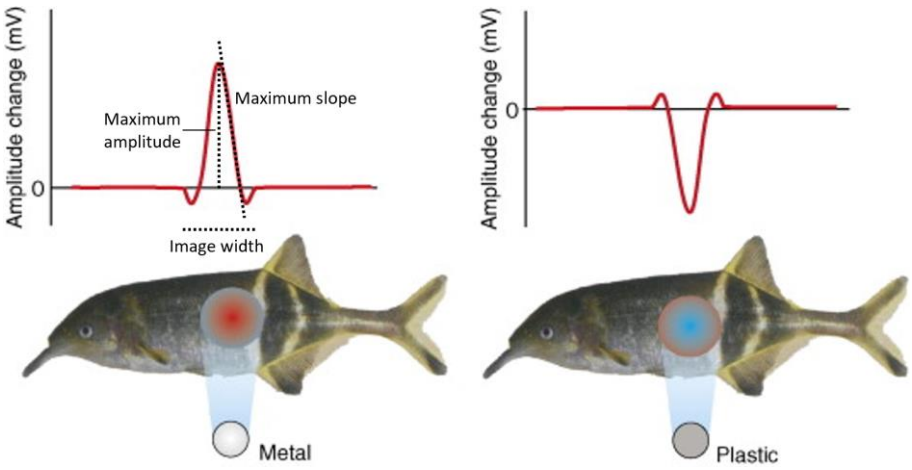


Fig. 3. Objects projecting electric images onto the flanks of *G.petersii.* The metal object (shown on left) projects an image on the skin which consists of a large central region where there is an increase in local EOD amplitude, shown in red, followed by a small surrounding region where local amplitude decreases, shown in blue. The plastic object (shown on right) evokes the opposite pattern. Both electric images have the characteristic 'Mexican Hat' profile (shown above the two fish) when local EOD amplitude change is plotted against position along the midline of the body. The dotted lines indicate three key image parameters from a static electric image that can be used to encode object properties (von der Emde, 2006). Adapted from von der Emde (2010).

Capacitance refers to the ability to collect and store electric charge (Collins Dictionary, 2021). Being able to detect both capacitive and resistive properties of an object is important for evaluating an object's complex impedance, which in turn, can be used for determining whether an object is living or not. Living matter tends to have a greater capacitive component, and so capacitance detection is essential for tasks like prey detection (von der Emde, 1990; von der Emde 1992; von der Emde and Bleckmann, 1992b; von der Emde and Ringer, 1992; von der Emde and Schwarz, 2002; von der Emde, 2006). Recent work suggests that *G.petersii* can recognise objects according to their 'electric colour' (Gottwald *et al.,* 2018). These 'colours' are derived from how an object modifies the electric field in terms of its amplitude and waveform, specifically

the waveform-to-amplitude modulation ratio. This means that certain types of objects (e.g. prey) are associated with certain 'electric colours', regardless of distance and size, allowing for rapid and reliable recognition (Gottwald *et al.,* 2018). Resistive and capacitive components of an object cannot be extracted with other sensory cues making electrosensing unique in that respect. As such, individuals possessing this sense have an advantage over non-electrosensing animals by being able to utilise this information from their environment.

Distance can also be estimated from a single electric image by taking the ratio between the maximal slope and maximum amplitude of the image (von der Emde *et al.,* 1998; Fig. 3). This ratio is dependent only on distance (so long as the rostral slope of the image is used) and not on size, shape, and material for most objects- more on this later (von der Emde *et al.,* 1998). The slope of the electric image is determined by the 'fuzziness' of the image's edges, with nearer objects making the electric image appear sharper, whilst further away objects will make it appear more blurred (von der Emde *et al.,* 1998; von der Emde, 2006).

6.2. Difficulties with extracting information from a single electric image using shape recognition as an example

However, not all object properties can be explained by either one or more image parameters within an electric image. Shape is one such example. We know that *G.petersii* can recognise three-dimensional shapes but it has been difficult to attribute this to known parameters of the electric image (von der Emde and Schwarz, 2000, 2002; von der Emde, 2004, 2006; von der Emde and Fetz, 2007; von der Emde *et al.,* 2010; Schumacher *et al.,* 2016b). The shape of an electric image cannot simply be taken as the shape of the object projecting that image, due to distortion relating to the way the electric field is generated and maintained (Caputi and Budelli, 2006; von der Emde, 2006; Pusch *et al.,* 2008). The mechanism(s) behind shape recognition and discrimination will be the focus of data chapters two and three (parts A and B), but it is sufficient

to say that individuals likely favour the use of object features to characterise and discern between them (von der Emde and Fetz, 2007; von der Emde *et al.,* 2010; Schumacher *et al.,* 2017a)- potentially extracting the necessary information from the analysis of multiple electric images (Hofmann *et al.,* 2013b; Schumacher *et al.,* 2016b; Fujita and Kashimori, 2019). This could occur as a result of movements around the object of interest- like the probing motor acts described previously (Toerring and Belbenoit, 1979, Toerring and Moller, 1984).

6.3. Potential neural pathways involved in active electrolocation (object recognition)

G.petersii certainly have the neuronal circuitry to cope with feature extraction- the process where an individual learns to recognise an object from a set number of characteristics (von der Emde and Fetz, 2007). Like many mormyrids, *G.petersii* show extreme encephalisation thought to relate to their electrosensory abilities (Carlson *et al.,* 2011; Carlson and Arnegard, 2011; Sukhum *et al.,* 2018). The brain of *G.petersii* makes up approximately 3% of this species' body mass and consumes 60% of the total oxygen used (Nilsson, 1996). The brain consists of specialised regions devoted to the active electrosensory system that occupy a large amount of space (von der Emde and Ruhl, 2016 cited in Schumacher *et al.,* 2017a; Schumacher *et al.,* 2017a). These include (but are not limited to) the electrosensory lateral line lobe (ELL), a cerebellum-like structure, and parts of the cerebellum (e.g. valvula). Each of these regions is associated with particular cell types and neuronal pathways that facilitate different aspects of electrical information processing (e.g. Meek *et al.,* 1999*,* 2008; Bell e*t al.,* 2005, 2008; Zhang *et al.,* 2007; Shi *et al.,* 2008). Research is continuing to update our understanding of how the various parts of the brain work together during different aspects of active electrosensing (e.g. spatial learning, see review by Engelmann *et al.,* 2021). For example, it seems that electrosensory inputs are processed in two parallel somatotopically ordered maps of the ELL, with phase and amplitude elements of the electric field processed separately. The information from both these maps seem to then terminate within the

nucleus lateralis of the midbrain. Here, information from both threads of the ELL seem to be processed together in a detailed somatotopic map (Hollmann *et al.,* 2016). All this highlights the advanced processing power of the active electrosensory system in the brain. Clearly *G.petersii* have invested a lot in their active sense, despite its significant metabolic costs, presumably because the improvements in sensory acquisition makes a difference to their survival and reproductive success (Nilsson, 1996; Carlson *et al.,* 2011; Markham *et al.,* 2016; Sukhum, *et al.,* 2016).

7. Recent progress in understanding the involvement of behaviours in sensing in weakly electric fish

As we have mentioned earlier, movements seem to have a significant role in sensory perception in *G.petersii.* In recent years, the function of some of their locomotor behaviours have become better defined, with studies making use of both empirical and modelled data in tandem. Hofmann *et al.* (2013a) found a novel cue for estimating an object's distance, referred to as the temporal slope-amplitude ratio, which could be extracted from a succession of electric images and required a very limited sensory surface (a single electroreceptor was sufficient). This method was less susceptible to ambiguity than the static measure of distance previously described (von der Emde *et al.,* 1998; Hofmann *et al.,* 2013a). Another study found that during an object detection task, *G.petersii* learned to adjust their behaviour in a way which seemed to improve the distance at which objects could be detected (Pedraja *et al.,* 2020). Recently, Engelmann *et al.* (2021) reviewed egocentric navigation in gymnotiform and mormyrid fish, finding that the behaviours which aided navigation and the putative neural pathways involved in this type of task were similar across both lineages, despite their independence. This suggests that there are principles of sensory-motor integration which transverse taxonomic groups, and perhaps sensory modalities.

8. Aim of thesis and questions being used to interrogate this aim

The aim of this thesis is to further investigate sensory-motor integration in weakly electric fish, using *Gnathonemus petersii* as a model. We plan to study the sensory and motor patterns of *G.petersii* during active electrolocation using techniques borrowed from electrophysiology and animal behaviour. Our main questions are as follows:

- **Are there quantifiable differences in the sensory information received by *G.petersii* during forwards and backwards swimming? (Data chapter one)**

To answer this first question, we recorded the EODs of freely moving *G.petersii*, modifying a setup used for extracellular recordings. At the same time, we filmed their swimming behaviour as the fish explored their surroundings. By analysing both components simultaneously, we hoped to better understand the mechanisms behind the integration of electrical information collected from the active electric sense during object exploration.

- **Does egocentric movement play a significant role in shape discrimination? (Data chapter two)**
- **What evidence is there for the use of movement-induced modulations (MIMs) for shape recognition? Is MIMs robust? (Data chapter three, part A)**
- **What evidence is there for the use of other movement related cues during shape discrimination? (Data chapter three, part B)**

To answer these last questions, we used a two-alternative forced-choice (2AFC) setup, where we trained individuals to discriminate between two differently shaped objects. We then manipulated how the objects were perceived by the manner in which they were presented, and the space available for sensing movements. We did this to interrogate the role of self-driven movements in extracting shape information from the environment, and to determine the consequences of altering these movements.

By answering these questions we hope to better understand how sensory and motor systems work together to allow *G.petersii* to behave optimally during active electrolocation, and appreciate how this interaction helps the species to cope with ecological challenges. The findings of this thesis will contribute to the understanding of active sensing more generally, since some of the general principles will apply not only to electrosensing, but other sensory modalities with an active component- e.g. echolocation.

Data Chapter One: Are there quantifiable differences in the sensory information received by *Gnathonemus petersii* during forwards and backwards swimming?

1. ABSTRACT

Elucidating the interactions between sensory and motor systems are crucial for understanding animal behaviour. Sensory systems help inform individuals on how to behave in a given situation, and egocentric movements can modify available sensory information. To understand the interdependency of these systems, we studied the sensory and motor patterns of the weakly electric fish, *Gnathonemus petersii.* This species produces pulse-type electric organ discharges (EODs) to detect objects in their local environment. Like other weakly electric fishes, they swim both forwards and backwards. We tested whether there were significant differences in the electrical information gathered by *G.petersii* during forwards and backwards swimming. The swimming behaviour and EODs of three individuals were recorded in the presence and absence of objects. We were able to extract 121 forwards and 107 backwards swimming sequences from 18 videos, and then matched these to their corresponding EOD traces. The time between EODs (inter-discharge intervals or IDIs) was calculated as a proxy for sensory activity. We found that the fish produced EODs when swimming both forwards and backwards, indicating that both swimming modes are important for information gathering. There were highly significant differences in IDI duration between forwards and backwards swimming, but only during object exploration and not when the objects were absent. This indicates that swimming is an important component of sensory acquisition. Forwards swimming had the shorter mean IDI in three out of four conditions tested; indicating that the fish sampled the environment more frequently in this mode. Functionally, this might be because the highest density of electroreceptors is found on the head, and so by moving forwards these fish can maximise the amount of information they gain

over a set distance or time. This makes sense given the costs in producing EODs. Visit number also had a significant effect on IDIs but object location did not. The first visit always had the shortest IDI mean, suggesting that fish sampled objects more when they were relatively novel. This would be important in allowing an individual to identify quickly what something is and how to respond. Given EOD costs, sampling should decline with subsequent visits, as by this stage the object and its possible intentions will be known. Together, our results support the idea that body movements are important for sensory acquisition in these fish as they enhance the information content obtained through the active electric sense.

2. INTRODUCTION

2.1. Principles of sensory-motor integration

Knowledge of the interaction between sensory and motor systems is crucial for understanding an animal's behaviour. Sensory systems help to inform an individual on how they should behave in a given situation and egocentric motion can modify what sensory information is available to them (Hofmann *et al.,* 2013b). An individual needs to be able to detect and respond to environmental signals appropriately, as misinterpretation can have serious consequences on an individual's survival or reproductive success. For example, failure to initiate an escape response in the presence of a predator could lead to injury or death. Alternatively, failure to respond to a courtship signal from a conspecific could lead to a loss of a mate and potential offspring.

Active sensing strategies are good systems for studying these interactions as they represent processes where sensory flow is modulated by movement (Hofmann *et al.,* 2013b). There are many active sensing strategies known of in the animal kingdom. For example, bats are known to modify their echolocation call by moving body parts associated with either call creation or

detection. They can direct the maximum slope of their echolocation beam to improve localisation of their target but this comes at the cost of wider detection (Yovel *et al.,* 2010).

2.2. Weakly electric fishes are excellent candidates for studying sensory-motor interactions

Weakly electric fishes are excellent models for unravelling sensor-motor relationships as they have a well described electric sense and exhibit a number of behaviours thought to be associated with electrosensing (Toerring and Belbenoit, 1979; Toerring and Moller, 1984; see review by Hofmann *et al.,* 2013b). These fish have evolved the ability to produce and detect low voltage electric fields, which can be modified by the environment. This is known as active electrolocation (Lissmann, 1951, 1958; Lissmann and Machin, 1958; Heiligenberg, 1973; reviewed by von der Emde, 2006). There are two independent lineages of weakly electric fish that have developed their own electrosensory systems: the Gymnotiformes from South America and the Mormyriformes from Africa (e.g. Bullock *et al.,* 1983; Winemiller and Adite, 1997; Lavoué *et al.,* 2012). Mormyrids use discrete electric pulses (known as electric organ discharges, or EODs) to generate their fields (Fig. 1c). These pulses tend to be stereotyped but the time between these pulses (known as inter-discharge intervals, or IDIs) can be quite variable (Fig. 1b). As such, they use timing for encoding environmental information (Sawtell *et al.,* 2005; von der Emde, 2006).

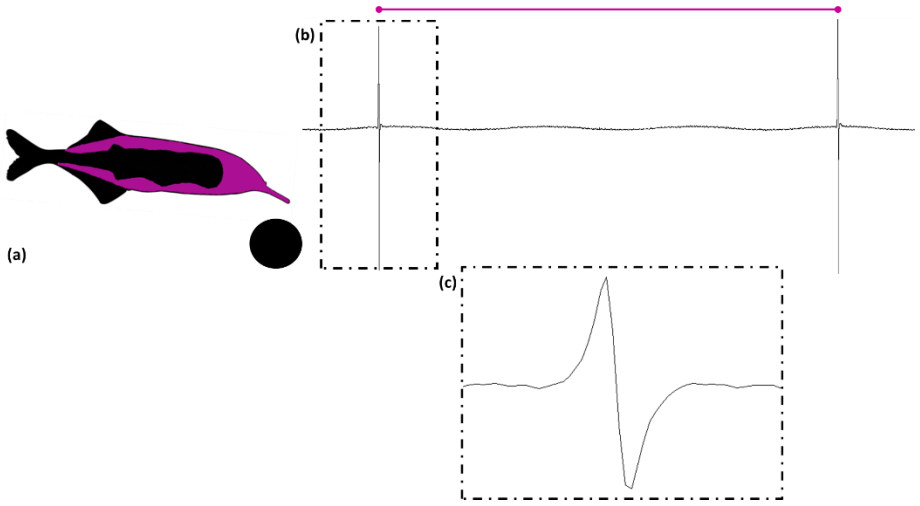


Fig. 1. Electrosensory system of *G.petersii*. (a) Fish exploring a spherical object, with electroreceptor sites highlighted in purple- adapted from von der Emde (2006). (b) Two consecutive electric organ discharges (EODs) with the inter-discharge interval (IDI) marked in purple. (c) Close up of a single EOD. *G.petersii* produce pulse-type EODs which form low voltage electric fields around them. Objects within the field distort and change its properties, which are detected by specific electroreceptors on the skin. Most electroreceptors are found on the head and chin appendage, some on the sides, but there are none on the tail. Since EODs are so stereotyped, *G.petersii* will use IDIs to encode information about nearby objects.

Various active sensing strategies have been identified in weakly electric fish. The gymnotiform *Apteronotus albifrons* are known to orientate their body so that the dorsal surface faces towards a target. Since this region is densely packed with electroreceptors, it is likely the alignment allows these animals to acquire more information from the environment (MacIver *et al.,* 2001; Snyder *et al.,* 2007). Similarly, the mormyrid *Gnathonemus petersii (G.petersii)* are known to orientate in a stereotypical manner during swimming, probably so that their two 'electric foveae' (receptor hotspots) are in the right position for detection (von der Emde and Schwarz, 2002; von der Emde, 2006; Bacelo *et al.,* 2008; Hollmann *et al.,* 2008; Pusch *et al.,* 2008; von der Emde *et al.,* 2008). Several 'probing motor acts' have been identified in mormyrids, with one being *'va-et-vient',*

where the fish swims back and forth past an object (Toerring and Belbenoit, 1979; Toerring and Moller, 1984). Similar back and forth motions have also been observed in gymnotiformes (Lannoo and Lannoo, 1993; Nanjappa *et al.,* 2000; MacIver *et al.,* 2001; Snyder *et al.,* 2007; Pedraja *et al.,* 2018). Recent work suggests that this kind of motion changes the sensory (electrosensory) flow, by altering the spatiotemporal patterns of the self-generated electric field (reviewed by Hofmann *et al.,* 2013b). This seems to enable weakly electric fish to tune their behaviour to the spatiotemporal receptive field properties of their nervous system, as well as increase their sensory volume, and can even help them to extract environmental information unavailable by other means. This all contributes to a greater level of perception (MacIver *et al.,* 2001; Snyder *et al.,* 2007; Stamper *et al.,* 2012; Hofmann *et al.,* 2013a). Sensory flow is thought to provide a cue for continuous distance estimation which might help individuals navigate around objects in real time (Hofmann *et al.,* 2013a, 2013b, 2017). Distance perception through motion parallax is possible due to properties of the electric field combined with the movement of the individual (Pedraja *et al.,* 2018). Despite these examples, we don't fully understand the precise effects of movement on sensing and how this is represented in the brain (Hofmann *et al.,* 2013b; Hollmann *et al.,* 2016; Pedraja *et al.,* 2018, 2020; Engelmann *et al.,* 2021). There is very limited research on how smaller, discrete swimming components might contribute towards this sensory flow (e.g. MacIver *et al.,* 2001; Hofmann *et al.,* 2014). In this chapter, we focussed on answering this question with the weakly electric elephantnose fish, *G.petersii.* The aim was to determine what electric information this species might obtain during periods of forwards and backwards swimming.

2.3. Using G.petersii as a model and aims of study

G.petersii is an excellent model for studying sensory-motor interactions as their active electric sense and behaviour are well characterised (e.g. Toerring and Belbenoit, 1979; Toerring and Moller, 1984; von der Emde, 2006). These fish also possess an interesting behaviour which can be used to interrogate these interactions: like other weakly electric fish they spend a significant time swimming backwards. This behaviour is highly unusual in fish, and as such, has long been of interest, particularly in relation to their active electric sense (Lissmann, 1951, 1958; Lissmann, 1974 cited in Lannoo and Lannoo, 1993). In gymnotiformes, this behaviour is speculated to optimise prey capture. By allowing the fish to scan the target across receptors of increasing density, it can get a better electric image of its prey, meaning that it can be captured with a short forwards lunge more accurately (Lannoo and Lannoo, 1993; MacIver *et al.,* 2001). In mormyrids, backwards swimming has been considered in the context of *va-et-vient,* where fish explore objects by repeatedly moving back and forth beside them, but not often as a separate component (e.g. Toerring and Belbenoit, 1979; Toerring and Moller, 1984; Hofmann *et al.,* 2014).

The aim of this study was to investigate whether backwards swimming played a role in sensing, and whether it was an important component of sensory acquisition. Specifically, our objective was to assess whether there were quantifiable differences in the sensory information received by *G.petersiii* during forwards and backwards swimming. To test this, we recorded the movements of three individuals in the presence and absence of objects whilst simultaneously recording their EODs. We matched swimming sequences with EOD traces from the same time frame. We then calculated the IDIs for all traces, which provided a proxy for the temporal changes occurring in sensory activity.

Together, this study is important for understanding the consequences of simple swimming movements on active electrolocation and for inferring the mechanisms behind the integration of sensory and motor information in these fish. This information will contribute towards developing

principles that can be generalised to other active sensing systems, and towards the goal of predicting behaviour given the sensory context, and vice versa.

3. MATERIALS AND METHODS

3.1. Subjects

We recorded the swimming behaviour and EODs of three individuals in a home tank using a camera and electrophysiology equipment. These fish were obtained from a licenced fish dealer (The Goldfish Bowl, Oxford, UK). They came from Nigeria (either Edo, Delta, or Lagos state) and ranged in length from 13.9-14.3 cm (tip of Schnauzenorgan to tip of dorsal part of caudal fin). We were unable to sex the individuals as captivity can alter and even reverse hormone-dependent sex differences (Landsman, 1991, 1993). We were also unable to age them for similar reasons. Individuals were tested separately. Each fish was housed in the same standard two-foot tank (61.1 cm x 31.0 cm x 30.5 cm) throughout their participation in the experiment. The tank contained reverse osmosis water treated with a remineralisation formula (Tropic Marin, Tropical Marine Centre UK). Water conductivity ranged from 404 to 500 µS/cm and temperature ranged from 22.8 to 26.5°C. These parameters stayed consistent within individuals, although temperature fell gradually over the course of a session. This was attributed to the necessary removal of the heater to minimise noise during electric signal recording. We set overhead lights to a 12:12-hour light-dark cycle. When experiments were not in progress, tunnels and other objects were added to the tank for shelter and enrichment. Individuals were fed a mixture of mini and normal bloodworms (Gamma, Tropical Marine Centre UK) once per day, five times a week. All experiments were conducted in accordance with the University of Oxford's animal ethics guidelines.

3.2. Sample size

Three individuals participated in this experiment (N=3). This sample size represents a drive to limit the number of animals studied in a laboratory setting, whilst also ensuring enough animals were studied that satisfactory conclusions could be drawn from the data set (reviewed by Animal Behaviour, 2018). The time-consuming nature of analysing IDIs and swimming behaviour also meant we were limited in the number of individuals we could study.

3.3. Experimental setup

We mounted a camera (Logitech HD Pro Webcam c920) overhead, approximately 95 cm from the floor (Fig. 2). At this height, activity across the whole tank could be recorded. Additionally, we fixed two pulleys to a cage to allow objects to be lowered into the water carefully (Fig. 2). The cage was grounded to the Earth to limit electrical noise in the setup. We placed two silver-tipped recording electrodes in guards on the two short sides of the tank. The plastic guards were fixed to the centre of each side, 1 cm from the top of the tank. The electrodes were placed inside with their tips submerged, 2 cm from the water meniscus (Fig. 2). The information gathered from these electrodes was sent to a World Precision Instrument DAM 50 Differential Amplifier (settings: input= A-B, low filter= 0.1 Hz, high filter= 10 kHz, mode= AC, gain= 100 AC) to be processed (Fig. 2). A speaker (BXL-GA10BL, Basic XL) was connected to the amplifier so that the EODs could be 'heard' (Fig. 2). The output from the amplifier was then digitalised with an analog-to-digital converter (Micro 1401-3, Cambridge Electronic Design Limited). We recorded the digitalised data in *Spike2 8.10* (Cambridge Electronic Design Limited) at a sampling rate of 50kHz (Fig. 2). The data was processed and then exported to statistical packages, e.g. *Excel 2013* (Microsoft) and *SPSS version 24* (IBM).

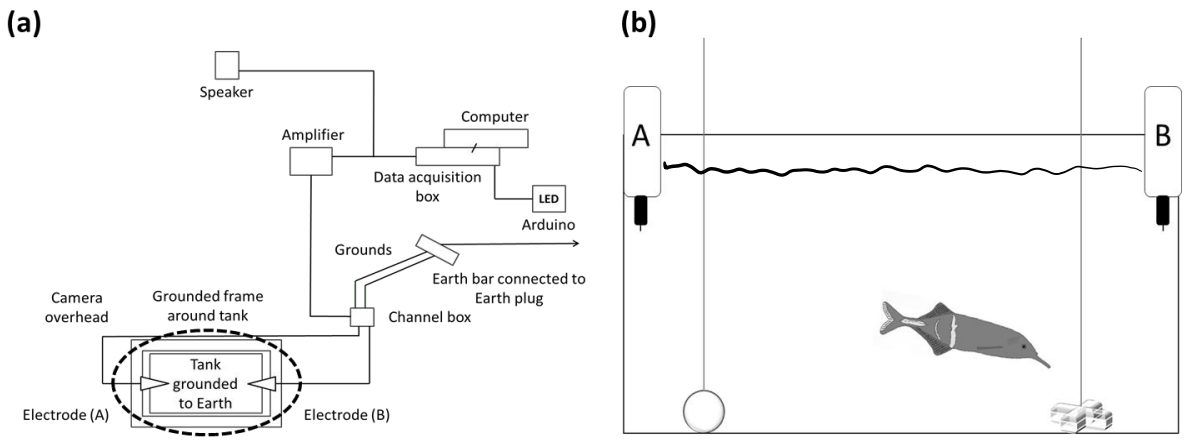


Fig. 2. EOD-kinematics experimental setup. Schematic of setup is shown in (a). (b) is a side on view of the dotted section in (a). It shows a free-swimming *G.petersii* examining one of the objects lowered down by a pulley. An overhead camera captured the swimming behaviour, and the electrodes ('A' and 'B') captured the EODs produced by the fish. *Not drawn to scale.*

3.4. Data collection

We recorded swimming behaviour during trials using a camera controlled by an application of *Spike2* (*Spike2 Video Recorder*). The frame rate was set to 20 frames per second and the image resolution was set to 640 x 480 pixels. This provided the minimum detail required for analysing backwards and forwards swimming movements. Later, we were able to increase the resolution to 1280 x 720 pixels without compromising on the EOD sampling rate. As the video and electrical recordings were controlled by the same programme, they were triggered to start at the same time and so drift between the two could be kept to a minimum. To check for synchronicity, we programmed an *Arduino Uno* microcontroller board (www.arduino.cc) to flash with a delay of 50 ms (equivalent to 20 frames per second; Fig. 2). On average, recordings with objects were 286 s in length and those without were 226 s. Computer issues at the start meant that there were some delays between the swimming and EOD recordings collected, particularly relating to fish A. To ensure this did not skew our results, we removed any recording pairs which had a difference of

more than six seconds. We decided on this cut off limit as it was consistent with the expected error of about 1 s drift for every 50 s of video. For fish B, the delays were smaller (up to 4 s). We were able to align each EOD recording with its associated video. We did this by subtracting the time difference (which represents the video buffering time) from the video's start and end time to get the start and end time for the corresponding EOD sequence. No adjustments were required for fish C since delays were <1 s.

We filmed the swimming behaviour and EODs of individuals in the presence and absence of two objects (a cross and sphere). The objects were made from the same material (plastic) and had the same 'colour' (frosted clear) and approximate volume (64.98 cm^3 and 65.45 cm^3 for the cross and sphere, respectively). This meant that they only differed in their shape. We were interested to see if shape had an influence on the sensory and motor activity patterns of these fish during trials, because recent work suggests that movement-induced modulations, are involved in shape recognition (Schumacher *et al*., 2016b)- more on this in subsequent chapters. Recordings made in the absence of objects acted as controls, providing baseline measurements for us to compare sensory and motor patterns, and determine whether these could be attributed to object exploration.

A single trial consisted of a fish exploring an empty or enriched tank (details above). Trials were completed in treatment blocks and ceased when an individual stopped exploring the objects. We considered object location, and the effect of experience within a trial by comparing sensory and motor activity at the beginning, middle, and end.

During trials with the cross and sphere, video and EOD recordings began before both objects were added. They were lowered into the water at similar speeds using manually controlled pulleys. Trials averaged five minutes depending on how keen the fish were to explore. Individuals were given a minimum of five minutes between trials to rest and allow us to prepare for the next trial. During trials without objects, the fish were free to roam in the tank (four minutes on

average). In total, 120 trials were recorded from the three fish across 23 days, and up to 10 trials were undertaken in a day. 18 of these videos and their associated EOD traces were then chosen for detailed analysis. We chose recording pairs with minimum drift and noise to limit interference.

3.5. Data processing

3.5.1. Selecting swimming sequences

Forwards (N=121) and backwards (N=107) swimming bouts were manually extracted from the videos (N=18). In videos where an individual was presented with objects, no swimming sequences were chosen until the objects had settled to their final resting positions in order to compare sequences across trials and fish. Forwards swimming sequences were defined as those where the fish moved in the direction it was facing for at least 1 s and in approximately 2 cm radius from the object of interest. Backwards swimming sequences were defined as those where the fish moved in the direction of its rear for at least 1 s in the radius described above. A laminated sheet with squares (2 cm by 2 cm) was placed underneath the tank to help us determine how far the fish was from the object at a given point. These criteria were adjusted according to context, e.g. radius threshold was increased in some cases where the fish exhibited probing motor acts beyond the original threshold (Toerring and Belbenoit, 1979; Toerring and Moller, 1984).

3.5.2. Extracting the relevant EODs

Once the swimming sequences were obtained, each one was matched with its associated EOD trace as described earlier. We then extracted the relevant EODs from the trace using *Spike2*. The time of each EOD could then be exported to *Excel* for further processing.

3.5.3. Calculating IDI duration

We calculated IDI duration by taking the difference between two consecutive EODs (Fig. 1b).

3.6. Statistics

We plotted IDI duration against time for each EOD sequence extracted to see whether any temporal trends in sensory activity could be observed within a single swimming bout (forwards or backwards). No trends were observed so these plots have been omitted for brevity. We also produced an IDI histogram for each swimming sequence. Both plot types were useful for outlier checking. We were interested in pooling the IDI data gathered from the three fish to see if IDI duration differed between forwards and backwards swimming during no object and object (cross or sphere) exploration on a group level. Histograms were produced for each of these conditions to accomplish this (see results). To determine whether the trends observed were only noticeable during encounters with certain shapes, or were seen more generally, histogram plots were compiled which combined the cross and sphere data. Histograms were also produced to look at whether IDI distributions changed over time. This was done for the same four conditions: cross, sphere, no object, and cross and sphere (see results).

Kruskal-Wallis tests *(IBM SPSS Statistics 24)* were run on the data to see whether forwards and backwards swimming differed significantly in terms of their IDI distributions. We also looked at whether IDI duration varied significantly with time. We chose a non-parametric test due to the skewed nature of the data and its robustness (see results section). Results were considered statistically different when $p<0.05$. Means, standard deviations, and sample sizes were calculated, and are given in the following format: $X \pm SD$, N. Note that N refers to the number of IDIs collected, and not fish number.

Visit histograms were constructed more recently with *IBM SPSS Statistics 28.*

4. RESULTS

4.1. Comparing IDI duration between forwards and backwards swimming: are there any notable differences during object vs. no object exploration?

IDIs from all individuals were pooled. We found a highly significant difference in IDI duration between forwards and backwards swimming along a cross-shaped object (Kruskal-Wallis test: H_1=151.810, p<0.001; Fig. 3). Forwards swimming had the shorter mean IDI compared with backwards swimming, indicative of increased sampling in this mode (forwards swimming: 89.743 ± 48.735 ms, N=1945; backwards swimming: 113.103 ± 57.178 ms, N=1110; Fig. 3). We also found a highly significant difference in IDI duration between forwards and backwards swimming along a sphere (Kruskal-Wallis test: H_1=34.067, p<0.001; Fig. 4). In this case, backwards swimming had the shorter IDI mean of the two (forwards swimming: 101.889 ± 54.000 ms, N=1572; backwards swimming: 90.330 ± 48.811 ms, N=1149; Fig. 4). We pooled the cross and sphere data sets to look at object exploration generally, and we found a highly significant difference in IDI duration between the two swimming modes (Kruskal-Wallis test: H_1=21.184, p<0.001; Fig. 5). Forwards swimming had the shorter mean IDI compared with backwards swimming (forwards swimming: 95.172 ± 51.503 ms, N=3517; backwards swimming: 101.520 ± 54.283 ms, N=2259; Fig. 5). However, we did not find a significant difference between forwards and backwards swimming in an empty tank (Kruskal-Wallis test: H_1=2.997, p=0.083; Fig. 6). Forwards swimming had the shorter IDI mean here though (forwards swimming: 162.629 ± 80.073 ms, N=945; backwards swimming: 168.312 ± 80.875 ms, N=1039; Fig. 6).

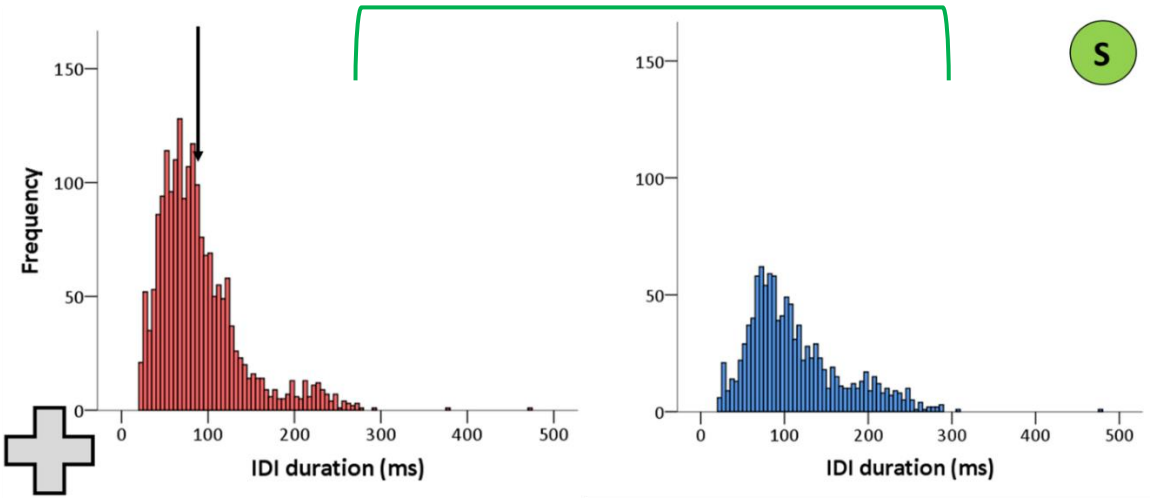


Fig. 3. IDI distribution for forwards (left, pink) and backwards swimming (right, blue) along a cross-shaped object. The green circle and bracket indicate that a significant difference was found between the distributions. The downward pointing arrow shows the position of the mean for the swimming mode with the shortest mean.

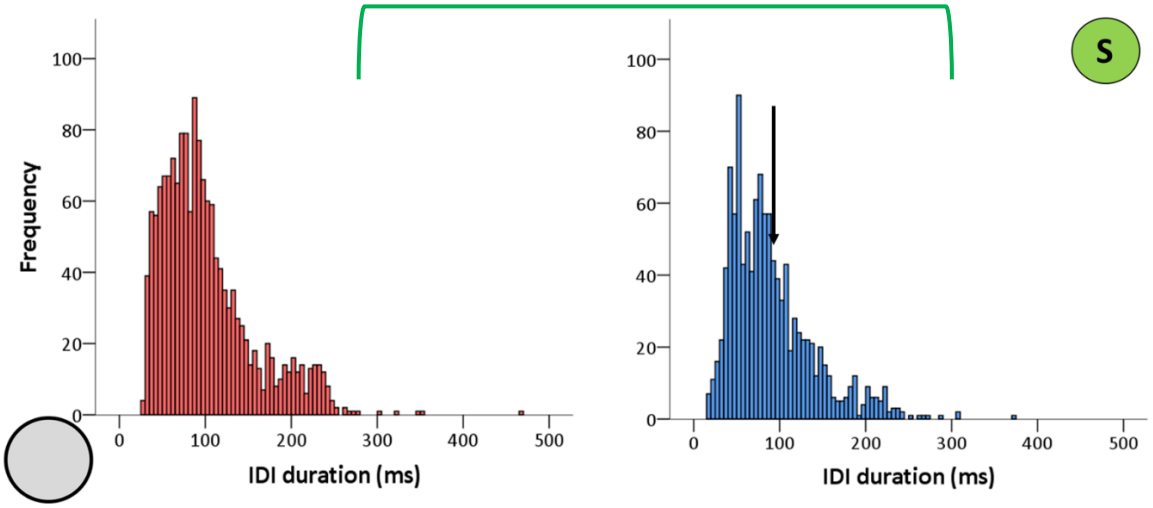


Fig. 4. IDI distribution for forwards (left, pink) and backwards swimming (right, blue) along a sphere. The green circle and bracket indicate that a significant difference was found between the two swimming modes. The downward pointing arrow indicates which one had the shortest mean.

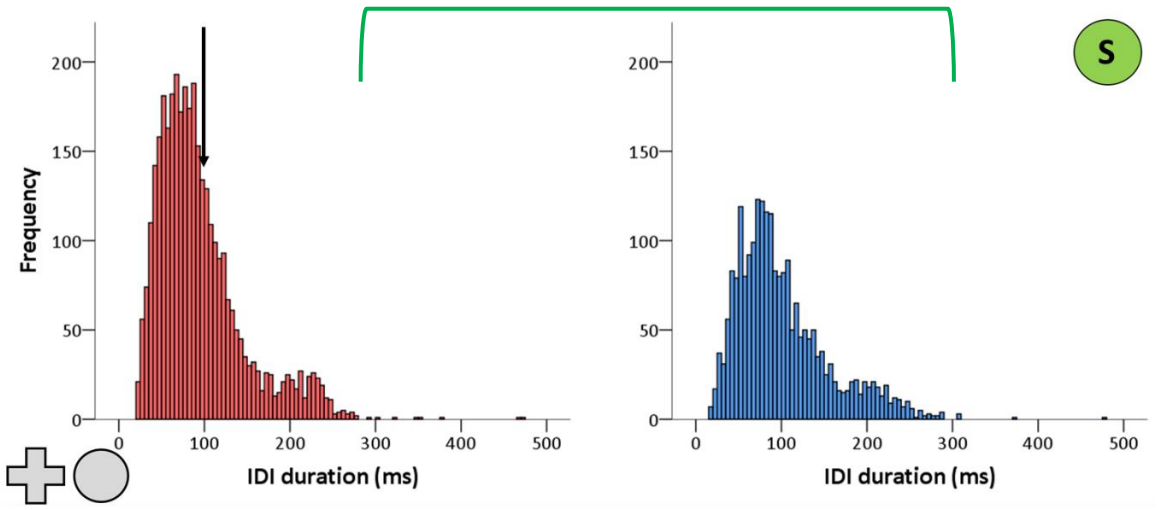


Fig. 5. IDI distribution for forwards (left, pink) and backwards swimming (right, blue) when the cross and sphere data were pooled. The green circle and bracket indicate that a significant difference was found between the swimming modes. The downward pointing arrow highlights which swimming mode had the shortest mean.

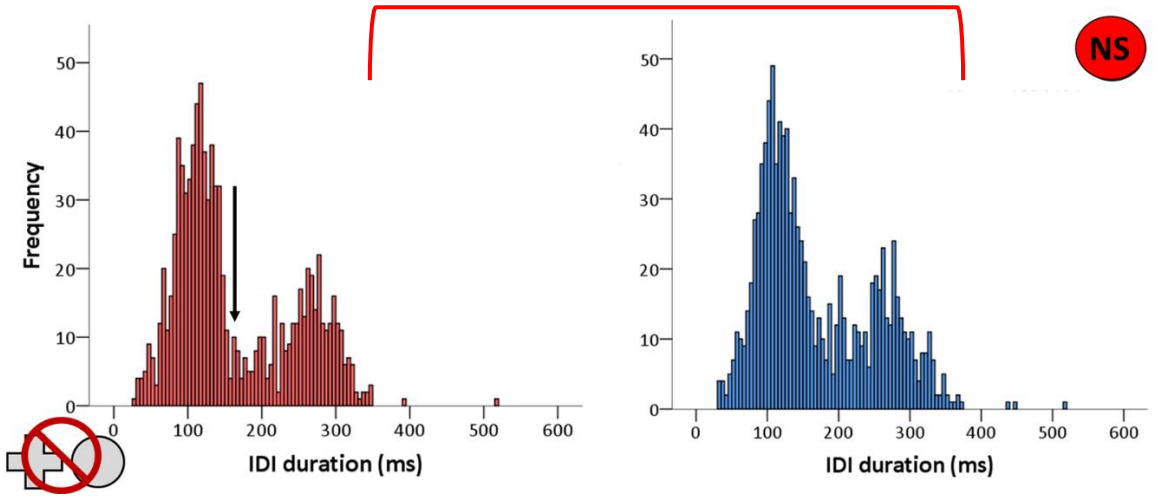


Fig. 6. IDI distribution for forwards (left, pink) and backwards swimming (right, blue) when no objects were presented to the fish. The red circle and bracket indicate that no significant difference was found between the two distributions. The downward pointing arrow shows which swimming mode had the shortest mean.

4.2. How does IDI duration change over time?

We found highly significant differences in IDI duration between visits regardless of object present and swimming mode, and in all cases the first visit had the shortest mean IDI.

4.2.1. Cross exploration

We found highly significant differences in IDI duration between visits during forwards (Kruskal-Wallis test: H_2=85.144, p<0.001; Fig. 7i) and backwards swimming along a cross-shaped object (Kruskal-Wallis test: H_2=152.043, p<0.001; Fig. 7ii). Closer observation of the forwards swimming data showed that the first visit had the shortest mean IDI, and the middle and final visits had similar means to one another (first visit: 77.733 ± 43.433 ms, N=680; middle visit: 96.848 ± 48.563 ms, N=612; final visit: 95.591 ± 51.720 ms, N=653; Fig. 7i). Closer observation of the backwards swimming data showed that IDI duration increased with subsequent visits (first visit: 92.029 ± 47.466 ms, N=496; middle visit: 123.349 ± 55.807 ms, N=334; final visit: 138.213 ± 61.096 ms, N=280; Fig. 7ii).

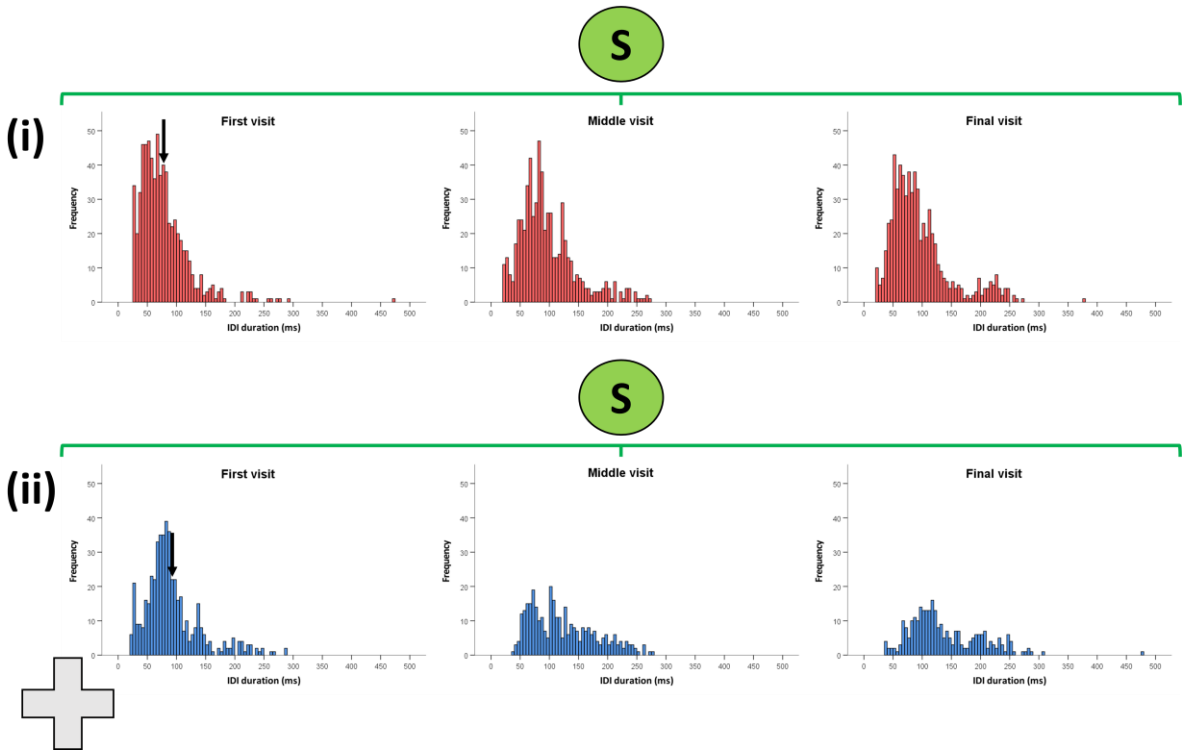


Fig. 7. IDI distributions for forwards (pink, upper) and backwards swimming (blue, lower) gathered during exploration of the cross-shaped object, but categorised according to visit number (first, middle, or final). The green circles and brackets indicate that a significant difference was found between the IDI distributions within each swimming mode. For each swimming mode, the downward pointing arrow denotes which visit had the shortest mean and its location.

4.2.2. Sphere exploration

We found highly significant differences in IDI duration between visits during forwards (Kruskal-Wallis test: H_2=120.125, p<0.001; Fig. 8i) and backwards swimming along a sphere (Kruskal-Wallis test: H_2=177.180, p<0.001; Fig. 8ii). Closer inspection of the forwards data showed that IDI duration increased with subsequent visits (first visit: 86.324 ± 49.105 ms, N=619; middle visit: 109.087 ± 50.516 ms, N=412; final visit: 114.216 ± 57.551 ms, N=541; Fig. 8i). Closer inspection of the backwards data showed that the first visit had the shortest mean (like all other conditions)

but that the middle visit had a greater mean than the final visit (first visit: 72.592 ± 39.197 ms, N=545; middle visit: 112.330 ± 50.628 ms, N=287; final visit: 100.910 ± 50.951 ms, N=317; Fig. 8ii).

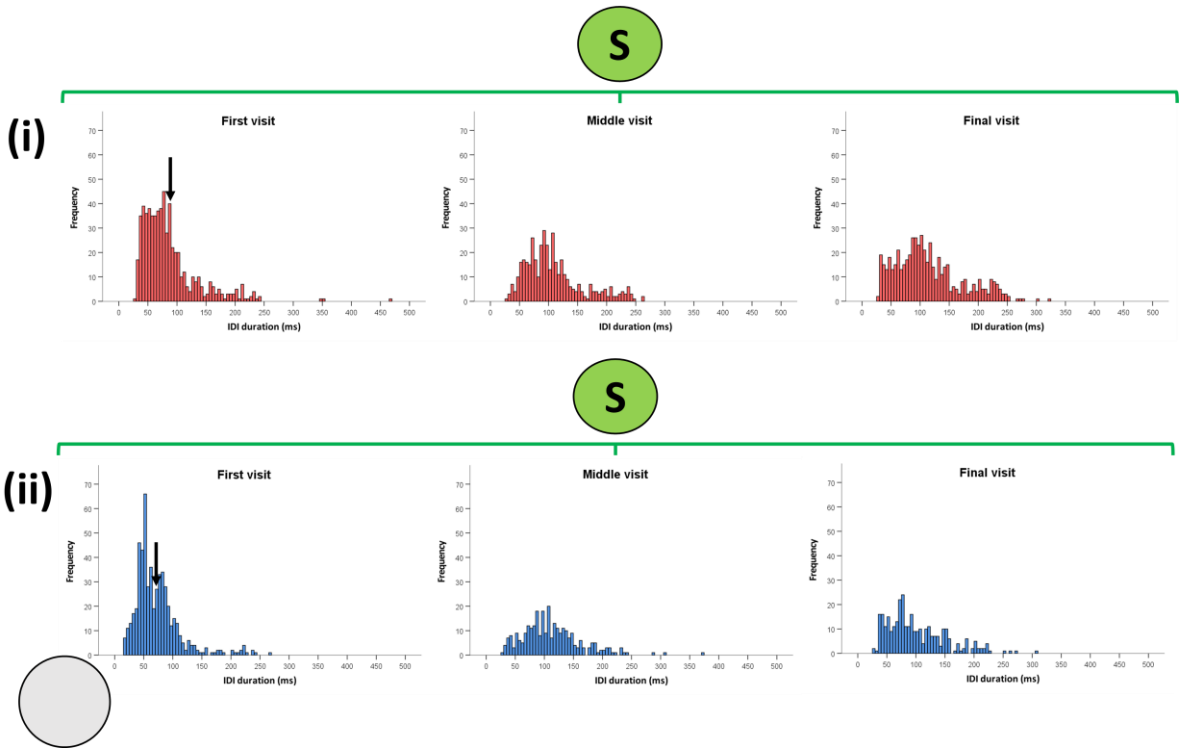


Fig. 8. IDI distributions for forwards (pink, upper) and backwards swimming (blue, lower) gathered during sphere exploration, but categorised according to visit number (first, middle, or final). The green circles and brackets indicate that a significant difference was found between the IDI distributions within each swimming condition. For each swimming condition, the downward pointing arrow shows which visit had the shortest mean and where.

4.2.3. General object exploration (cross and sphere data pooled)

We found highly significant differences in IDI duration between visits during forwards (Kruskal-Wallis test: H_2=188.341, p<0.001; Fig. 9i) and backwards swimming when the cross and sphere data were pooled, and no longer separated out according to shape (Kruskal-Wallis test: H_2=305.926, p<0.001; Fig. 9ii). Closer inspection of the forwards data found that IDI duration increased with subsequent visits (first visit: 81.827 ± 46.404 ms, N=1299; middle visit: 101.772 ± 49.698 ms, N=1024; final visit: 104.030 ± 55.201 ms, N=1194; Fig. 9i). Closer inspection of the backwards data found that the first visit had the shortest mean IDI, and the middle and final visits had similar means (first visit: 81.853 ± 44.389 ms, N=1041; middle visit: 118.256 ± 53.715 ms, N=621; final visit: 118.406 ± 58.914 ms, N=597; Fig. 9ii).

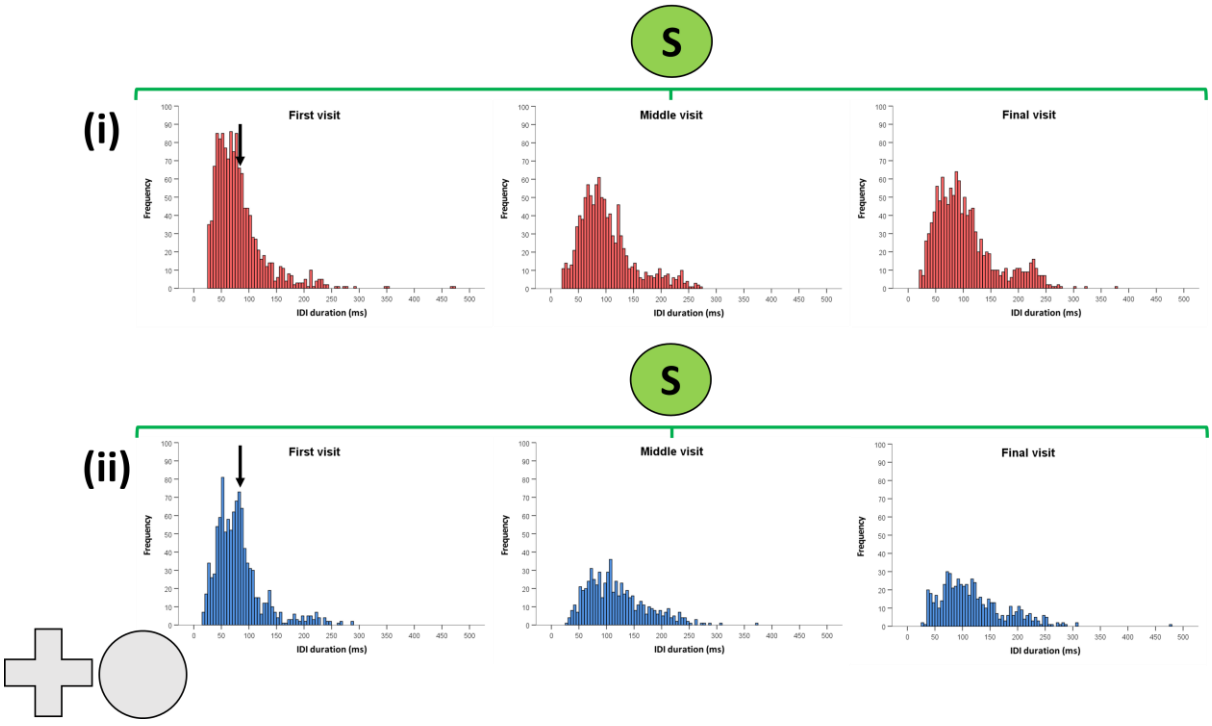


Fig. 9. IDI distributions for forwards (pink, upper) and backwards swimming (blue, lower) when the cross and sphere data were pooled, and categorised according to visit number (first, middle, or final). The green circles and brackets show that a significant difference was found between the IDI distributions within each swimming mode. For each swimming mode, the downward pointing arrow shows which visit had the shortest mean.

4.3. Does location affect IDI distributions?

Object location did not impact IDI duration (Kruskal-Wallis test: H_1=2.977, p=0.084).

5. DISCUSSION

We recorded the swimming movements and EOD patterns of three individuals in the presence and absence of objects to better understand the mechanisms and functions of sensory-motor integration in *G.petersii.*

5.1. Highlights

We found that fish produced EODs during forwards and backwards swimming which suggests that both modes play a role in information gathering. IDI duration differed significantly between the two swimming modes during object exploration, and not in empty tank controls. This further supports the hypothesis that body movements are important for sensory acquisition. IDIs were shorter when fish were swimming forwards in three out of four conditions examined, suggesting that individuals sampled their environment more frequently in this mode. Visit number had a significant effect on IDI duration: fish sampled objects more when they were relatively novel. Object location did not affect IDIs produced. We will now expand upon each of these points below to explain our results more fully and to suggest what might be driving these results.

5.2. G.petersii produce EODs when swimming in both directions

We found our fish produced EODs during forwards and backwards swimming, indicating that both swimming modes are important in sensory acquisition. This also implies that *G.petersii* have the neural circuitry to collect information bidirectionally. There is evidence in other weakly electric

fish species that there are neurons in the brain that can encode different movement directions by changes in their activation and firing patterns (e.g. Khosravi-Hashemi *et al.,* 2011; Khosravi-Hashemi and Chacron, 2012; Clarke *et al.,* 2014; Wallach *et al.,* 2018; Fotowat *et al.,* 2019). This would enable an individual to discriminate between objects moving in different directions within their field of view and know how to respond to each appropriately (Khosravi-Hashemi and Chacron, 2012). In some species, we are starting to get a better understanding of the neural circuitry involved in coding motion reversal and how this information is processed in different parts of the brain (e.g. Clarke and Maler, 2017; Wallach *et al.,* 2018; reviewed by Engelmann *et al.,* 2021).

What neuronal circuitry *G.petersii* uses is currently unknown and should be a target for further investigation, especially since the directionality studies described above focussed primarily on wave-type gymnotiforms (Pedraja *et al.,* 2018). Since *G.petersii* is a pulse-type electric fish, it uses a temporal code rather than a frequency code to encode information (Sawtell *et al.,* 2005). As such, perhaps the coding for motion is fundamentally different for them compared with wave-type fish. Nevertheless, both pulse and wave-type electric fish do exhibit similar motor behaviours, like *va-et-vient* (Toerring and Belbenoit, 1979; Toerring and Belbenoit, 1984; Lannoo and Lannoo, 1993; MacIver *et al.,* 2001; Stamper *et al.,* 2012; Hofmann *et al.,* 2014; Pedraja *et al.,* 2018). This type of motion is thought to help in the extraction of important signals from the most cluttered and noisy of environments (Babineau *et al.,* 2007) by perhaps matching sensory feedback to the demands of the nervous system (Stamper *et al.,* 2012). Additionally, the movement is likely to provide information unavailable by other means (Fechler *et al.,* 2012). For example, movement faciliates depth perception via motion parallax (Pedraja *et al.,* 2018). As such, despite their evolutionary divergence, gymnotiformes and mormyriformes might share ways of processing this kind of information, given the fundamental nature of movement in shaping their behaviour, perhaps in terms of the 'hardware' (e.g. cell types) or 'software' (chemical reactions) involved. This sentiment is shared by Engelmann *et al.* (2021) who found

similarities between both lineages in the sensory and motor processes involved in spatial navigation.

It is likely that the 'switch' between forwards and backwards swimming in our study would be important, as it would allow an individual to scan an object effectively. Since electric images are inherently blurry (Caputi and Budelli, 2006; von der Emde, 2006; Babineau *et al.,* 2007) it is unlikely a single pass would provide an individual with enough information to identify an object. In order to obtain more information on the object, the fish would need to repeatedly scan small sections quickly so that they could respond appropriately and at the right time. To do this, they would have to be able to gain electrical information both forwards and backwards.

5.3. Comparing IDI duration between forwards and backwards swimming: object vs. no object exploration

We found that there was a highly significant difference in IDI duration between forwards and backwards swimming, but only when the fish were exploring the objects (Figs. 3, 4, and 5). This suggests the sampling differences related to the acquisition of information from these objects.

If *G.petersii* gathered information about the environment from both swimming modes with similar efficacy, we would have expected to have found no significant difference between the IDIs captured during forwards and backwards swimming.

However, if *G.petersii* gathered information from one swimming mode more than the other, we would have expected to have seen a significant difference in the IDI distributions associated with forwards and backwards swimming. We would have also expected the swimming mode that facilitated greater sensory acquisition to be characterised by shorter IDIs, as this is indicative of increased sampling, which leads to the build-up of more detailed electric images of the environment.

Forwards swimming had the shorter IDI mean in three out of four conditions studied (Figs. 3, 5, and 6). Shorter IDIs mean that more EODs can be produced in a given time period. This has the potential to enable greater sampling of the environment by increasing the amount of information gained per unit time and/or distance (Post and von der Emde, 1999; Hofmann *et al.,* 2013b). *G.petersii* are known to change their IDI length depending on context. The reduction in IDI duration is most noticeable however when they encounter an object for the first time. *G.petersii* show a 'novelty response' which is characterised by a sudden, temporary increase in EOD rate and serves to orientate the animal to a new sensory input (von der Emde and Zelick, 1995; Post and von der Emde, 1999; Engelmann *et al.,* 2009).

Greater sampling during forwards swimming would be beneficial, as these fish often move towards an object head first. This tendency likely relates to them having the highest density of electroreceptors here, as well as possessing mechanisms (both passive and active) which enhance the sensory information received on the head (Bacelo *et al.,* 2008; Engelmann *et al.,* 2008; Hollmann *et al.,* 2008; Pusch *et al.,* 2008; von der Emde *et al.,* 2008; Pedraja *et al.,* 2020). Thus, by sampling frequently in this swimming mode, *G.petersii* would feasibly be able to maximise what they could obtain from the environment at a given point in time (reviewed by von der Emde, 2006). As previously mentioned, these fish have two electric foveae located on their head. One is the nasal region and the other is the Schnauzenorgan: both seem important for different aspects of detection (von der Emde, 2006; Bacelo *et al.,* 2008; Hollmann *et al.,* 2008; Pusch *et al.,* 2008; von der Emde *et al.,* 2008; Engelmann *et al.,* 2009). Since they are so distal, these parts will be the first to detect and encounter any object in front of the fish. On the other hand, fewer receptors are found on the sides and none on the tail, so these fish are more limited in the amount of information they can gather whilst swimming backwards (reviewed by von der Emde, 2006; Bacelo *et al.,* 2008; Hollmann *et al.,* 2008; Pusch *et al.,* 2008; von der Emde *et al.,* 2008; Fig. 1a). This is supported by our sample sizes for forwards and backwards swimming. More IDIs were collected during forwards swimming compared with backwards swimming along objects even

though similar numbers (and lengths) of forwards and backwards sequences were analysed (121 and 107, respectively). Additionally, when Hofmann *et al.* (2014) studied the relationship between motor patterns of various complexities and sensory flow, they found that during backwards swimming *G.petersii*'s EOD rate was low but regularised and not tightly coupled with object proximity. Again, this suggests that information gathering during this mode is somewhat limited.

Although weakly electric fish possess some strategies to reduce the cost of EOD production, there is still an inherent energetic cost that is associated with using this sensory modality (Nelson and MacIver, 2006; Salazar and Stoddard, 2008; Reardon *et al.,* 2011; Stoddard and Salazar, 2011; Salazar *et al.,* 2013; Markham *et al.,* 2016; Sukhum *et al.,* 2016). This energetic cost is difficult to quantify precisely, as we do not fully understand the processes involved and studies on this have primarily focused on gymnotiform rather than mormyriform fish (Salazar *et al.,* 2013). We also know that there are significant movement costs associated with active sensing that need to be considered (MacIver *et al.,* 2010; Stamper *et al.,* 2012). As such, it might make sense for these fish to invest in forwards swimming if it can provide them with more information in a given period. This would be especially important if an individual is to identify quickly what an object is, and how they should respond. However, it still begs the question why backwards swimming is so prevalent in these fish. Is this a consequence of it being multi-functional?

In wave-type gymnotiformes, backwards swimming is considered to be an important component of foraging as it allows an individual to scan and then capture prey with a short lunge forwards (Lannoo and Lannoo, 1993; Nanjappa *et al.,* 2000). In pulse-type gymnotiformes, it is considered more as a repositioning tool (Nanjappa *et al.,* 2000). In both gymnotiformes and mormyriformes, it is associated with behaviours like *va-et-vient,* seemingly enhancing the extraction of information from electrosensory flow (Belbenoit, 1970; Toerring and Belbenoit, 1979; Toerring and Moller, 1984; Lannoo and Lannoo, 1993; MacIver *et al.,* 2001; Babineau *et al.,* 2007; Snyder

et al., 2007; Stamper *et al.,* 2012; Hofmann *et al.,* 2013a, 2013b). It also seems to be associated with escape in both *G.petersii* and *A.albifrons* (Skeels, personal observations). Backwards swimming may therefore represent a compromise between sensing and safety, where detection is reduced in favour of minimising risky encounters. Indeed, our anecdotal observations suggest that *G.petersiii* exhibited greater bouts of backwards swimming when either encountering something unfamiliar (and so was uncertain about whether it was beneficial, dangerous, or benign) or when it encountered a threat.

5.4. How does IDI duration change over time?

We found highly significant differences in IDI duration between object visits, and, in all conditions, the first visit was shown to have the shortest mean IDI.

We studied the EOD patterns of *G.petersii* examining the same object on multiple occasions, as we suspected time would have an effect on IDI distributions, given how these fish encode information (Sawtell *et al.,* 2005) and their response to novel objects and their subsequent habituation (Post and von der Emde, 1999).

If *G.petersii* gathered equal amounts of information from each visit, we would have found no significant difference in IDI distributions across visits. However if *G.petersii* gathered different amounts of information from each visit, we would have found a significant difference in IDI distributions across visits. We would expect IDI duration to increase with subsequent visits, as the fish obtains more information about the environment and so can afford to sample less frequently.

As mentioned earlier, we found highly significant differences in IDI duration between visits regardless of object and swimming mode (Figs. 7, 8, and 9). In all cases, the first visit had the shortest mean which supports our hypothesis that *G.petersii* gathers different amounts of information with each visit. In three conditions, we saw IDI duration increase with subsequent

visits. In the other conditions, the means for the middle and final visits were still greater than for the first visit, but the middle visit either had a greater mean than the final visit, or they were similar.

The first visit having the shortest IDI mean for all conditions makes sense functionally, as one would expect *G.petersii* to sample the environment more when they encounter something novel, especially given what we know about their 'novelty response' (Toerring and Belbenoit, 1979; von der Emde and Zelick, 1995; Post and von der Emde, 1999). These changes in sensory and motor output would help an individual to build up electric images of their environment quickly so that they can identify what the novel thing is, and how to respond (Post and von der Emde, 1999). Once they have enough information, sampling should decline with subsequent visits as EOD production and associated locomotion can be energetically costly and should be minimised where possible (e.g. MacIver *et al.,* 2010; Stamper *et al.,* 2012; Salazar *et al.,* 2013; Markham *et al.,* 2016). This pattern was observed in three out of six conditions. In the three remaining conditions only one diverged. This is likely to be a consequence of other factors affecting IDI distributions. It is difficult to say what these factors may be, as despite the improvements in our understanding of active sensing through empirical and computational work, there is still much we don't understand in terms of how certain behaviours affect sensory flow, what these changes are, and what the consequences are for the animal- both sensory and behaviourally (Hofmann *et al.,* 2013a, 2013b, 2014, 2017; Pedraja *et al.,* 2018, 2020; Engelmann *et al.,* 2021).

5.5. What other factors might influence IDI duration?

Object location did not have a significant impact on IDI distribution, which makes sense given that the positon of an object is indicated by where on the fish the peak amplitude of the field is (reviewed in von der Emde, 2006). The shape of the object did not seem to have a significant effect on the IDI distributions gathered during forwards and backwards swimming and across time (Figs. 3, 4, and 5). This does not mean there were no shape effects to observe. Shape is a difficult

parameter to assess as we are still determining how *G.petersii* encode this kind of information (von der Emde, 2006; von der Emde and Fetz, 2007; von der Emde *et al.,* 2010; Schumacher *et al.,* 2016b; Fujita and Kashimori, 2019). Recent work suggests that they may use movement-induced modulations (MIMs) to distinguish between different three-dimensional shapes. In brief, MIMs can be defined as 'changes in the electrical images that occur as a fish swims past an object' (Schumacher *et al.,* 2016b). This means for example a sphere would have a very different MIMs signature to a cylinder, even if it was inspected from a similar set of angles. By manipulating an object's shape, they found that fish could discriminate objects up to further distances when there was a large difference in MIMs- in other words when the objects looked the most different. Other variables such as material and volume were controlled for and so could not be used for discrimination (Schumacher e*t al.,* 2016b). In the future, a potential avenue of research could be to investigate the influence of object shape on IDI patterns through modelling which we did not have the time to complete when conducting this study. Further modelling would also help us to quantify the effect of fish identity on IDI duration which was not the focus of this study.

5.6. Comments on analysis

At the time, we chose to use Kruskal-Wallis tests to analyse our forwards-backwards swimming data set, given the non-normal distributions of our data (Figs. 3-9), and to ensure the few large IDI values we recorded did not have a disproportionate effect on our results (Dytham, 2011). However, we were not able to account for repeated samples from individuals due to unforeseen circumstances that limited the time and resources available when this study occurred. This means our results must be considered with some caution, as we do not know how individual fish might have driven the results. Nonetheless, the plots illustrate a stark difference in IDI distributions between the two swimming modes when exploring objects compared to when they were absent (Figs. 3-6). This provides support for a real difference exisiting. In retrospect, the Kruskal-Wallis

tests were not the most suitable for delving into the complex interactions occuring between sensory and motor systems in this experiment. However, the time and resource pressures we experienced meant we were limited in the scope of tests we could exploy at the time. In the future, we plan to rectify this by running generalised linear mixed models (GLMMs) similar to those used in data chapters two and three to understand the factors driving IDI patterns during various types of exploration. Note that while medians were used when running the Kruskal-Wallis tests, we have quoted means and their standard deviations in this text, as they have more utility in describing the functional value of the differences in sampling across forwards and backwards swimming.

5.7. Concluding remarks

Our study found that EODs were produced in both forwards and backwards swimming, which suggests that *G.petersii* have the neural circuitry to gather bidirectional electrical information. Furthermore, we found highly significant differences in IDI distributions between forwards and backwards swimming during object exploration only. Both provide support that body movements play an active role in sensing. The next step is to identify the role movements play in shape discrimination, which will be discussed in data chapters two and three (parts A and B).

Data Chapter Two: Exploring the role of egocentric movement for shape discrimination

1. ABSTRACT

Body movements are known to play an active role in sensing. However, it is not fully understood what information is provided by these movements. The weakly electric fish *Gnathonemus petersii* senses their environment through active electrolocation during which they perceive object-induced distortions of a self-produced electric field with epidermal electroreceptors. The analysis of electric images projected on their skin enables them to discriminate between three-dimensional objects. While we know the electric image parameters used to encode numerous object properties, we don't understand the parameters for shape. It is thought that 'movement-induced modulations' (MIMs) evoked by body movements might be involved in shape discrimination. MIMs cause movements of electric images over the body surface as a fish swims near an object. Our study aimed to understand whether body movements are important for shape discrimination during active electrolocation. We trained fish to complete a shape discrimination task in a two-alternative forced-choice setup, and then manipulated the space available to individuals for scanning movements to see if this led to a change in their discrimination performance. We found that if enough space was available, fish were very good at discriminating objects of different shapes. However, performance decreased strongly when the space was reduced so that scanning movements were impaired. This shows that the ability to discriminate between shapes is underpinned by movement. Our study demonstrates the importance of body movements for gaining complex environmental information such as object shape through active electrolocation.

2. INTRODUCTION

2.1. Basic principles of active electrolocation

Weakly electric fish, such as *Gnathonemus petersii (G.petersii),* possess an active electric sense that they use to detect objects in their environment, termed active electrolocation (Lissmann, 1951, 1958; Lissmann and Machin, 1958; Heiligenberg, 1973). These fish generate electric signals (referred to as electric organ discharges, or EODs) from their electric organ which consists of modified muscle cells known as electrocytes (Lissmann, 1958; Bennett, 1971; Moller, 1980; Markham, 2013). Each EOD produces a three-dimensional electric field around the fish with a set geometry according to properties of the fish itself (Schwarz, 2000 cited in von der Emde 2006; Caputi and Budelli, 2006; von der Emde, 2006). Objects close to the electric field (on the centimetre range) will distort it, with the type of distortion dependent on the intrinsic properties of the object itself. For example, a conductive object will increase the density of field lines, increase the amplitude of local EODs, and even distort their waveforms (von der Emde, 2006). Specialised electroreceptors on the skin of the fish, known as mormyromasts in mormyrid fish like *G.petersii*, detect these object-induced changes (Bell *et al.,* 1989). This area of change on the skin is known as 'the electric image' of the object, since it represents an area where the voltage pattern is different to elsewhere on the body due to the presence of the object (Caputi and Budelli, 2006; von der Emde, 2006).

2.2. Sensory ecology of G.petersii

G.petersii reside in turbid rivers and lakes across Central and Western Africa, and are largely nocturnal, hunting an array of invertebrate prey buried in sediment on the river or lake bed (Moller *et al.,* 1979; von der Emde and Bleckmann, 1998; Olaosebikan, *et al.,* 2020). In these environments, visual information is more limited, and *G.petersii* mostly rely on other sensory

systems to navigate daily challenges (von der Emde and Bleckmann, 1998; Schumacher *et al.,* 2017b). These fish have an array of sensory systems they can call upon, but among them the active electric sense is the most dominant, particularly at short distances (von der Emde and Bleckmann, 1998; Moller, 2002; Rojas and Moller, 2002; Schumacher *et al*., 2016a, 2017a, 2017b). This is not because active electrosensing simply acts as replacement for vision in these poorly lit environments, but it can provide more fine scale spatial information at this working range, and enable *G.petersii* to acquire unique information about objects that would otherwise be missed, such as their capacitance, which is a good indicator for determining whether an object is alive or inanimate (von der Emde, 1990; von der Emde and Bleckmann, 1992b; von der Emde and Ronacher, 1994; von der Emde, 2006; von der Emde e*t al.,* 2010; Schumacher *et al.,* 2016a, 2017a, 2017b).

2.3. Object recognition during active electrolocation

Object recognition using the active electric sense has been studied extensively in these animals (e.g. von der Emde and Schwarz, 2000, 2002; von der Emde and Fetz, 2007; von der Emde *et al.,* 2010; Schumacher *et al.,* 2016a, 2016b, 2017a). Research has shown that different image parameters from a single electric image can be used to work out a whole array of object properties. In mormyrids*,* a typical electric image projected onto the skin surface has a centre-surround ('Mexican hat') profile, (Caputi *et al.,* 1998; Caputi and Budelli, 2006; von der Emde, 2006; Fig. 3 in general introduction). From the electric image, various parameters can be extracted, such as: EOD waveform modulations, image width, maximal slope, and peak amplitude. Using one or more of these parameters, weakly electric fish like *G.petersii* can detect where an object is located, how far away it is, the size of the object, and the object's resistance as well as its capacitance (reviewed by von der Emde, 2006). For example, distance can be calculated by taking the ratio between the maximal slope and maximum amplitude of a single electric image

(von der Emde *et al.,* 1998; Fig. 3 in general introduction). This measure is dependent only on distance (but only if the rostral slope of the image is utilised) and not on size, shape, and material for most objects- apart from spheres- more on this later (von der Emde *et al.,* 1998).

2.4. Understanding shape recognition

2.4.1. Difficulties in measuring shape with the active electric sense

Shape, however, has long been a mystery. No parameters or collection of parameters in a single electric image can explain three-dimensional shape (von der Emde, 2006; Schumacher *et al.,* 2016b). Shape is a complex cue to extract from the environment, since it is made up of a collection of features that all contribute towards an object's overall shape (e.g. object height, the presence or absence of corners, the number, length, and orientation of sides; von der Emde and Fetz, 2007).

Moreover, shape is more difficult to extract from an electric image than from a visual image, as unlike with vision, there is no such mechanism within the electrosensory system to focus the electric image (Caputi and Budelli, 2006; von der Emde, 2006). In addition, the asymmetric nature of the electric field generated around the fish means that the electric image will be distorted and so will not reflect the true dimensions of a given object (Caputi and Budelli, 2006; von der Emde, 2006; Engelmann *et al.,* 2008; Pusch *et al.,* 2008). The amount of distortion can vary depending on the part of the body projected onto, with electric images appearing most warped at the trunk, where image shape is dependent on the positioning of the object along the rostral-caudal axis (Babineau *et al.,* 2007; Engelmann *et al.,* 2008; Pusch *et al.,* 2008). As such, unlike with vision, *G.petersii* cannot take the shape of an electric image to be a true expression of the object's actual shape, since there is no clear geometric relationship between the two (Schwarz, 2000 cited in von der Emde and Fetz, 2007; Caputi and Budelli, 2006; von der Emde and Fetz, 2007).

2.4.2. Shape discrimination capabilities and their benefits

Nevertheless, through behavioural experiments, we have known for some time that *G.petersii* can recognise three-dimensional shapes (even virtual ones) and accurately discriminate between objects differing in their shape (von der Emde and Schwarz 2000, 2002; Graff *et al.,* 2004; von der Emde and Fetz, 2007; von der Emde *et al.,* 2010; Schumacher *et al.,* 2016a, 2016b*,* 2017a). Note that Graff *et al.* (2004) generated virtual objects by placing electrodes within their setup. They were able to change the configuration of these electrodes (and hence the shape they projected) by changing the way in which they were connected. In the wild, it would be advantageous for *G.petersii* to determine an object's shape accurately, so that they could use this information to assess what the object is and determine what the most appropriate response would be, e.g. to escape from a nearby predator.

The ability to discriminate shape might help the fish to compensate for 'electric illusions' which can develop when assessing the distance of certain objects (von der Emde and Schwarz, 2000; Schwarz and von der Emde, 2001). *G.petersii* have difficulty estimating the distance of spheres, often judging them to be further away than other objects placed at the same distance. This might be the result of the fish producing smaller slope-to amplitude ratios than other objects of equivalent distance, presumably because of their curvature (von der Emde *et al.,* 1998). However, fish trained for a prolonged time with a variety of objects no longer made these mistakes, perhaps because they were able to recognise the shapes of the objects and then take into account the errors associated with estimating their distances (von der Emde and Schwarz, 2000; Schwarz and von der Emde, 2001). Shape would be a useful cue to extract from objects to improve their identification and subsequent categorisation.

2.4.3. Possible mechanisms behind shape recognition

2.4.3.1. Feature extraction

Although the mechanism of shape recognition itself has been difficult to ascertain, a number of observations highlight the features of shape that are attended to during active electrolocation. *G.petersii* can determine the shape of an object from their contours alone, even when these are interrupted, suggesting that *G.petersii* can perceive something like 'illusionary contours' (von der Emde and Fetz, 2007)- a phenomenon previously described in the context of vision (Nieder, 2002). However, if the outline of an object is too disrupted (i.e. corners only), *G.petersii* are unable to determine its true shape, suggesting that a single feature is insufficient to recognise shape (von der Emde and Fetz, 2007). Together, these observations suggest that *G.petersii* have the capability of identifying individual features from an object and using them to create an internal representation of it to aid shape recognition (Graff *et al.,* 2004; von der Emde and Fetz, 2007; von der Emde *et al.,* 2010; Schumacher *et al.,* 2017a). This theorised mechanism of recognition, whereby an animal extracts and memorises particular cues found in a learned stimulus, and then uses the information to decide how to react to a stimulus, is called 'feature extraction' and has been observed in animals such as the honey bee, where they have been found to abstract a feature from a visual pattern and use it to distinguish between novel patterns (Srinivasan, 1994; von der Emde and Fetz, 2007; von der Emde *et al.,* 2010; Schumacher *et al.,* 2017a).

2.4.3.2. Template matching

Another theorised mechanism of object recognition is known as 'template theory' or 'image matching' which has been considered extensively in the context of insect vision (e.g. Wehner and Räber, 1979; Cartwright and Collett, 1982, 1983; Collett, 1992; Collett *et al.,* 1992; Graham *et al.,*

2004; Collett, 2010). In template theory, an animal will learn and store a visual snapshot of part of their environment which is of interest to them. They will then compare current snapshots with this stored template. Snapshots that resemble the stored representation will be recognised as a match (Schuster and Amtsfeld, 2002). Insects are known to use template matching (or image matching) during visually guided navigation (Collett *et al.,* 2013). They can compare current views of their environment with remembered views to maintain a familiar direction (alignment image matching) and to head to a familiar location (positional image matching)- both surmised in Collett *et al.* (2013).

Desert ants seem to use alignment image matching when travelling along habitual routes, using the changing direction of a route landmark to activate a series of associated learned heading directions to keep them on the right course (Collett, 2010). Bulldog ants can use positional image matching to return to the nest, even from a location previously unknown to them (Narendra *et al.,* 2013). Template matching is a good navigational strategy for them to employ, as only a limited number of snapshots need to be learned and memorised, and as such, simplifies the process (Stamp Dawkins and Woodington, 2000; Schuster and Amtsfeld, 2002). Template matching works best when travel routes are kept constant and stored snapshots are accessed and assessed at the same points along these routes (Schuster and Amtsfeld, 2002; Collett *et al.,* 2013). Although this strategy has often been associated with invertebrates, *G.petersii* have been found to use template matching during recognition of visual patterns (Schuster and Amtsfeld, 2002; Schumacher *et al.,* 2017a). It is likely that this strategy is employed due to their visual system having poor spatial resolution (Schuster and Amtsfeld, 2002; Landsberger *et al.,* 2008; Kreysing *et al.,* 2012; Pusch *et al.,* 2013a, 2013b; Francke *et al.,* 2014). Indeed, brain regions involved in processing visual information (e.g. parts of the diencephalon, optic tectum) are much reduced in *G.petersii* compared to other teleost fish (Wullimann and Northcutt, 1990; von der Emde and Ruhl, 2016 cited in Schumacher *et al.,* 2017a; Schumacher *et al.,* 2017a). As such, a simpler mechanism of recognition would be desirable when using vision (Schuster and Amtsfeld, 2002). It

is possible that template matching could also be used during active electrolocation, where electric images of encountered objects are compared to stored electric images of known objects (von der Emde, 2004; von der Emde and Fetz, 2007).

2.4.4. How is shape encoded within electric images?

2.4.4.1. Difficulties of using a single static electric image to encode shape

Often object properties have been considered in the context of a single, static electric image. Being able to extract information from individual electric images can be advantageous, since it is a quick method, requiring only a single two-dimensional receptive surface and no additional movement of the animal (von der Emde *et al.,* 1998; von der Emde, 2006).

However, object shape cannot simply be taken as the shape of a single electric image due to the amount of distortion the electric field can experience (Caputi and Budelli, 2006; von der Emde, 2006; Engelmann *et al.,* 2008). Image parameters, like the slope of an electric image, have been considered as possible cues for shape recognition (Pusch *et al.,* 2008). It was demonstrated that cubes produced electric images with steeper slopes when positioned towards flat surfaces, like the trunk of the fish, and shallower slopes when positioned towards curved surfaces, like the head (Pusch *et al.,* 2008). Conversely, spheres were found to have the opposite effect on the maximal slope (Pusch *et al.,* 2008). The difference in the gradient of the slopes can be explained by the 'edge effect' (Sicardi *et al.,* 2000). Objects with a flat face (e.g. cube) will project an electric image distinguished by a plateau when opposing the fish's trunk. The plateau will stop suddenly when the ends of the object are reached, however. This results in high values for the slope at the edges of the electric image (Pusch *et al.,* 2008). Conversely, if objects with flat faces project onto the head region, no such plateau develops. This is due to the head being highly curved. As a result, electric images with shallower slopes form, since current draining at the edges of the

object leads to voltages spread more smoothly over a wider distribution (Pusch *et al.,* 2008). Spheres have the opposite effect with respect to the electric images that form on the respective surfaces (flat and curved). The edge effect is less pronounced for curved objects, meaning that the images they produce are reduced. When projected onto curved surfaces, these objects produce images that are smaller and with steeper slopes, since local amplitude is affected by the curvature of both the object and sensory surface (Pusch *et al.,* 2008).

Taking this all into account, it is doubtful whether the slope from a single electric image could be used as an unequivocal measure of shape, given how much the electric image is influenced by the object's positioning relative to the body, meaning a single object can appear very differently at one part of the body to another (e.g. Pusch *et al.,* 2008). The ambiguous nature of this measure means that it would be difficult to reconcile single snapshots of the same object taken from different points of the body, let alone discriminate between two objects of varying shape. Other cues would need to be considered to resolve the ambiguity, and/or the approach to the objects would have to be controlled in a way which meant that the two objects could be 'viewed' the same (i.e. from the front) and therefore be compared fairly.

2.4.4.2. Advantages of using multiple electric images to encode shape

We know that *G.petersii* will behave in a stereotypical fashion when exploring nearby objects and these behaviours might help reduce the ambiguities arising from static cues during object recognition (Toerring and Belbenoit, 1979; Toerring and Moller, 1984). However, it is likely that the egocentric motion is more useful in modulating the electric information in a dynamic way, by providing a series of electric images of a given object from numerous angles that can allow the extraction of more complex information, like shape (von der Emde and Fetz, 2007; Fechler *et al.,* 2012; Hofmann *et al.,* 2013b; Schumacher *et al.,* 2016b; Fujita and Kashimori, 2019). Shape is made up of a combination of features that make it a more complicated environmental cue. A

method of analysing a series of electric images from a given object may consequently be of use in assessing and evaluating the various features that contribute to shape.

Object shape might instead depend on analysing changes in the electric field over a series of successive electric images. These changes would occur if an individual was to swim around and scan an object from different viewpoints (Caputi and Budelli, 2006; von der Emde, 2006). If the object was stationary or slow moving, this behaviour would potentially help an individual to resolve any ambiguities which may have arisen when measuring other object parameters from static electric images, such as distance (Aloimonos and Rosenfeld, 1992 as cited in Caputi and Budelli, 2006; von der Emde *et al.,* 1998).

Recent research has moved away from understanding how weakly electric fish extract information from single static electric images and now focusses on how they might extract information more dynamically from a series of electric images (Hofmann *et al.,* 2013a, 2013b, 2014, 2017; Schumacher *et al.,* 2016b; Pedraja *et al.,* 2018, 2020). This strategy has many advantages over the analysis of single electric images. Firstly, it provides access to environmental information that can only be extracted when an animal moves relative to an object (reviewed by Hofmann *et al.,* 2013b). This method is also less susceptible to some of the ambiguities described for single electric images, though not immune (von der Emde *et al.,* 1998; Pusch *et al.,* 2008; Hofmann *et al.,* 2013a). Finally, this method of analysis can require fewer electroreceptors if looking at features that change temporally (Hofmann *et al.,* 2013a).

G.petersii might be able to obtain shape information about an object of interest from multiple electric images elicited by movement of the animal around that object (von der Emde and Fetz, 2007; Schumacher *et al.,* 2016b; Fujita and Kashimori, 2019). Fujita and Kashimori (2019) examined what shape related cues could be obtained from a series of electric images by creating a model which predicted the electric field generated by a hypothesised fish and how nearby objects might perturb this field. Fujita and Kashimori (2019) looked at multiple electric images

that were generated by rotating differently shaped objects rather than the movement of the fish itself. They found that differently shaped objects display similar Gaussian shapes within a single electric image and were not significantly different from one another, providing further support that shape is unlikely to be encoded within a single electric image (Fujita and Kashimori, 2019). Although various electric image parameters changed when an object was rotated, Fujita and Kashimori (2019) found that the integrated effect of peak amplitude and half-maximum width of electric images provided the best measure of an object's shape, feasibly being extracted during exploratory swimming (Fujita and Kashimori, 2019). However, more research is required to validate this putative cue given that the model made several assumptions that do not hold true (objects were two-dimensional, body shape and skin conductivity were uniform, and electric organ fired in a simple way across space and time; Fujita and Kashimori, 2019). The last three variables will vary according to the weakly electric species being studied. Given this, it is unknown whether *G.petersii* would be able to extract shape information via this mechanism.

Schumacher *et al.* (2016b) also proposed that *G.petersii* might be able to extract shape information from an object by moving alongside it, since examining an object from different positions would lead to changes in the voltage pattern across a series of electric images and the magnitude of these changes would be dependent on the object's shape (Hofmann *et al.,* 2013a, 2013b). As such, individuals should be able to tell differently shaped objects apart from analysing these electric images (Schumacher *et al.,* 2016b). For example, a sphere when viewed from a constant distance, will appear similar from different angles due to its symmetrical nature. However, an elongated object, like a cylinder, will appear differently depending on whether it is viewed from its short end compared to its long end (Schumacher *et al.,* 2016b). Hence, an individual should be able to reliably distinguish between objects based on the modulations of these electric images, so long as the objects differ enough in their shape (Schumacher *et al.,* 2016b). The changes in the electric image that occur as a fish swims past an object is referred to as 'movement-induced modulations' or 'MIMs' (Schumacher *et al.,* 2016b). Previous work

examining the role of MIMs in shape recognition found that fish could discriminate two objects across a further range when there was a large difference in their MIMs (which occurred when the two objects differed a lot in their shape). Objects that were more similarly shaped (and as such elicited similar levels of MIMs) could not be recognised for so long, with discrimination breaking down at shorter distances (Schumacher *et al.,* 2016b). These results, together with observations of probing motor acts during object investigation, indicate a possible role of MIMs for shape recognition. Nevertheless, the evidence is not robust, as only a very limited number of individuals were studied (N=2) and the use of non-movement cues derived from a single electric image could not be discounted (Schumacher *et al.,* 2016b). Furthermore, this study only manipulated the sensory component (the objects that could be perceived) and not the motor component (swimming space was not constrained) despite the likely importance of movement in sculpting the electric information available (Hofmann *et al.,* 2013b; Schumacher *et al.,* 2016b).

2.5. Aim of study

Our study aimed to test the role of MIMs in shape recognition by manipulating the motor component of the behaviour. To do this, we trained individual fish in a two-alternative forced-choice (2AFC) setup to discriminate between two objects differing only in shape. Once learning was confirmed, we varied the space available for them to undertake scanning movements next to the objects, and we tested whether this led to a change in discrimination performance. If MIMs were involved in shape discrimination, we would expect an individual's performance (indicative of their ability to discriminate) to decline as swimming space was restricted. This study is not only important for examining the role and importance of egocentric movement in sensory acquisition, but also the consequences of disrupting this type of behaviour.

3. MATERIALS AND METHODS

3.1. Subjects

We trained individual *G.petersii* to complete a shape discrimination task in a 2AFC setup (N=6). This species cannot be bred readily in captivity (Landsman, 1991, 1993) but is widely available through the international aquarium trade. We sourced our fish from a licenced fish dealer (The Goldfish Bowl, Oxford, UK). These were originally from Nigeria (Edo, Delta, or Lagos state) and had an approximate total length of 11.6-14.2 cm (excluding Schnauzenorgan) and 13.1-16.2 cm (including Schnauzenorgan). We could not age or sex the fish due to hormonal changes triggered by captivity (Landsman, 1991, 1993). Individuals were housed separately as they are territorial and will show aggression to conspecifics, especially when kept in small groups (reviewed in Moller, 1995; Terleph, 2004; von der Emde *et al.,* 2012). Each fish was housed in a standard two-foot tank (approx. l x w x h: 60.2 x 35.2 x 31.5 cm) which also served as their experimental space. The fish were maintained under strict conditions due to their sensitivity to environmental pollutants and sudden changes in water parameters (Geller, 1984; Lewis *et al.,* 1990, 1992a, 1992b; Prabhakar and Landsman, 1994 as cited in Olaosebikan *et al.,* 2020; Alazemi *et al.,* 1996). Reverse osmosis water was treated with a remineralisation formula to ensure fish well-being and stabilisation of the aquarium systems (Tropic Marin, Tropical Marine Centre, UK). Water conditions were controlled to replicate natural conditions as much as possible. Water conductivity was 415 ± 35 µS/cm, water temperature was 25.5 ± 0.5 °C, and pH 7.3± 0.3. We set the overhead room and tank lights to a 12:12h light-dark cycle. Experiments were conducted during the light half. Light levels ranged from 20-30 lux, as measured above each tank using a light meter (LX1330B, Dr. Meter). Night time light levels were assumed to be close to 0 lux due to the lack of light sources at this time (Landsberger *et al.,* 2008). Fish were fed bloodworms (Gamma, Tropical Marine Centre, UK) only during training when participating in the study. Outside of experiments, fish were given bloodworms twice a day, six times a week. We also supplemented

their diet with brine shrimp (twice weekly) and additional vitamins (once a week) to ensure they got the nutrients they required for healthy body function. In addition, we provided plenty of enrichment to keep them engaged outside of experiments. Note that this study was conducted in accordance with the University of Oxford's animal ethics guidelines.

3.2. Experimental setup

Each tank was made up of four different compartments: the waiting area (l x w: 14.0 x 35.2 cm) where the fish began trials, the sensing area (26.2 x 35.2 cm) where the fish decided which object to choose, and the two experimental areas, A and B (each being 18.0 x 17.1 cm) which contained either the positive or negative object (Fig. 1). Outside of experiments, the fish could access all compartments of their tank, but these areas now contained normal enrichment rather than experimental apparatus.

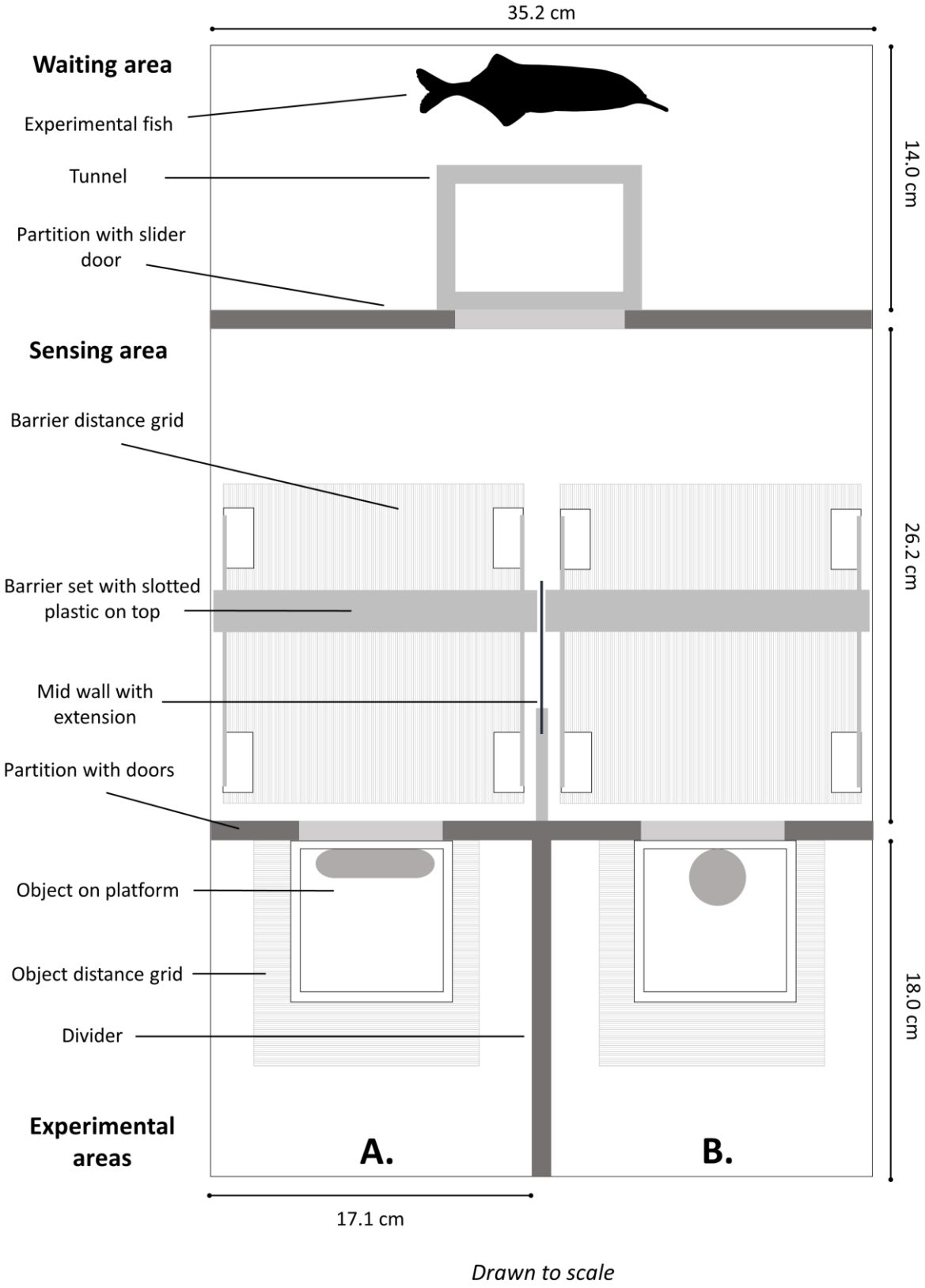


Fig. 1. Top view of the experimental (2AFC) setup used during shape discrimination trials.

Materials for the apparatus were carefully chosen to minimise the effect on the electric fields generated by the fish, as well as for their durability in water (see appendix A for more setup details).

An acrylic mid wall (h x w x d: 28.5 x 6.0 x 0.6 cm) extended from the partition separating the sensing area from experimental areas A and B (Fig. 1). The purpose of this wall was to prevent fish taking shortcuts when exploring the objects by the openings to A and B in the sensing area. We wanted to control the area that the fish moved through and sensed, since we were interested in determining if changing this space had an impact on an individual's ability to discriminate between the two objects correctly. As such, it was important to limit behaviour outside of the boundaries we set, since these fish will tend to take the simplest routes to reach their goal, including taking cut-throughs (Skeels, personal observations).

A slider door controlled access into and out of the waiting area (Fig. 1). The slider door was made of white polypropylene (h x w x d: 9.7 x 9.0 x 0.2 cm) with a nylon mesh insert (7.0 x 6.9 cm). A clear acrylic tunnel (28.9 x 10.9 x 7.7 cm) was placed at the entrance of the door to ensure the fish did not leave the waiting area at too much of an angle in case this had a bearing on choice (tunnel opening facing fish: 27.7 x 10.1 cm). This was particularly important at the start of training when we needed to override pre-existing side biases. We found that fish tended to pick the same side/compartment (A or B) consistently regardless of the object placed. This preference was different for each individual and likely developed over time. This behaviour is not surprising given these fish exhibit sensory lateralisation, which describes asymmetric sensory input relating to task specialisms of different brain regions (Kareklas *et al.,* 2018).

Four barriers were placed in the sensing area (Fig. 2). Two were placed either side of the opening to A, and the same for B (Fig. 1). Each barrier comprised of a clear acrylic surround and a nylon mesh window (mesh size: 0.71 mm per square). The overall size of each barrier was 18.4 x 14.4 x 0.2 cm, and the mesh window was 14.8 x 7.9 cm. Barriers were placed in the sensing area to limit

the amount of space that the fish could occupy and consequently scan using their electrosensory system (Fig. 2). During training, the barriers were placed 15.9 cm apart (D1). In testing, the barriers could be placed at either 15.9 (D1), 12.7 (D2), 9.5 (D3), or 6.3 cm apart (D4).

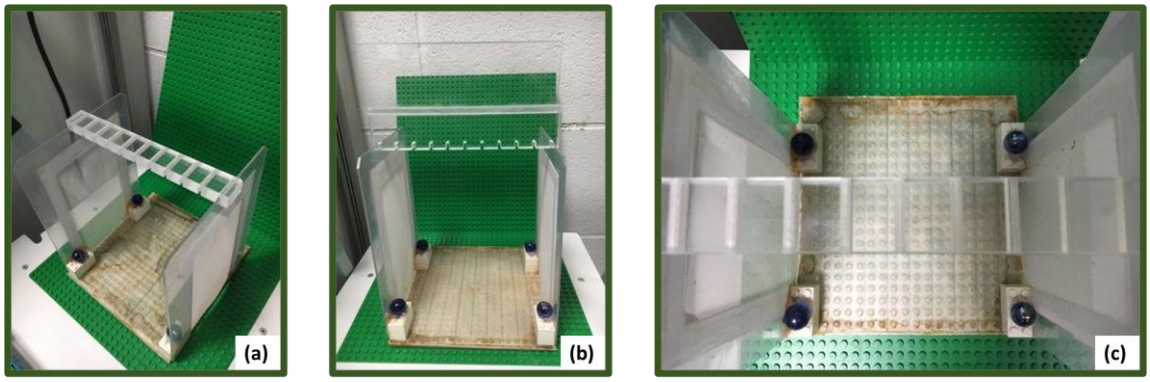


Fig. 2. A typical barrier placed in the sensing area. (a) Two barriers were connected by a slotted piece of plastic to form a barrier pair. One barrier pair was placed by the entrance to A, another by B. (b) Some barrier pairs were connected by taller slotted pieces of plastic. This was to encourage those individuals more inclined to swim over the barriers to swim through the channel created by each pair instead. (c) Top view of barrier pair. The barriers are aligned using the distance grid (seen in background) and the distance between the two is maintained with the slotted plastic (seen in foreground). Note that in all photos the barriers were placed at their widest extent, 15.9 cm, or D1.

During training, each object was positioned on a platform (overall height ca. 10.8 cm, base: l x w x d: 8.6 x 8.6 x 0.5 cm and top: 7.6 x 7.6 x 0.5 cm). Objects were placed ca. 1.6 cm behind the doors if fish were trained without the doors and ca. 1.9- 2.5 cm away from if trained with the doors (had to also account for door depth and door not being flushed). This distance was chosen since it sits within the range at which *G.petersii* can successfully discriminate objects (von der Emde *et al.,* 2010; Fechler and von der Emde, 2013; Schumacher *et al.,* 2016a, 2016b). To get into these experimental areas, an individual was required to move the doors using their Schnauzenorgan.

Each door comprised of a white polypropylene surround (Ø= 7.6 cm, total d=0.3 cm) and a nylon mesh window (Ø= 6.4 cm). We trained and tested two individuals with the doors removed to check that the doors themselves did not influence performance.

An Apeman A70 Action Camera recorded the behaviour of the fish during training and test trials. It was placed 31.50 cm above the surface of the water to ensure the whole tank could be seen, albeit with a slight fishbowl effect. This was not an issue for us since we were not using the footage for tracking purposes. We set the frame rate to 30 FPS and the image resolution was set at 1920 x 1080 pixels (frame width x height). Playback of the recorded sessions was useful in checking data collected during trials and for collecting supplementary information.

3.3. Training regime

Pre-training sessions were conducted before training fully commenced. These sessions allowed individuals to become familiar with the setup. They learned to swim out of the waiting area and into the sensing area, swim through the channels made by the barriers, open the doors leading to A and B, enter A/B to get a reward, and then return to the waiting area. They were also given time to become familiar with the object platforms.

The fish (N=6) were trained separately in a 2AFC setup to swim past the door and into the area with the positive object (which was associated with a food reward- bloodworm) and to avoid the area with the negative object (which was associated with a mild punishment- being shooed back to the waiting area). Similar reward and mild punishment schemes have been used in the past to train *G.petersii* with great success (e.g. von der Emde and Fetz, 2007; Schumacher *et al.,* 2016a, 2016b, 2017a).

Bloodworm was given as the food reward and dispensed using a plastic pipette. A plastic 30 cm ruler with a metal washer attached (ca. outer Ø= 2.9, inner Ø= 0.9, d=0.5 cm) was tapped against

the side of the tank and used to shoo the fish back (see appendix A). The combination of the sound and movement was aversive to the fish but not enough to inflict stress.

The position of the positive object was randomised using a true random number generator (random.org, 2021) albeit within predefined limits. This meant that the positive object appeared 50% of the time in A and 50% of the time in B. We did this to prevent choice being determined by object location rather than the intrinsic properties of the objects themselves. This was important since we know that *G.petersii* and other mormyrid species prefer to use egocentric cues when navigating familiar environments (Walton and Moller, 2010; Schumacher *et al.,* 2017b). Generally, each fish completed 24 trials in a training/testing session. One training/testing session was conducted with each fish per day. A total of 3214 training trials were conducted during the training phase.

3.4. Experimental Objects

The fish were split equally into two training groups. All fish received the same training and testing regime, but they differed with respect to the objects that they were trained with (Fig. 3). All fish were trained with the same positive object (S+) but each group was provided with a different negative object (S-).

S+ was a sausage-shaped object (h x w x d: ca. 1.5 x 6.3 x 1.5 cm). S- was either a cube (side length 2.42 cm) or sphere (Ø 3 cm). The sausage-shaped object was presented with its longest side facing the door and the cube was presented with one of its sides facing the door. All objects were made from the same material (aluminium) and of the same approximate volume (sausage-shaped object ~14.18 cm^3 or ~13.35 cm^3 depending on the calculation, sphere~14.14 cm^3, and cube~14.17 cm^3). We did two volume calculations for the sausage-shaped object. The first assumed it was a cuboid and the second assumed it was a capsule. Neither quite encapsulated

our object, so it's likely the true volume was somewhere between the two estimates. Regardless, the volumes of the objects were sufficiently similar that it should not have impacted on choice (von der Emde and Fetz, 2007). As such, the objects differed only in their shape (with object height being a feature of this). Aluminium objects were chosen to stand out and contrast with the surrounding equipment. Since aluminium is a good electrical conductor, it would have increased stimulus amplitude, whereas the surrounding plastic apparatus would have decreased this (Rojas and Moller, 2002; von der Emde and Fetz, 2007).

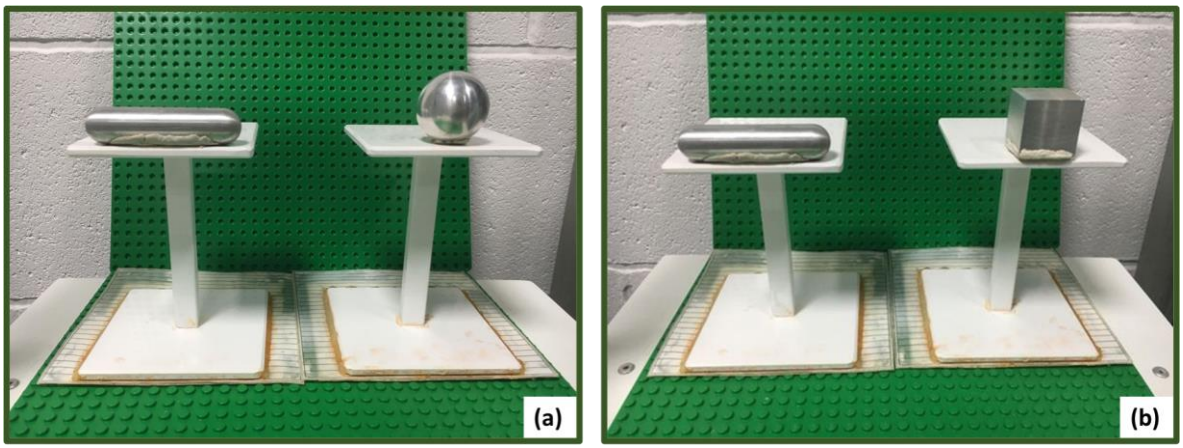


Fig. 3. Object pairs used in the shape discrimination task. (a) Fish in group one (N=3) were trained to discriminate between a sausage-shaped object (S+) and sphere (S-). (b) Fish in group two (N=3) were trained to discriminate between a sausage-shaped object (S+) and a cube (S-). Objects were made from aluminium and had approximately the same volume. They were positioned at approximately the same distance from the doors, and as such, they only differed in their shape. Note that the sausage-shaped object was S+ for both training groups.

3.5. Testing regime

Testing commenced once individuals had reached the pre-assigned learning criterion of 75% correct over three consecutive training sessions (as described in Schumacher *et al.,* 2016a, 2016b). If MIMs are involved in shape discrimination, we would expect an individual to find the

task harder and make more mistakes when swimming space beside the objects is restricted. This is because at shorter distances an individual would be more limited in their ability to perform scanning behaviours, and as such they will be less able to obtain shape information about each object through active electrolocation, making it harder for them to differentiate between the two, resulting in S- being chosen more often.

Test trials were introduced every third trial. During these trials, the position of the barriers by the doors were changed. The position was the same for both barrier pairs (one by A and one by B) and chosen randomly, albeit on a predetermined scale. The barriers could be placed at four possible positions: 15.9 (D1), 12.7 (D2), 9.5 (D3), or 6.3 cm apart (D4). Since there were eight test trials, we were able to test each distance twice in a single testing session. During test trials, fish were neither rewarded nor punished to prevent further learning (von der Emde and Fetz, 2007; Schumacher *et al.,* 2016b, 2017a). Testing was interspliced with training trials to avoid frustration developing by the lack of reward in test trials, maintain discrimination performance, and keep motivation high (von der Emde and Fetz, 2007). On average, we conducted 30 testing trials at each distance for every individual studied. This ensured sampling was approximately even across distances and that we had enough repeatability in our dataset, within and across individuals. In total, we conducted 1460 training trials and 720 test trials in the testing phase alone (see appendix A for more details).

During both training and testing trials, we made a note of the location of S+, the barrier distance, whether a correct choice was made (our metric of performance/success), how long it took for the choice to be made, and the fish's movement patterns during that trial. We did this to help us understand the decision-making process during the task under various conditions. This will be examined further below.

3.6. Posthoc analyses

3.6.1. Latency of response during test probes

Latency (response time) is defined here as the time it took an individual to choose an object (measured from when the slider door was opened to when the fish entered either A or B completely). We were interested in looking at latency as a measure of certainty, with the idea that an individual would take less time if they were more certain of their choice, and conversely more time if they were less certain.

As such, if MIMs were used for shape discrimination, we would not only expect to see a change in performance with decreasing barrier distance, but a change in latency too. We would expect correct object selection to decline as swimming space becomes more restricted. The increased amount of uncertainty in identifying S+ would translate into longer latencies, since repeated inspections of the objects would be needed before an individual would be able to make an informed decision on which object was the correct one to pick.

3.6.2. Side switching behaviour during test probes

Side switching is defined here as the number of times an individual went between the two sides of the sensing area before making a choice. Side switching was examined as another measure of certainty. We hypothesised that individuals would make fewer side switches when they were more certain of their choices, and more when they were less certain.

If MIMs were involved in shape discrimination, the increased uncertainty as scanning space narrows would not only have a consequence on their performance in trials (assessed as % correct) but also on side switching. We would expect an individual to switch between the two sides more when the distance between barriers is narrower, as limited manoeuvrability will make individual scans less informative. Conversely, when barrier distances are increased, fewer

switches would be expected, since an individual would have enough space to scan the objects more optimally (as learned through training). As such, fewer visits to each side would be necessary to make an accurate assessment on which object to choose.

We studied the amount of side switching (no, single, or multiple) across each of our testing distances. No side switching meant that the fish chose the side first visited. Single side switching meant that the fish went to one side then visited another, ultimately choosing this second side. Multiple side switching meant that the fish went between the two sides numerous times before making a choice. For each testing distance, we split trials according to their outcome (either correct or incorrect). We did this to determine whether side switching behaviour differed between trials which resulted in a correct choice being made to those where an incorrect choice was made. We thought fish might employ different strategies to complete the task when encountering higher or lower levels of certainty (Fig. 9). Note that this was a qualitative analysis as we were time constrained.

3.7. Statistics

3.7.1. Shape discrimination performance during testing

We used binomial generalised mixed models (GLMMs) to test for an effect of barrier distance on shape discrimination performance. We initially created two models to predict correct object selection, with the first being a full model which contained two fixed effects and one random effect. The fixed effects were barrier distance (our main parameter of interest, left as a numeric) and object pairing (coded as a two-level factor, since fish were trained with two different object sets). Our random effect was fish ID and was classed as a factor. The second model was very similar to the first, but our main parameter of interest (barrier distance) was dropped. We then compared the two models using a likelihood-ratio test (LR) to determine which one was a

significantly better fit to our data. We then investigated the preferred model to determine what was driving the discrimination performance of our fish and the effect size of these predictors (see appendix A for further details).

3.7.2. Latency of response during test probes

We used gamma generalised mixed models (GLMMs) to determine whether barrier distance had a significant effect on latency. We chose this type of model due to the distribution of our data (Fig. 8). We initially created two models. Our first model stated that latency depended on barrier distance and the outcome of the trial (both fixed effects) and fish identity (our random effect). The second model omitted the key variable but left in the other variables. Like above, a LR test was then done to compare the two models to assess which one best fitted our data. The better fitting model was then run and investigated to determine what was influencing response times and the strength of the influence (see appendix A for more information).

Statistics were run in *R Studio, R version 3.6.2* using the 'lme4' package.

3.8. Maintenance of task performance

We continually assessed how well an individual remembered the task by examining the training trials interspliced with test probe trials during testing. An individual was said to have a good understanding of the task if they got 75% or more correct in these training trials (see appendix A for more details on criteria).

4. RESULTS

4.1. Task acquisition

All fish learned to discriminate between S+ and S- regardless of the training group they were assigned to (Fig. 4). However, they all learned at different rates (Figs. 4a-4f). Note that the fish did not start the experiment completely naïve. They had some experience with the apparatus having been trained in a similar setup previously. However, they had never reached the learning criterion of 75% correct on three consecutive sessions until this current iteration of the experiment.

Fish one to four accessed A and B by moving a door with their Schnauzenorgans (Figs. 4a-4d). For fish five and six, we removed the doors so that they could simply swim into A or B without moving a door (Figs. 4e and 4f). We found that the doors did not have a negative effect on task acquisition (Fig.4).

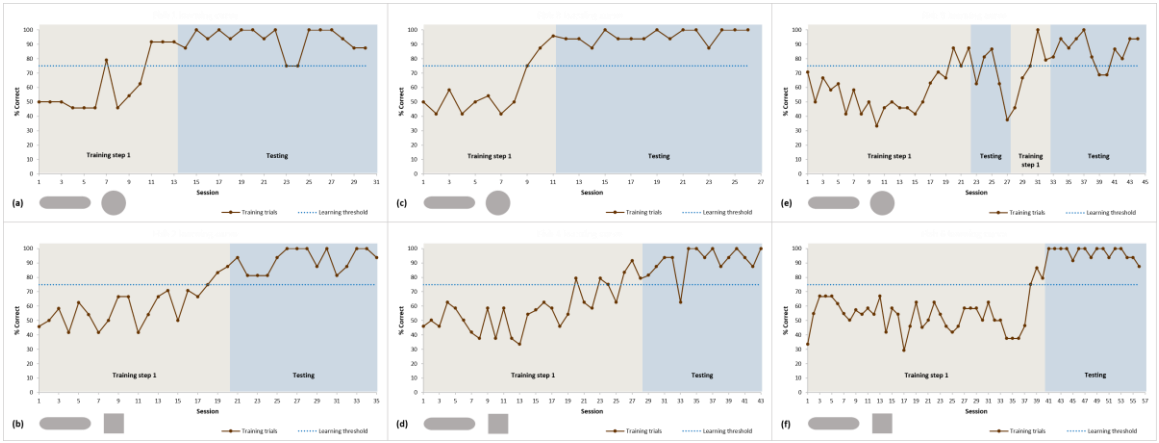


Fig. 4. Learning curves for fish one to six (a-f). Each fish learned to discriminate between two objects. Fish were all trained with the same S+ (a sausage-shaped object) but differed in their S- (either a sphere or cube). Fish one (a), three (c), and five (e) had the sphere as their S-, whereas fish two (b), four (d), and six (f) had the cube. S+ was associated with a food reward during training and S- with a mild punishment, being shooed back to the starting area. Fish one to four were trained with doors, whereas fish five and six were not. Training step 1 (shaded in light brown) involved the fish learning to discriminate the objects with the long side of S+ facing

towards the fish. The cube was placed so one of its sides faced the fish. The orientation of the sphere did not matter due to its symmetrical nature. A session usually consisted of 24 trials, but performance scores were converted into percentage of trials correct, so sessions of different lengths could be compared. Once the fish had reached or exceeded the learning threshold of 75% on three consecutive sessions (dotted line) they moved onto testing (shaded blue). There was great variability in how long this took between fish. During testing, training trials were interspliced with test probes to maintain motivation and performance. The scores from these are plotted here. Performance rarely dropped below 75% for most fish. Note that test probes could not be plotted here since the conditions were not the same as for the training trials (variable barrier placement and no food reward or mild punishment). Total N=4722 training trials across both experimental phases (fish one=584, fish two=720, fish three=504, fish four=895, fish five=893, and fish six=1126).

4.2. Shape discrimination performance during testing

We created two models to predict success at choosing S+ (see methods section). We found that our first model best fitted the data (LR test: χ^2=43.403, df=1, p<0.001) and since the only difference between the models was the inclusion of barrier distance, this suggests that barrier distance was a significant explanatory variable. We examined model one to confirm the influence barrier distance had on discrimination performance and to quantify its effect. We also examined what other variables may have contributed to performance.

We found that an individual's ability to discriminate differently shaped objects was significantly influenced by how far apart the barriers in the sensing area were (GLMM: Z=6.184, df=716, p<0.001; Figs. 5, 6, and 7; Table i). Fish found it harder to correctly identify the positive object as the distance between the barriers was reduced (Figs. 5, 6, and 7; Table i). This was regardless of which objects the fish were trained and tested with (GLMM: Z=0.795, df=716, p=0.426).

The modelled effect of distance on performance was similar to what we observed in the raw data collected during testing, and this supports distance being a significant factor in how well the fish were able to discriminate between the two objects (Figs. 6, and 7; Table i). Additionally, we found that performance did not decline steadily across distance but dropped more sharply at the shorter distances (Table i; see appendix for further model details). This sharp drop likely relates to a discrimination threshold being reached, where the reduction of space means that these fish cannot scan the objects adequately, so are unable to discriminate between the two objects reliably (see discussion).

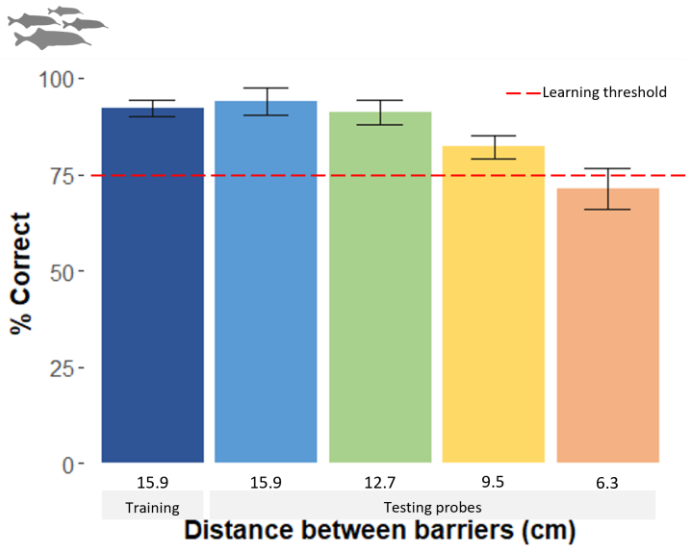


Fig. 5. Group mean performance during the testing phase of the MIMs experiment. Both training and test probe trials were conducted in this phase (every third trial was a test). Performance was taken as the percentage of trials during a particular condition that were correct. Group mean performance was calculated by pooling performance data from all individuals studied (N=6). Group means are represented by the coloured bars. The dark blue bar (on the far left) shows group mean performance during the training portion of testing and is here as a baseline to compare against. The remaining bars show group mean performance during test probes when barrier distance was varied by a set amount, and no reward or punishment was given. The black

error bars indicate the standard errors of the mean for each condition studied, whilst the dotted line denotes the learning threshold of 75% for each barrier distance.

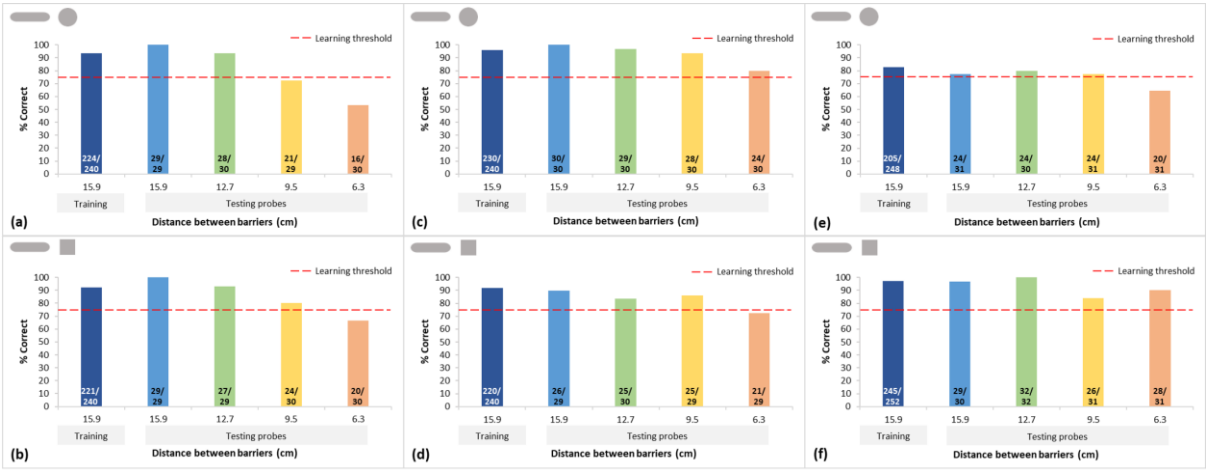


Fig. 6. Performance of fish one to six during the testing phase of the MIMs experiment (a-f). Performance was determined by the proportion of trials for each condition that were correct. Training trials completed in testing are shown in dark blue. The rest of the bars show how well they were able to complete the discrimination task at different barrier distances. The numbers within each bar show how many trials were correct against how many were run. The dotted line represents the learning threshold of 75%. Fish one, three, and five were all trained with the sphere as their S- (a, c, and e). Fish two, four, and six had the cube as their S- (b, d, and f). Note that fish one to four (a-d) accessed A and B by moving a door, whereas fish five and six did not (e and f).

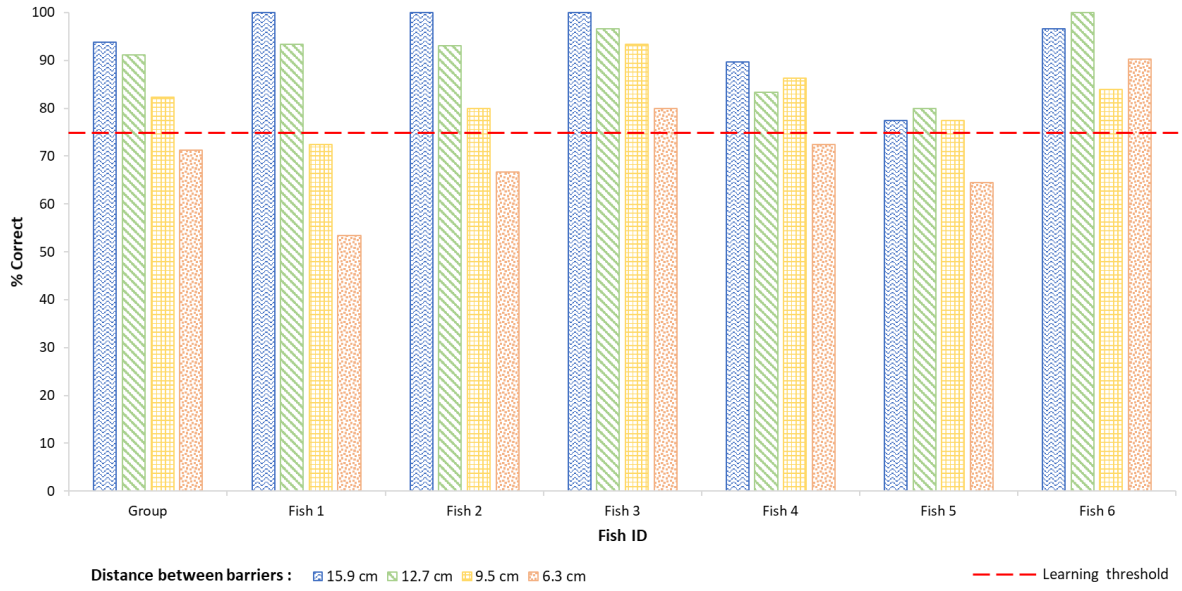


Fig. 7. Performance scores shown for test probe trials on a group (far left) and individual level (rest of bars). Group performance was calculated by combining data from the six fish tested at each barrier distance and calculating the proportion of trials that were correct under each condition. Performances at each barrier distance are colour and pattern coded. Learning threshold is shown by the red dotted line. 720 test trials were conducted in total (fish one: 118, fish two: 118, fish three: 120, fish four: 117, fish five: 123, and fish six: 124)

Table i. Predicted and observed values of success for a test trial given barrier distance

	Predicted probability of success %		**GLMM**			**Observed success[1] %**	**Predicted (observed) performance change %**
Distance	**Estimate**	**95% CI**	**Z**	**df**	**p**		
D1 (15.9 cm)	94.12	[84.28, 98.10]	6.184*	716	<0.001	93.82	
D2 (12.7 cm)	89.23	[69.08, 97.04]				91.16	D1-D2= -4.89 (-2.66)
D3 (9.5 cm)	81.07	[48.21, 95.41]				82.22	D2-D3= -8.16 (-8.94)
D4 (6.3 cm)	68.89	[27.94, 92.95]				71.27	D3-D4= -12.18 (-10.95)

N=6 individuals; 720 observations (test probe trials); [1]Observed success was determined after pooling together data from all individuals tested. For each barrier distance, success was then calculated as: (total number of correct trials/total number of trials run) x 100; *modulus of Z

4.3. Posthoc analyses

4.3.1. Latency of response during test probes

Latency increased as barrier distance decreased. We created two models to predict latency (see methods section). We found that model one best described the latency data (LR test: χ^2=378.79, df=1, p<0.001). Since the only difference between the two models was the inclusion of barrier

distance as a fixed effect, this suggests that barrier distance is a significant explanatory variable in explaining the latency patterns we observe (Fig. 8). We found that barrier distance did indeed have a significant effect on latency (GLMM: T=19.949 (modulus); df=703, $p<0.001$). However, trial outcome did not have a significant effect on latency, which is also supported by the plots (GLMM: T=0.677, df=703, p=0.499; Fig. 8).

We found that latencies were skewed strongly to the left regardless of barrier distance and trial outcome: short response times were most common with longer response times being much rarer (Fig. 8). However, as barrier distance narrowed, the 'tails' of the plots lengthened. This was most evident at D4 (Figs. 8di and 8dii) where the tails were significantly longer (ca. five to eight times) than what was observed at D1 (Figs. 8ai and 8aii). At D4, not only were the plot tails longer, but even the 'short' responses were lengthier than at other distances (Fig. 8). Our plots support the model's conclusion that trial outcome had no significant effect on response times, but it is important to treat this with some caution, since there were many more data points for correct than incorrect trials. This could not be helped as incorrect trials were rarer events (Fig. 8). This trend was consistent across individuals, though some showed this trend more subtly.

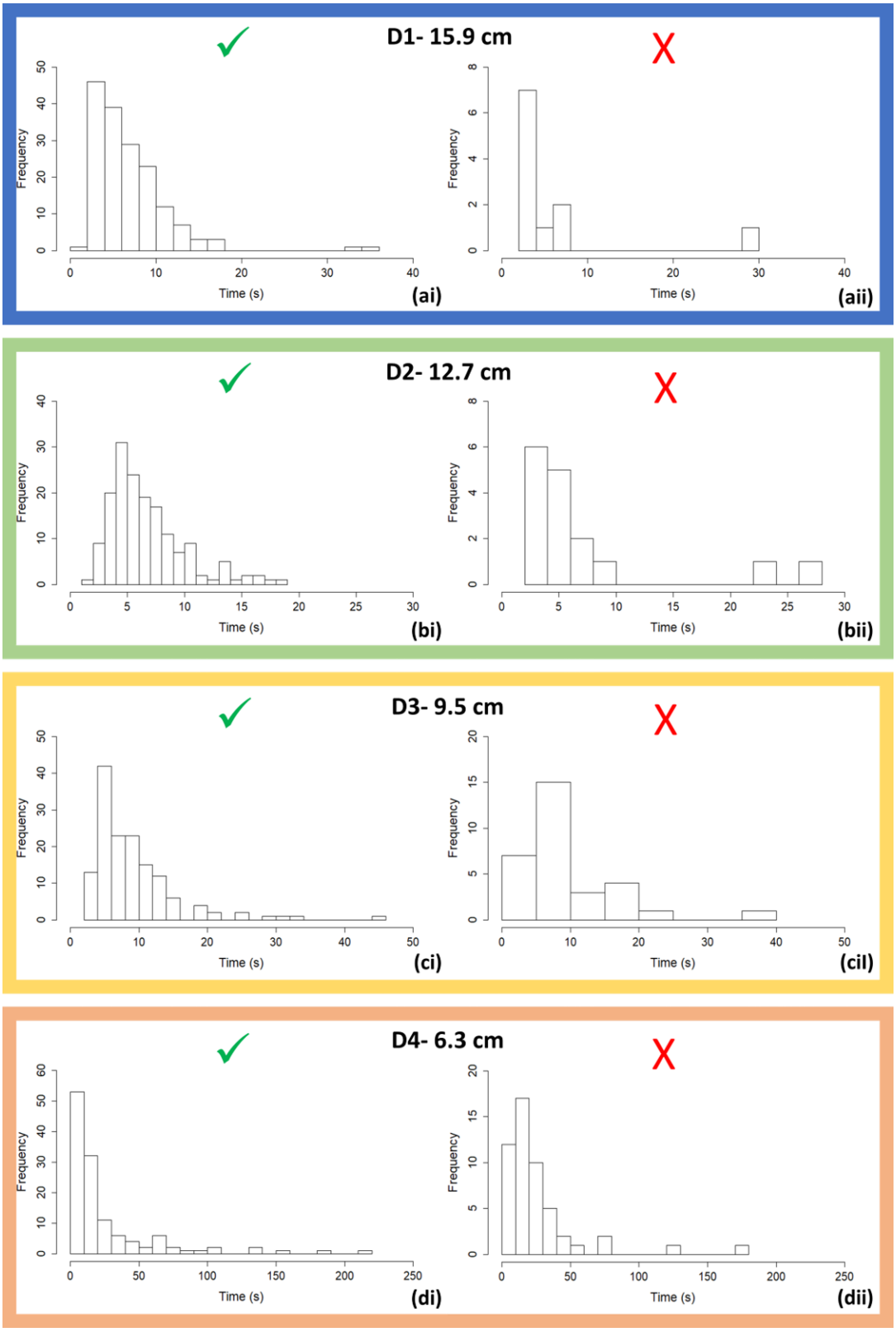


Fig. 8. Response times for completing the shape discrimination task during test probe trials at barrier distances one to four (a-d). Each data point represents the time it took an individual to make a choice in a single trial (given in seconds, s). Times have been pooled together here to

show responses on a group level. For each distance, response times have been categorised according to whether the trial resulted in a correct (i; tick) or incorrect choice being made (ii; cross). N=708 trials (ai=165, aii=11, bi=163, bii=16, ci=146, cii=31, di=125, and dii=51).

4.3.2. Side switching behaviour during test probes

Multiple side switching increased with decreasing barrier distance (Fig. 9). We found that, as a group, no side switching was the most common of the three behaviours observed regardless of testing distance and trial outcome (Fig. 9a). Single side switching followed next, and then multiple side switching (Fig. 9a). Multiple side switching seemed to occur more often in trials where a correct choice was made regardless of the testing distance (Fig. 9a). Indeed, trials where an incorrect choice was made showed higher levels of no side switching compared to their correct counterparts (Fig. 9a). We cannot quantify a distance effect on switching behaviour as we did not model this due to time constraints. Nevertheless, a noticeable increase in multiple switches can be observed at the shortest barrier distance when looking at the group plot (Fig. 9a). Interestingly, at this distance (D4) there was less difference in the proportion of the different switching behaviours between correct and incorrect trials (Fig. 9a).

In general, we observed a range of switching behaviours during test probe trials. However, individuals tended to prefer to limit their side switching where possible, indicated by the dominance of no and single switching behaviours across barrier distances (Fig. 9). There was variation in the type of switching behaviour preferred by the fish, with some individuals displaying a stronger preference for no switching than others (Fig. 9). Multiple switching tended to be most observed when fish were completing trials at the shortest barrier distance, D4 (Fig. 9). Correct trials tended to be more diverse in terms of the types and proportions of side switching behaviours noted than their incorrect counterparts (Fig. 9). Fish five and six showed lower levels

of multiple switching compared to the other fish studied (Fig. 9). Both these fish were trained and tested without the doors.

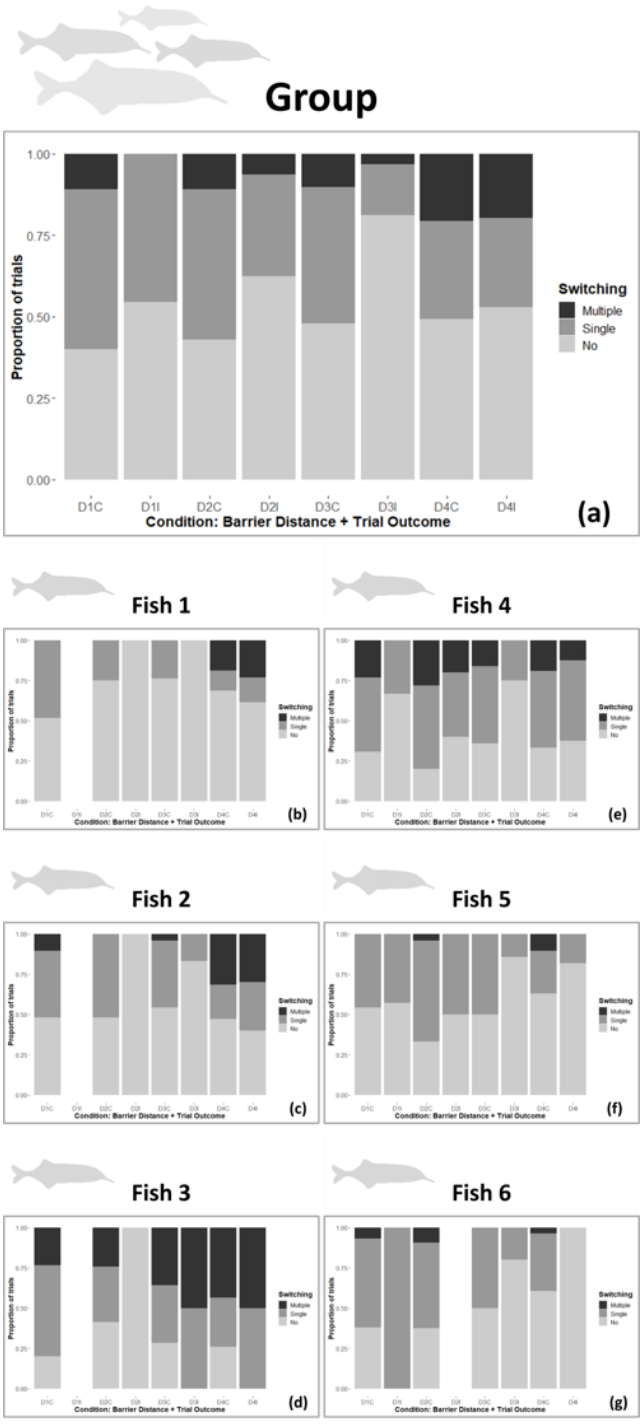


Fig. 9. Side switching behaviour of *G.petersii* during test probe trials. Amount of side switching that occurred under different testing conditions for both the group as a whole (a) and on an individual level (b-g). Trials were placed into one of eight conditions. The condition was

dependent on the barrier distance used for that trial (D1-D4) and the outcome of the trial at that testing distance (C=correct trial and I=incorrect trial). Greyscale bars indicate the type of side switching behaviour observed (lightest grey=no side switching, middle grey=single side switch, and lastly, black=multiple (two or more) side switches). The size of each bar represents the proportion of trials which fitted each category. Blank spaces denote conditions where no trials were available to assess. Total N=716 trials (fish one=117, fish two=117, fish three=119, fish four=117, fish five=122, and fish six=124).

5. DISCUSSION

G.petersii have the remarkable ability of being able to recognise complex three-dimensional shapes using active electrolocation (von der Emde and Schwarz 2000, 2002; von der Emde and Fetz, 2007; von der Emde *et al.,* 2010; Schumacher *et al.,* 2016a, 2016b, 2017a). However, it has been difficult to investigate how *G.petersii* do this, since no image parameter or collection of image parameters in a static electric image has been shown to encode shape reliably (von der Emde, 2006; Schumacher *et al.,* 2016b). Recently, research has focussed on whether shape information could be extracted dynamically by comparing a succession of electric images during movement (von der Emde, 2006; von der Emde *et al.,* 2010; Fechler and von der Emde, 2013; Hofmann *et al.,* 2013b; Schumacher *et al.,* 2016b; Fujita and Kashimori, 2019).

Here, we investigated the role of egocentric movement in shape discrimination in *G.petersii.* We were interested in determining whether movement-induced modulations could provide a useful method for extracting shape information. Previous research suggests that shape information could be obtained from these modulations and used to discriminate objects of differing shape; with objects being discriminated from further distances when they differed more in their modulations, however the conclusions were not robust (Schumacher *et al.,* 2016b).

To achieve this, we trained fish to complete a shape discrimination task in a 2AFC setup. We manipulated the space available for scanning movements to determine whether this had an impact on their ability to distinguish the objects correctly.

5.1. Task acquisition

All fish learned to discriminate between S+ and S- (Fig. 4). We would expect this given that the objects were placed within the distance range that active electrolocation can successfully be used for object recognition- 4 cm or less (von der Emde e*t al.,* 2010).

From our plots, we observed variation in how long it took our fish to learn the task (Fig. 4). Object pairing may have influenced learning rate (von der Emde and Fetz, 2007) but this seems unlikely given that object pairing had no effect on discrimination performance. Individual approaches to learning may have factored in (von der Emde and Fetz, 2007) as well as age. However, we can only speculate as our study was not designed to answer these questions.

Generally, our fish took longer to learn the task than those examined by von der Emde and Fetz (2007). This could be attributed to how *G.petersii* weight different object properties (von der Emde and Fetz, 2007), or perhaps differences in the two experimental setups, as well as their water conductivities (Knudsen, 1974, 1975; Bell and Russell, 1978; von der Emde, 1993; Rasnow, 1996; MacIver *et al.,* 2001; von der Emde and Fetz, 2007). Again, it is difficult to definitively answer this since our study was not designed to interrogate these factors. However, they would make for interesting avenues for future research.

5.2. Shape discrimination performance during testing

5.2.1. Key findings

We found that barrier distance had a significant effect on an individual's ability to discriminate between the two objects (Figs. 5, 6, and 7; Table i). Individuals found it more difficult to complete the discrimination task as the barrier distance narrowed and space around the object became more limited- regardless of the objects they were trained and tested with (Figs. 5, 6, and 7; Table i). This supports our hypothesis that *G.petersii* can and does use movement to facilitate the extraction of shape information from the environment.

5.2.2. Discrimination performance worse at short barrier distances

We found that the effect of distance was not steady; the biggest drops in discrimination performance tended to occur between the shortest barrier distances (Figs. 5, 6, and 7; Table i). As mentioned earlier, this drop likely represents a discrimination threshold being reached at these distances, where the amount of space available was not enough to reliably scan the objects, and so mistakes became more frequent, potentially because individuals were relying on other cues to complete the task. These cues might have been more reliable than MIMs in this situation, but less suited to picking up the fine details required for object discrimination. A more gradual drop-off in performance may have been observed if we had used finer increments when narrowing the distance between barriers. This was not possible with the current setup but worth bearing in mind if the experiment was repeated. The idea that the barriers shaped the movement of the fish is supported by our observations at the shorter distances where we found individuals tended to change their movements by the door and the way in which they interacted and opened the doors. These movement changes were also seen in fish trained without the doors, suggesting the changes were not just due to the doors themselves (see appendix).

5.2.3. Fish performed better at set object distance than in other studies

Generally, performance levels were higher than what we would expect for objects placed 1.6 cm (no doors) and ca. 2.0-2.5 cm away (with doors). This is especially noticeable when the distance between barriers was wide (Fig. 6). Schumacher *et al.* (2016b) found that one individual could discriminate between a sphere and an ellipsoid (a similar object pairing to what was used in this study) with an accuracy of 80-90% correct (nearer to 90%) at an object distance of 2 cm, and between 70-80% (nearer to 80%) at an object distance of 3 cm. Another performed similarly at 2 cm (just under 90% correct) but performed worse at 3 cm when performance dropped to just over 60% (Schumacher *et al.,* 2016b). The difference in success rate observed between the two individuals at an object distance of 3 cm might relate to how each fish tackled the task, or the order in which they were trained with the various objects. Another object recognition study found that electrically trained fish using just their electric sense scored ca. 70-75% correct at 2 cm. Modelled performances at 2.5 cm dropped to ca. 60-65% correct (Schumacher *et al.,* 2016a). Both studies exhibited some variation in performance scores, but nevertheless, they showed performance declining with object distance, which makes sense given the dominance of the active sense at short range, and the fact its efficacy declines strongly with distance (Bastian, 1986 as cited in Caputi and Budelli, 2006; von der Emde *et al.,* 2010; Schumacher *et al.,* 2016a, 2016b).

Our fish might have performed better at these distances because they often exhibited chin probing near to the doors/openings (see appendix). As such, it is likely that they were able to close the distance on the objects, allowing the active electric sense to work more effectively- it works best when the fish are as close to the object as possible (reviewed in von der Emde, 2006). In addition, our fish had all their sensory modalities available to them throughout, so perhaps the higher performances were related to them accessing information from multiple senses (e.g. active electric sense and vision). Schumacher *et al.* (2016a) found that fish performed better when electrical and visual information were available, scoring ca. 90-95% correct. These scores

are generally in line with what we obtained in training and testing at D1- though some individuals in our study surpassed these scores and one performed much worse (Fig. 6). D1 is the most comparable condition to those used by Schumacher *et al.* (2016a, 2016b) given that the barriers were at their widest extent at this distance, meaning motor volume was restricted only minimally. In the studies conducted by Schumacher *et al.* (2016a, 2016b), no restrictions were made on the scanning space around the object, as the focus was on manipulating the features of the objects themselves. We chose to manipulate the sensory and motor spaces, rather than manipulating the fish, to minimise the unintended effects of blocking one or more sensory systems out (Schumacher *et al.,* 2016a). Despite our fish having multiple senses available to them, we were confident that they were predominately using their active electric sense when completing the task, given their inherent preference for this sense over short distances, presumably because it is best equipped for object recognition at this range (von der Emde *et al.,* 2010; Schumacher *et al.,* 2016a, 2016b, 2017a). We will discuss the use of active electrolocation and other senses in this task in more detail later.

5.2.4. Individual variation in test probes

There was variation amongst individuals in how well they performed in the test probe trials. We were not able to test for this, but we observed it from our results (Fig. 6). This might have been driven by a variety of factors including fish size, motivation, and door setup (Knudsen, 1975; von der Emde, 1992; Caputi and Budelli, 1995; Caputi *et al.,* 1998; von der Emde and Fetz, 2007). However, the most likely explanation is that the variation arose from each fish approaching the task in their own preferred way. Even with this variation, the overall results were robust (Fig. 6).

5.2.5. Object discrimination using the active electric sense and other putuative cues

It is highly likely that in situations of uncertainty (as described above), individuals relied on other sensory cues when completing the task. We know that animals will use different sensory modalities to obtain similar information from the environment, so that if one sense was to fail, another one can take its place, with minimal loss of information in the process. This is often referred to as 'redundancy' and is a key reason for why animals have multiple senses; helping them to cope with an ever-changing environment (Rojas and Moller, 2002; Wilgers and Hebets, 2011; Schumacher *et al.,* 2017a). For example, wolf spiders are known to use visual and seismic cues during mating. However, males will adjust which cues they use according to which signals will transmit the best in their current environment (Wilgers and Hebets, 2011).

We know *G.petersii* possess multiple senses that can also be used flexibly. These fish exhibit cross-modal object recognition, where information about an object obtained with one sense can be accessed and recognised by another (Schumacher *et al.,* 2016a). In the case of *G.petersii,* we know that they can use the active electric sense and vision in this manner (Schumacher *et al.,* 2016a). How much they use each sense is dependent on its reliability which varies with distance. Active electrolocation provides individuals with fine spatial resolution information about their environment which is useful for object detection and recognition (von der Emde, 2006; von der Emde *et al.,* 2010). However, this sense attenuates strongly with distance, meaning that it works most reliably as a short-range sense- on the centimetre range (Snyder *et al.,* 2007; von der Emde *et al.,* 2010; Pedraja *et al.,* 2014; Schumacher *et al.,* 2016a, 2017a, 2017b). On the other hand, vision works best at distance, since the eyes of *G.petersii* have poor spatial resolution, and are instead specialised for detecting large, moving objects in dimly lit and turbid environments (Landsberger *et al.,* 2008; Kreysing *et al.,* 2012; Pusch *et al.,* 2013a, 2013b; Francke *et al.,* 2014). As such, we find that active electrolocation dominates during object recognition at short range (when it provides the most reliable sensory input) but is replaced by vision at longer range (when

visual cues are more reliable; Schumacher *et al.,* 2016a, 2016b, 2017a, 2017b). The dominance of the active electric sense and the use of vision when the electric sense is compromised is observed in other weakly electric fish species, like *A.albifrons and Eigenmannia virescens* (Dangelmayer *et al.,* 2016; Sutton *et al.,* 2016).

This dynamic weighting of sensory inputs based on reliability allows *G.petersii* to cope with uncertainty in the environment, like the kind created in this experiment, where shape information extracted through movement potentially becomes more difficult to obtain with narrowing barrier distance (Dangelmayer *et al.,* 2016; Schumacher *et al.,* 2016a; Sutton *et al.,* 2016). In these situations, individuals may switch to using other sensory modalities that provide shape information, but are less compromised than MIMs, even if they are less optimised for the task- e.g. vision. This may contribute to individuals performing worse than previously observed at these shorter distances (Figs. 5, 6, and 7). Performance differences amongst individuals might therefore relate to the way in which they order and prioritise information from other sensory modalities (von der Emde and Bleckmann, 1998).

Given that we did not block out any senses, how can we be sure that our fish are primarily using their active electric sense for shape discrimination and not another sense? In principle, an individual could get shape information about the two objects using another sense which provides this information, such as vision (e.g. Schumacher *et al.,* 2016a). However, as mentioned previously, *G.petersii's* visual system is not specialised for object recognition. The grouped retina adaptation enables sensitivity in low light conditions, but spatial resolution is limited (Landsberger *et al.,* 2008; Kreysing *et al.,* 2012; Pusch *et al.,* 2013a, 2013b; Francke *et al.,* 2014). As such, their eyes are optimised for spotting large, fast-moving objects (e.g. predators) in poor optical environments, as found in their native environment, and not for picking up the finer details of an object- especially static objects- such as those used in this experiment (Landsberger *et al.,* 2008; Kreysing *et al.,* 2012; Pusch *et al.,* 2013a, 2013b; Francke *et al.,* 2014; Olaosebikan *et*

al., 2020). In addition, previous work has shown that even if multiple senses are available, *G.petersii* will favour their electric sense during object recognition at short distances (Schumacher *et al.,* 2017a). The object distances we used in our experiment ca. 2.0-2.5 cm fits within this range, so we can be confident that the dominance of the active electric sense over other sensory modalities (termed 'electrosensory capture') occurred in our experiment as well (Schumacher *et al.,* 2017a).

Nevertheless, we cannot exclude a contribution from vision as we did not block out this sense. It is likely that vision was employed to complement or replace other senses (Moller, 2002; Rojas and Moller, 2002; Dangelmayer *et al.,* 2016; Schumacher *et al.,* 2016a, 2017a; Sutton *et al.,* 2016). For example, visual landmarks (e.g. the doors, openings, and barriers) could have been used by an individual for initially orientating and moving towards A and B from the waiting area, since these were more than one fish length away, a distance considered to be outside the working range of the active electric sense during pure object detection (Moller, 1995; von der Emde *et al.,* 2008; von der Emde *et al.,* 2010; Francke *et al.,* 2014; Pedraja *et al.,* 2020) but within detection range of the visual system (Landsberger *et al.,* 2008). Individuals might have used visual cues in situations where electric information was potentially compromised as well. For example, during decision making at the narrower barrier distances. In these trials, fish were restricted in their movements by the objects, and so may have resorted to using non-movement cues instead to extract shape information. Vision would have been a suitable sensory modality to use here as it is possible that shape information could have been extracted through template matching- though this looks to be harder for them (Schuster and Amtsfeld, 2002). Additionally, the visual system can access and recognise shape information previously collected and learned through the active electric sense via cross-modal object recognition, and this might occur innately, but more likely through learned experience (Schumacher *et al.,* 2016a). The latter might potentially work by an individual learning to associate the visual and electric images generated for defining features of an object's shape. They could then generalise these associations to assess the value of novel

objects and/or objects in novel scenarios- such as when the barrier distance was narrowed as in our study (Schumacher *et al.,* 2016a). Regardless of the mechanism, this method of recognition enables individuals to respond appropriately and swiftly to environmental stimuli even if cues change in their reliability and subsequent usefulness (Schumacher *et al.,* 2016a; Solvi *et al.,* 2020).

Fish often poked their Schnauzenorgans through into A and B. They would then sometimes use their Schnauzenorgan to get close to and/or briefly touch the objects (see appendix). This behaviour has been recorded in other object recognition studies (von der Emde and Schwarz, 2002; von der Emde and Fetz, 2007; Fechler *et al.,* 2012). Cain (1992) also found evidence that *G.petersii* can localise by touch using their Schnauzenorgan. In the case of our study, tactile cues could potentially be useful for informing an individual on object features, such as curves and corners. These features can define and dominate an object's shape (von der Emde and Fetz, 2007). Recent work suggests that the Schnauzenorgan does contain structures akin to human touch receptors, so it is feasible that touch could be used in part to detect and potentially aid discrimination between objects (Amey-Özel *et al.,* 2019).

It is unlikely that touch was the primary sense used for shape recognition here, however. For touch to be the main driver of shape recognition, one would have expected an individual to touch all round the object, so that they could get a better idea of its perimeter, and, by proxy, its general shape. However, the touches were not necessarily directed at these features. Touches tended to be confined to a particular place on the object. The non-reliance of tactile cues for object recognition is supported by von der Emde and Fetz (2007). They found that individuals would occasionally touch the objects during trials. To ensure that they were not using tactile cues during object discrimination, control experiments were run where objects were covered in electrically transparent agar, meaning that the fish could still detect the objects electrically, but not by touch (or vision). von der Emde and Fetz (2007) found that individuals continued to

discriminate between the objects well, indicating that their success was not reliant on touch, but active electrolocation.

Instead, it is likely that touch plays more of a supporting role, complementing and/or adding to the information obtained through electrosensing, especially since there seems to be a coupling between the two sensory systems (Amey-Özel *et al.,* 2019). Touch might be used as a kind of 'check', allowing an individual to determine whether the inputs received from both sensory systems are consistent with one another. This might help an individual to decide with greater certainty on what the most appropriate response would be in a given context. In some situations, touch may act as a replacement sense when other sensory modalities are compromised (Walton and Moller, 2010). In the case of object detection and recognition, touch is likely to be less efficient at capturing the relevant information compared to the active electric sense which is well suited to detecting fine spatial details (von der Emde *et al.,* 2010; Walton and Moller, 2010).

Certainly, the role of the Schnauzenorgan in electrosensing is better understood than its role in touch (Amey-Özel *et al.,* 2015). The Schnauzenorgan is an 'electric fovea'- a region highly specialised for electrosensing (von der Emde and Schwarz, 2002; Bacelo *et al.,* 2008; Hollmann *et al.,* 2008; von der Emde *et al.,* 2008; Amey-Özel *et al.,* 2012). The sensory surface of the Schnauzenorgan is over-represented in the brain*,* not only because its surface is densely packed with electroreceptors (particularly mormyromasts) but because a lot of neuronal resources are dedicated to processing their sensory inputs (von der Emde and Schwarz, 2002; Bacelo *et al.,* 2008; Hollmann *et al.,* 2008; von der Emde *et al.,* 2008). The Schnauzenorgan also shows structural and morphological differences that seem to be adaptations for electrosensing (Hollmann *et al.,* 2008; von der Emde *et al.,* 2008; Amey-Özel *et al.,* 2012). Additionally, *G.petersii* display distinct movements of the Schnauzenorgan seemingly to focus a stimulus onto its receptive surface (von der Emde and Schwarz, 2002; von der Emde *et al.,* 2008). Together, these specialisations allow the Schnauzenorgan to be highly sensitive to changes in the components of

the self-generated electric field, such as capacitance and resistance, and so make it useful for prey detection (von der Emde and Schwarz, 2002; Bacelo *et al.,* 2008; Hollmann *et al.,* 2008; von der Emde *et al.,* 2008). Note that the movements do not modulate the sensory input (Pusch *et al.,* 2008; Engelmann *et al.,* 2021). Altogether, these findings support the idea that the Schnauzenorgan movements we observed likely related to its role in active electrolocation rather than touch. This strengthens our argument that the active electric sense was the dominant sensory modality used in our task despite not blocking out other sensory modalities. This preference is understandable given that the active electric sense is the more reliable and best equipped sense for the task of object recognition (von der Emde *et al.* 2010).

5.2.6. Summary

Our analysis so far suggests that individuals completed the shape discrimination task primarily with their active electric sense, and that object shape information was extracted from movement, presumably from analysing successive electric images as the fish moved alongside the object. In other words, MIMs seem to have played an important role in shape discrimination (von der Emde *et al.,* 2010; Fechler and von der Emde, 2013; Hofmann *et al.,* 2013b; Schumacher *et al.,* 2016b; Fujita and Kashimori, 2019). The following sections will look at interpreting other behaviours occurring during choice making in the hopes of understanding the changes that occur with decreasing barrier distance.

5.3. Posthoc analyses

5.3.1. Latency of response during test probes

We were interested in examining response times as an indicator of the level of certainty an individual experienced during trials under a set number of conditions. Our model and plots show that barrier distance had a significant effect on latency (Fig. 8).

5.3.1.1. Latencies suggest optimisation of behaviour

We found that the peak of responses was very much skewed to the left regardless of barrier distance tested at and the outcome of the trial (Fig. 8). This shows that 'short' responses were more common, perhaps indicative of individuals having optimised their behaviour for completing the task as a result of learning in the training phase. Our fish were trained for a prolonged period so would have had time to optimise their behaviour (Fig. 4). Once the task was learned, individuals tended to tackle it in the same manner each time (see appendix). We know that *G.petersii* will modify their behaviour when learning a task to maximise what they are able to extract from their active electric sense (Pedraja *et al.,* 2020). A by-product of this optimisation might be quicker response times, resulting from less time needing to be spent on scanning the objects and/or more efficient movements requiring less time to complete. Indeed, Pedraja *et al.* (2020) found that fish became more efficient with their use of space during their object detection task. Certainly, fish would be motivated to complete the task quickly so that they can receive the food reward earlier. Additionally, it would make sense for an individual to want to complete the task in less time given the energetic costs associated with electrosensing even taking into account adaptations to limit these costs (e.g. Reardon *et al.,* 2011; Stoddard and Salazar, 2011; Salazar *et al.,* 2013; Sukhum *et al.,* 2016).

5.3.1.2. Latencies lengthened with decreasing distance

Notably, we found that the 'tails' of the plots lengthened with decreasing barrier distance. This was most evident when you compare response times at D1 (the widest barrier distance) and D4 (the narrowest barrier distance; Fig. 8). At D4, we see that 'long' response times have become more frequent (although still relatively rare) and are much longer in length than they were at other distances (Fig. 8). Even the 'short' responses are longer in length (Fig. 8). This trend towards an increase in frequency of longer response times with decreasing barrier distance suggests that individuals were more uncertain during trials at shorter distances, and so needed more time examining the objects before they could make a choice. This is likely because a discrimination threshold with MIMs was reached. As discussed earlier, this would occur when the space is restricted to such a degree that an individual is unable to scan the objects as effectively with their active electric sense, meaning that extra scans are required to resolve any ambiguities that exist between the two objects. Even with these additional passes, an individual may not have sufficient information to make a well-informed choice, and so they still might end up making a mistake despite taking the extra time. In these situations, an individual might rely on other cues with perhaps limited success due to them being less suitable for the task at hand.

5.3.1.3. Latencies suggest a range of strategies employed according to context

As mentioned previously, which cues are used is likely to be specific to an individual and might relate to innate preference or experience through learning (von der Emde and Bleckmann, 1998; von der Emde and Fetz, 2007; von der Emde *et al.,* 2010; Schumacher *et al.,* 2016b). The range in response times we observe might therefore relate to different behavioural strategies being employed which are context-dependent (in this case according to barrier distance). When individuals cannot rely on their preferred strategy, they might have multiple strategies that they call upon according to their perceived reliability (Schumacher *et al.,* 2016a, 2017a, 2017b).

We found that fish demonstrated a range of behaviours particularly during trials at the narrower barrier distances. These observed behaviours were relatively consistent across individuals and tended to be associated with different latencies. At the shorter barrier distances, fish were sometimes very 'hesitant' and would visit both sides of the sensing area several times before making a choice. As one might expect, these trials tended to have longer response times. This was likely the result of individuals attempting to gather enough information in the usual way to make an informed response, but needing more time to do so, due to the limitations imposed on by the barriers, meaning that movement related cues used for shape recognition were less informative, possibly to the extent that the fish resorted to using other cues instead. At other times, fish were more 'instinctual'. They would pick the first side visited and so these trials tended to have short latencies since individuals were covering less ground. von der Emde and Fetz (2007) observed the same behaviour- they found that in 30% of trials the first object encountered was chosen without consideration of the second object. One reason an individual might do this is if the first object encountered was S+. Another reason could be, if insufficient shape information was being generated from movement, then an individual might instead rely on their developed side preference when picking an object. Since there were only two possible objects to choose from, this method of preferential picking of one side would have some success even if it was limited (50%). It could also relate to an individual sometimes putting less attention in locating S+ to potentially seek out the area of low reward/food probability (Pedraja *et al.,* 2020). See side switching section for a further discussion of these behaviours.

5.3.1.4. Trial outcome had no effect on latency and the ambiguities of studying latency in isolation

Our analysis found that latency was not affected by the outcome of the trial. In other words, fish took similar amounts of time when making correct and incorrect choices (Fig. 8). This is

somewhat surprising considering we would expect fish to take less time making a choice when they were more certain of S+ (i.e. when the barriers were wide apart) and more time when they were less certain of S+ (i.e. when the barriers were close together). As mentioned already, von der Emde and Fetz (2007) found that individuals who chose one side without consulting the other tended to make more mistakes. In this case, short latencies might have been indicative of trials that resulted in errors. One reason that we did not observe a significant difference in latency with trial outcome might relate to a sampling imbalance. As mentioned previously, we had more data from correct trials than incorrect trials, since fish performed relatively well even at the shorter barrier distances (Figs. 6 and 8). As a result, we may not have captured a true representation of all the latencies possible during incorrect trials, thus making comparative assessment difficult.

Nevertheless, since our models and plots show latency lengthening as barrier distance decreases, seemingly due to the declining reliability of the active sense increasing uncertainty, perhaps the length differences of trial outcome which ought to result from uncertainty are indeed present, but somehow masked. How might this work? Perhaps a trial where an individual behaves in an optimal fashion and makes the right choice could be similar in length to a trial where an individual is instinctive and picks the first object encountered but ultimately makes a wrong choice. Both trials would look similar if latencies were just considered, despite an individual employing different strategies in the two trials. As such, looking at the latency data alone might not be sufficient in picking up the differences occurring between the two trial types- especially if they are subtle.

Since we cannot make a true assessment of the decision-making process taken by our fish by looking at the latencies alone, we must examine this alongside other data (such as levels of side switching) to get a more informed judgement on what differences in behaviour might exist between trials conducted at different barrier distances and with different outcomes. This is

needed to allow us to understand why certain behaviours have developed and what implications this might have on an individual's response to a task changing in difficulty.

5.3.2. Side switching behaviour during test probes

In conjunction with latency, we were interested in examining side switching behaviours as a measure of certainty. We expected individuals to perform fewer side switches (and take less time) when they were more certain of S+ but exhibit more side switches (and take more time) when they were less certain.

5.3.2.1. Drive to limit side switching suggests optimisation of behaviour

From our observations, we found that our fish displayed a range of side switching behaviours during testing (Fig. 9). Generally, we found that individuals preferred to take as few switches as possible (Fig. 9). Choosing the option which requires less movement and time makes sense given the costs associated with active electrolocation (Reardon *et al.,* 2011; Salazar *et al.,* 2013; Sukhum *et al.,* 2016) and swimming (MacIver *et al.,* 2010; Ruiz-Torres *et al.,* 2013). Both behaviours can be energetically expensive despite adaptations to minimise these costs (MacIver *et al.,* 2010; Reardon *et al.,* 2011; Ruiz-Torres *et al.,* 2013; Salazar *et al.,* 2013). In weakly electric fish, there is a tight coupling between sensing and movement; each shaping the action of the other (MacIver *et al.,* 2010; Hofmann *et al.,* 2013b). However, this means that the systems do not always operate optimally if you were to consider one in isolation. MacIver *et al.* (2010) found that *A.albifrons* will swim in an energetically costly way to improve sensory performance. In this case, the need to detect prey appears greater than minimising the drag forces on the animal which makes swimming more energetically intensive (MacIver *et al.,* 2010). This means that the way individuals moved around our setup might not have been motivated to minimise energetic costs, but to

enhance electrolocation- again highlighting the importance of this sense for navigating daily challenges (Lannoo and Lannoo, 1993; MacIver *et al.,* 2001; Snyder *et al.,* 2007; MacIver *et al.,* 2010).

Individuals may also have been motivated to be efficient with their use of time and space by the prospect of receiving a food reward. Our fish were strongly motivated by food and would 'beg' for food even in trials that were not reinforced with a reward or mild punishment. As such, it is likely an individual would be motivated to make a choice as soon as they detected S+ to potentially receive a food reward sooner, and to be in the position to start the next trial earlier and potentially receive another reward from that trial. In the wild, it would make sense for *G.petersii* to favour the most parsimonious option whilst foraging, not only to maximise prey capture, but to minimise exposure to electrosensitive predators, such as catfish (Hanika and Kramer, 1999, 2000).

5.3.2.2. Side switching implemented when there was a need for extra shape information

As mentioned earlier, multiple side switches were generally observed less frequently than the other behaviours; more often occurring during correct trials and trials at the shortest barrier distances (Fig. 9). Indeed, trials that resulted in a correct outcome tended to be more diverse than their incorrect counterparts, exhibiting a greater variety of switching behaviours (Fig. 9). These observations support the idea that the fish were primarily relying on MIMs to complete the shape recognition task, and that side switching was employed to add value in trials, for example to help resolve ambiguities between the two objects in situations of greater uncertainty. In these situations, each object would require a more thorough inspection before successfully being recognised. This is because the limited space would make gathering information with the active electric sense less efficient, so more passes would likely be required to make a correct choice. von der Emde and Fetz (2007) made similar observations in their study. As such, side switching likely plays an important role in minimising the chance of the wrong object being chosen. In these

situations, it would make sense for an individual to invest more in object exploration given the higher chance of making a mistake, which risks them potentially losing out on a food reward and instead receiving a mild punishment which they were keen to avoid- a shoo back to the waiting area.

5.3.2.3. Was side switching indicative of non-MIMs cues being used instead?

If the active sense was too compromised, individuals might have resorted to using other cues less optimised for close recognition of stationary objects- such as vision (Landberger *et al.,* 2008; Kreysing *et al.,* 2012; Pusch *et al.,* 2013a, 2013b; Francke *et al.,* 2014). Fish using these other cues would likely have needed more time and visits with the objects before having enough information to make an informed choice. However, this would not have guaranteed success, given that these cues were less suited to the task and fish were less experienced in using them. This might explain why multiple switches were also observed during incorrect trials, particularly at the shorter barrier distances when uncertainty seems to have been greater (Fig. 9).

5.3.2.4. Side preferences seemed to influence switching behaviour

During experiments, we noticed that each fish tended to prefer to visit a set side first. At the start of training, this preference determined which object was picked, and it took some time for this preference to be overridden and for the fish to pay attention to the objects (Fig. 4), presumably in part because this strategy offered individuals some success (50% of the time an individual could be expected to get a reward if the same side was consistently picked). Even when individuals paid attention to the objects and made their choice according to these objects, they still showed a side preference, especially in situations of uncertainty.

5.3.2.5. Individuals might have switched cues according to the part of the task they were completing

Since active electrolocation is a short-range sense, it is plausible that the fish used another cue to navigate out of the waiting area and into the sensing area (Fig. 1). We know that *G.petersii* can detect objects possibly ca. one fish length away but can only recognise objects when they are a few centimetres away (Moller, 1995; von der Emde *et al.,* 2008; von der Emde *et al.,* 2010; Fechler and von der Emde, 2013; Francke *et al.,* 2014; Schumacher *et al.,* 2016a, 2016b, 2017a; 2017b). Given that the waiting area was 14.0 cm long and the sensing area 26.2 cm long, our fish certainly wouldn't have been able to recognise the objects from that distance and use them as electrical landmarks (Fig. 1). Depending on the size of the fish and their position, some might have been able to detect the objects, but for most this would have been outside their perceptual range. It therefore seems likely that fish were using another cue to reach the objects. Each fish was very familiar with the basic configuration of their tank. As such, an individual might have learned over time to navigate this environment using internal (egocentric) cues. Egocentric navigation relies on an individual memorising the location of a goal according to their own position, and then using current positional information alongside learned movement patterns to reach that goal (Benhamou *et al.,* 1990; Walton and Moller, 2010; Schumacher *et al.,* 2017b).

As such, it is possible they might have used these egocentric cues to reach the doors/openings leading to A and B whilst they were out of range of the active electric sense. Indeed, past research has shown that not only does *G.petersii* use egocentric cues to navigate familiar surroundings, but these are preferred over both electric and visual landmarks (Schumacher *et al.,* 2017b). In the wild, *G.petersii* live in highly complex and variable freshwater environments, which means that egocentric navigation would be of benefit to this species since this strategy is not reliant on external cues (Moller *et al.,* 1979; Schumacher *et al.,* 2017b; Olaosebikan, *et al.,* 2020). It is likely that when the objects were in detectable range of the active electric sense, individuals then

switched back to using external (allocentric) cues to discriminate between the objects as these would have been more reliable for identifying S+ (von der Emde *et al.,* 2010; Schumacher *et al.,* 2016a, 2016b, 2017a, 2017b). If the first object encountered by an individual resembled S+, then it is likely they would have picked this object without considering the alternative. This might help explain why no side switching was such a prolific behaviour observed across conditions (Fig. 9) and fits with our idea that the degree of side switching might be an indicator of the level of certainty an individual experienced during a trial.

Being able to switch between using egocentric and allocentric cues means that *G.petersii* can choose the most appropriate strategy for a particular goal. Reaching the sensing area is likely to require a different set of cues to examining the objects in A and B, despite both being part of an overarching goal of selecting the correct object. In the wild, this flexibility would help *G.petersii* to successfully exploit their natural environments where information content is highly variable (Schumacher *et al.,* 2017b).

5.3.2.6. Importance of contextualising switching behaviour

Different strategies could potentially result in the same switching behaviour. This is why it was important for us to collect other data alongside the switching data (e.g. latencies). These help to contextualise the switching behaviour, but even so, they cannot always resolve the ambiguities. Short trials might be short in length because the fish was certain of the location of the positive object so made a decision quickly, or because the fish was uncertain so resorted to acting more instinctively with little consideration of the objects. Further cross-referencing against other contextual data (such as trial condition) will help resolve more of these differences. We did not have the time to examine these links in as much detail as we would have liked to, but this is something we could do in the future now that the data have been collected. Together, this would

give us a better picture of the reasons behind why certain switching behaviours are employed when they are.

5.3.2.7. Diversity in switching behaviours during correct trials suggests flexibility is key to success

We found that correct trials tended to show more variety of switching behaviours regardless of barrier distance (Fig. 9). This might indicate that individuals were more successful when they showed greater flexibility in their response. We know that *G.petersii* show flexibility in the senses they can use to detect changes in the environment and the strategies that they will employ to navigate those environments (Moller, 2002; Schumacher *et al.,* 2016a, 2017a, 2017b). By being flexible, individuals could be more adaptable to changes in the environment- such as the reliability of a particular cue. In our study, individuals that were able to adjust their behaviours to compensate for the difficulty in deriving shape cues from movement (at the short distances) likely performed better.

5.3.2.8. Summary

Examining side switching alongside latency data has been valuable for evaluating the changing certainty of the discrimination task with barrier distance. In general, the level of side switching increases with more uncertainty, resulting in longer response times (Figs. 8 and 9). Nevertheless, there is motivation to minimise side switching where possible for numerous reasons described above (Fig. 9). This also explains why most latencies recorded tended to be on the shorter side (Fig. 8). Nevertheless, some strategies resemble one another even when you take side switching and latency data together. Other information (e.g. trial condition) is required to contextualise the behaviour.

Another important thing to note when assessing the side switching data (and latency data) is that there is a sampling imbalance. Since fish generally performed highly, we have many more switching behaviours recorded for correct trials than incorrect ones (Fig. 9). As such, the patterns we see in the plot across incorrect trials may not be truly representative. In the future, modelling would help to quantify the effect of distance and outcome of trial on the level of side switching observed. Further study on the relationships not only between side switching and latency but other notable behaviours will also be fruitful for explaining the diverse ways individuals try to complete the shape discrimination in the face of greater uncertainty.

5.4. Other putative movement cues

It is possible that fish used some MIMs-like strategy, where instead of monitoring the modulation of a series of electrical images generated through movement, they took electrical viewpoints from the front and side of the head and used those stationary electric images to inform their choice (von der Emde, 2004; von der Emde and Fetz, 2007; Schumacher *et al.,* 2016b). As such, when barrier distance declined, individuals may have shifted their position to get similar viewpoints by which to recognise the objects. This type of behaviour has been noticed in invertebrates and birds (Collett, 1992; Stamp Dawkins and Woodington, 2000). In our experiment, fish did tend to approach A and B in particular ways but often they would move very quickly, making fine adjustments when close to the door/opening. As a result, it was unclear whether multiple viewpoints were being taken and if these provided similar electric images of the objects each time. It seems that *G.petersii* use template matching as a method of object recognition when considering visual stimuli (Schuster and Amtsfeld, 2002). However, when using their active electric sense, it seems more likely they are using a feature extraction model, where they are able to identify individual features of an object and connect these together to form some kind of representation of that object (von der Emde and Fetz, 2007; von der Emde *et al.,* 2010).

In our study, the key feature for the fish to pay attention to is shape, as this is the defining difference between our objects. No parameter from a single static electric image has so far been shown to encode shape (von der Emde, 2006). Instead, recent studies have theorised that this information could be extracted by an individual comparing and analysing the patterns of a series of electric images (Schumacher *et al.,* 2016b; Fujita and Kashimori, 2019). As such, it seems that MIMs would probably be more suitable for discriminating the two objects in our study. Nevertheless, it is possible that fish could employ both MIMs and MIMs-like strategies for completing the task, with the chosen strategy dependent on context (e.g. level of certainty).

5.5. Concluding remarks

Our study has shown that movement seems to be an important component for enabling *G.petersii* to recognise and successfully discriminate between objects that differ only in their shape. We found that performance worsened as the barrier distance was decreased. Our analysis supports the hypothesis that our fish were predominately relying on their active electric sense for object recognition at close range, and that movement-induced modulations (MIMs) of a series of electric images generated by swimming was likely the cue being used, since restricting the space between the barriers limited the amount of scanning movements that could be undertaken beside the object. It is likely that as barrier distance narrowed, fish were forced to rely on other cues relating to their innate and developed preferences. This explains in part the variation in performances and behaviours we observed. We found that examining response times and side switching behaviour provided us with evidence that individuals became more uncertain when completing the task as the distance between barriers narrowed. At the shortest distances, fish seemed to either attempt to use their favoured strategy to complete the task or fall back onto old rules/use new cues with limited success. Looking at these parameters alongside other notable behaviours gave us some indication of the range of strategies employed, but there was some

ambiguity which might be resolved with further modelling. We cannot exclude the possibility of non-movement cues being of use in our study. Nevertheless, our results indicate that movement allows more optimal detection and recognition of objects. This study therefore contributes to the growing body of evidence that movement can underpin sensory acquisition, and that there is indeed a tight coupling between sensory and motor systems. The next chapter will investigate the use of MIMs further in a similar behavioural paradigm, determining whether MIMs is a robust cue and if there is any evidence for the use of a MIMs-like (snapshot) strategy. Regardless, this study has shown that *G.petersii* are capable of shape discrimination even in the most difficult of situations, and this is likely related to their optimal use of movement to extract relevant information from their environment, and their flexibility, meaning that they have a hierarchy of cues available to them that they can rely upon if their favoured cue was to fail. In the wild, this probably helps them to evade predators, catch prey, and find mates in a highly complex environment that will vary according to the seasons.

Data Chapter Three: How robust is MIMs as a cue for shape discrimination (part A)? What evidence is there for a MIMs-like (snapshot) strategy being utilised (part B)?

1. ABSTRACT

We have shown that body movements can play a role in shape recognition during active electrolocation in the weakly electric fish, *Gnathonemus petersii (G.petersii).* However, to date, we have not been able to ascertain how the movement facilitates the extraction of shape information. From our previous study, we suspect that this species might be able to derive shape information from the changes that occur across multiple electric images as an individual swims past an object (MIMs). The idea is that objects of differing characteristics will elicit dissimilar MIMs which can be used for discrimination. Since MIMs seem to be important, we wanted to interrogate this differently. This study aimed to investigate whether MIMs are robust and if other movement cues could be used. For example, individuals might compare electric snapshots taken at known locations with remembered views (template matching) to determine whether the object facing them is the one they associate with a food reward- a MIMs-like strategy. To do this, we trained fish to complete a shape recognition task in a 2AFC setup, as in the previous study. In addition to moving the barriers, we rotated S+ 90° so that its short end faced the fish. We expected this to make the task harder if movement was the primary cue used, as it would cause the objects to elicit similar levels of MIMs and/or make it difficult to get snapshots comparable to past views. We found that individuals generally performed worse in this study. Barrier distance was a significant influencer of trial outcome, but the effect was weaker than in the MIMs experiment. This suggests that MIMs was less useful in this current study, and so it was possible that individuals resorted to other cues, with snapshots being a possibility so long as the viewpoints were uncompromised. Latency and side switching behaviour indicate an increased

level of uncertainty which fits with our hypothesis that the current task was harder. Nevertheless, latency and side switching can interact in complicated ways which makes it difficult to identify the mechanism of shape recognition with certainty. Further analysis of this study might help to resolve these ambiguities. Even so, this study has provided additional evidence of the importance of egocentric movement in complex object recognition whilst using the active electric sense, and has shown the possibility that movement could provide information about shape in different ways.

2. INTRODUCTION

2.1. Object recognition in G.petersii

It is well established that *G.petersii* have an extraordinary capacity to recognise and discriminate three-dimensional objects with their active electric sense (von der Emde and Schwarz, 2000, 2002; von der Emde, 2004; von der Emde and Fetz, 2007; von der Emde *et al.,* 2010; Schumacher *et al.,* 2016a, 2016b, 2017a). These fish can differentiate objects according to their capacitance, resistance, distance, size, volume, and shape (von der Emde and Bleckmann, 1992b; von de Emde and Ronacher, 1994; von der Emde and Zelick, 1995; von der Emde *et al.,* 1998; von der Emde and Schwarz, 2000, 2002; Schwarz and von der Emde, 2001; von der Emde, 2004, 2006; von der Emde and Fetz, 2007; von der Emde *et al.,* 2010; Hofmann *et al.,* 2013a, 2017; Schumacher *et al.,* 2016b; Pedraja *et al.,* 2018). These properties are encoded by image parameters derived from either a single static electric image, and/or by examining a series of successive electric images as would be generated by movement alongside an object (reviewed by von der Emde, 2006; Hofmann *et al.,* 2013b). This ability to detect and recognise objects via active electrolocation probably helps *G.petersii* to compete and exploit a number of environmental niches across their range, and has contributed more generally to the success of weakly electric fishes in Africa and

South America (Moller *et al.,* 1979; von der Emde, 2004; Markam *et al.,* 2016; Olaosebikan *et al.,* 2020).

2.2. Shape recognition in G.petersii: MIMs as a putative cue

As we have stated in data chapter two, shape is a feature that *G.petersii* can extract from an object so long as they are within a few centimetres of it (von der Emde and Schwarz, 2000, 2002; von der Emde and Fetz, 2007; von der Emde *et al.,* 2010; Schumacher *et al.,* 2016a, 2016b, 2017a). Nevertheless, shape is a cue which has been difficult to attribute to one or more image parameters within a single electric image (von der Emde, 2006; Schumacher *et al.,* 2016b). This is supported by Fujita and Kashimori (2019) who found through their modelling that differently shaped objects gave rise to similarly shaped Gaussian electric images, and so this could not be used as a feature to differentiate between objects.

Instead of individual parameters within a single electric image, focus has moved onto determining whether shape is encoded more dynamically- specifically by comparing the patterns of a series of electric images generated through movement alongside an object (Caputi and Budelli, 2006; von der Emde, 2006; Fechler and von der Emde, 2013; Hofmann *et al.,* 2013a, 2013b; Schumacher *et al.,* 2016b). Schumacher *et al.* (2016b) found some empirical evidence to support movement-induced modulations having a role in shape discrimination, but they only sampled a small number of individuals and were not able to discount the use of stationary cues. Through modelling, Fujita and Kashimori (2019) have theorised the potential cues that could be extracted through movement and be used to discern shape. They found that there was an integration effect of peak amplitude and half-maximum width that seemed to vary only with shape, and so feasibly could act as a reliable cue for shape discrimination (Fujita and Kashimori, 2019). The model, however, oversimplifies several key elements that can impact on the electric field generated (see previous data chapter for more details) meaning that further investigation is required to confirm whether

peak amplitude and half-maximum width combined could be used by *G.petersii* as an unequivocal cue for discriminating between three-dimensional shapes (Fujita and Kashimori, 2019).

Our own work has strengthened the hypothesis that movement helps with the extraction of shape information from the environment. In the previous data chapter, we hypothesised that, if MIMs were involved in shape discrimination, fish would find it harder to scan the objects when space was restricted, resulting in poorer performances. We found this to be the case. Our fish were generally very good at discriminating between differently shaped objects, but their discrimination performance declined significantly with decreasing barrier distance, with a notable drop off occurring at the shortest barrier distance of 6.3 cm (see data chapter two- Figs. 5, 6, and 7; Table i). Analysis of response times and side switching also suggests a shift in behaviour with barrier distance, which might reflect their increased level of uncertainty. Together, this supports the hypothesis that movement (likely MIMs) is important for shape recognition (see data chapter two- Figs. 8 and 9).

2.3. Shape recognition in G.petersii: a MIMs-like strategy as a putative cue

As discussed in the previous chapter, there is a possibility that *G.petersii* were using a strategy akin to MIMs but not actually MIMs- referred to here as a MIMs-like strategy. Instead of assessing the change in modulation patterns of successive electrical images generated when swimming, they might have taken electric snapshots from one or two locations (e.g. in front and to the side) and then used these static electric images to get a representation of the object, which they could then compare with a stored representation to determine if there is a resemblance (von der Emde and Fetz, 2007; Schumacher *et al.,* 2016b). This is akin to template matching which we have discussed in the context of visually guided navigation in invertebrates, where individuals will reach their goal (e.g. a nest or food source) by moving in a way which maximises the fit between

their current view (found on the retina) and remembered view of their goal (Collett and Land, 1975; Wehner and Räber, 1979; Cartwright and Collett, 1982, 1983; Collett, 1992).

Similar behaviours have been observed in other animals. Stamp Dawkins and Woodington (2000) studied visual pattern recognition in hens, finding that when they approach an object, the hens would move their head and body in set ways, unique to the individual. This not only allows the object to be fixated on from a similar distance, but to also be viewed with the same part of the eye during each investigation (Stamp Dawkins and Woodington, 2000). However, when these travel routes were disrupted by the addition of barriers, the hens found it hard to discriminate between the objects (Stamp Dawkins and Woodington, 2000). Unlike other animals studied in this context (e.g. bees) Stamp Dawkins and Woodington (2000) found that even though the objects were examined with the same part of the eye in each trial, the eyes did not trace the same route each time, meaning that the hens did not fixate the objects from set viewpoints (Collett, 1992). This might have been a consequence of the type of object used in the study, which could be recognised by basic features that did not vary with viewing angle, meaning that comparing snapshots at set positions with remembered views was unnecessary (Collett, 1992; Stamp Dawkins and Woodington, 2000). It makes sense for an individual to only use a viewpoint-dependent mechanism when necessary (i.e. for more complicated recognition that requires multiple views of an object) given that this would take more time (for fixation) and require a fair amount of memory to store reference snapshots (Srinivasan, 1994; Stamp Dawkins and Woodington, 2000). Nevertheless, this study demonstrates that consistent and repeatable movements of the viewer are important for facilitating object recognition by providing them similar views of the object each time they are visited (Stamp Dawkins and Woodington, 2000).

G.petersii use a view-dependent mechanism to recognise visual patterns (Schuster and Amtsfeld, 2002). Schuster and Amtsfeld (2002) demonstrated that *G.petersii* will memorise the view of a rewarded visual pattern learned through training, and in testing, they will choose a pattern if the

view it forms on the retina closely resembles the remembered view. However, template matching is not the only mechanism that *G.petersii* could potentially be using for visual pattern recognition. Template matching means that only a limited number of views of key locations need be remembered (Stamp Dawkins and Woodington, 2000). Nevertheless, animals often have to recognise an array of features in their environment which would require a lot of memory if everything needed to be stored as individual snapshots (Srinivasan, 1994). A more efficient method of pattern recognition might be one where an individual is able to learn and remember a key feature from a pattern of interest, and use this to make subsequent choices (Srinivasan, 1994). This was observed in honeybees, where they were found to abstract a feature from a rewarding pattern (its orientation) and use this to inform on whether a newly encountered pattern should be selected or not- with selection happening if the new pattern shared this feature (Srinivasan, 1994; Giger and Srinivasan, 1995). Since this process is not necessarily reliant on capturing the object of interest at a particular viewpoint, it can be considered as a method of recognition that can be viewpoint invariant and has been termed 'feature extraction' (Schuster and Amtsfeld, 2002; von der Emde and Fetz, 2007).

Schuster and Amtsfeld (2002) investigated whether *G.petersii* could use learned features in a pattern during visual recognition. They attempted to train fish to recognise patterns according to their shape but were unable to. The lack of success does not discount the possibility that individuals could use a viewpoint invariant mechanism for visual pattern recognition, but it does suggest that this might be more difficult, perhaps occurring under a select number of conditions (Schuster and Amtsfeld, 2002). However, it seems that during active electrolocation, individuals do rely on feature extraction, where an individual recognises an object according to a select number of features learned on the stimulus during training (Srinivasan, 1994; Giger and Srinivasan, 1995; Palmeri and Gauthier, 2004; von der Emde and Fetz, 2007; von der Emde *et al.,* 2010; Schumacher *et al.,* 2017a). It seems feasible that individuals were using this same mechanism in our experiments, given the dominance of the active electric sense during object

recognition at close range and the fact that the key difference between the two objects was in their shape (von der Emde and Fetz, 2007; von der Emde *et al.,* 2010; Schumacher *et al.,* 2016a, 2017a).

Nevertheless, as we have shown, just because an animal exhibits one mechanism of object recognition, this does not mean they are excluded from using another- indeed there may be multiple ways for an individual to complete the same task (Jolicoeur, 1985). There is now a move away from thinking about object recognition as being either viewpoint variant (e.g. Tarr *et al.,* 1998) or viewpoint invariant (e.g. Eley, 1982). Instead, these mechanisms lie on opposing ends of the same spectrum, with the chosen mechanism being determined by a number of factors, such as the objects being examined and the type of task employed (e.g. Edelman, 1995; Hayward and Tarr, 1997; Biederman and Bar, 1999; van Lier and Wagemans, 1999; Hayward and Williams, 2000; Vanrie *et al.,* 2002). Is it therefore possible that *G.petersii* could detect shape not only by comparing the modulations of successive electric images as they swam past the object (where the angles by which the object is engaged with does not matter, so long as they are variable) but from comparing electric images at set viewpoints as well?

2.4. Rotational invariance

G.petersii can recognise and discriminate objects with their active electric sense even when they have been rotated (von der Emde *et al.,* 2010). This ability to recognise objects from different viewpoints, even novel viewpoints, is referred to as 'rotational invariance' but has often been considered in the context of vision (Biederman and Gerhardstein, 1993 cited in von der Emde, 2004). von der Emde *et al.* (2010) found that individuals completed the discrimination task relatively fast, and their response times did not seem to vary according to the degree of object rotation. Similar results were found by Guyonneau *et al.* (2006) when they gave humans a visual object detection task to complete. In both cases, the speed of response suggests that 'mental

rotation' was not used to resolve either task, with mental rotation being a process by which the brain attempts to match what is being viewed with stored, internal representations of that object, which can take considerable time to compute (Shepard and Metzler, 1971; Guyonneau *et al.,* 2006; von der Emde *et al.,* 2010). Instead, it is more likely that objects were recognised by a series of features that characterised them- some of which may have been so easy to detect and/or distinguish that they could be noticed regardless of the view taken (Guyonneau *et al.,* 2006; von der Emde *et al.,* 2010). This makes sense, especially since von der Emde *et al.* (2010) found that fish only struggled to discriminate between rotated objects if they were very similar in appearance, but only the rotation of S- seemed to have a detrimental effect on performance. This indicates that individuals made their choice by evaluating features on both S+ and S-, but those features associated with S- seemed to have been lost when this object was rotated and might even have been weighted more strongly (von der Emde *et al.,* 2010). von der Emde and Fetz (2007) observed a similar evaluation of both objects and asymmetric weighting of features. It is possible that features relating to S- were given greater weight due to their association with situations that individuals were keen to avoid (e.g. receiving a mild punishment) or related to innate aversions that were there for adaptive purposes (von der Emde and Fetz, 2007; von der Emde *et al.,* 2010). Both studies provide support for feature extraction being an important mechanism of object recognition during active electrolocation- with rotational invariance thought to be facilitated by the use of easily recognisable features (von der Emde and Fetz, 2007; von der Emde *et al.,* 2010). Nonetheless, rotational invariance might not be possible if the objects are too similar in appearance, and if the features which are used to identify the objects are more complex, meaning they appear quite different depending on the angle of perspective taken (von der Emde *et al.,* 2010). In these cases, object recognition might be determined by comparing current and past views of the object.

2.5. Aims of study and rationale

Our study aimed to further investigate the role of MIMs in shape recognition by testing how robust it is as a cue (part A of chapter). We also investigated what evidence there was for other movement-related cues being used, for example a MIMs-like strategy (part B of chapter).

If MIMs are involved in shape discrimination, we would expect individuals to find it more difficult to complete the object recognition task in this setup compared to the previous one. This is due to the combined effect of making the task more complex (by making the objects more similar in their appearance- see Fig. 1) and restricting the sensing volume with the barriers. As a result, we would expect individuals to make more mistakes during testing, with performance likely to break down at a wider distance, though the extent of this breakdown might be related to the object pairing. Fish trained with the sausage-shaped object and sphere are likely to have greater difficulty with this task than those trained with the other pairing since the objects resemble each other much more.

If fish are recognising and discriminating the differently shaped objects by comparing electric snapshots taken at set points in the sensing area, we would expect individuals to shift their head and body movements to compensate for the rotation of S+. We would also expect that moving the barriers would interfere with this, ultimately resulting in a difficulty in discriminating the objects.

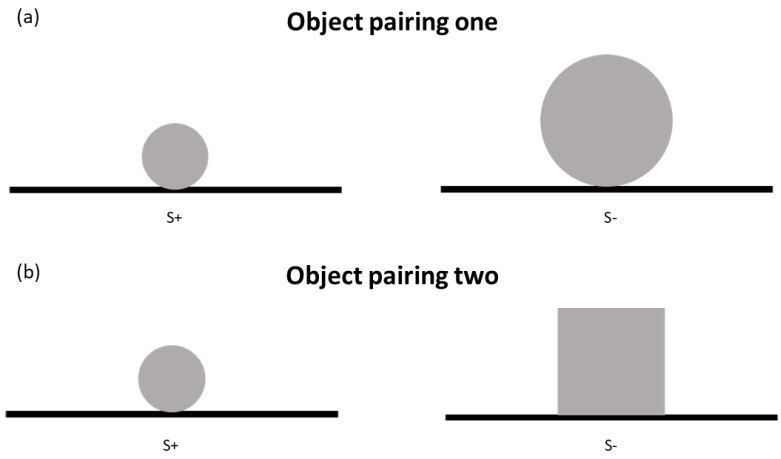


Fig. 1. Appearance of objects in the object rotation experiment. Individuals were assigned to either object pairing one (a) or two (b). In both pairings, S+ was the same object and it was rotated 90° so that its short end faced the fish while the position of S- was left unchanged. (a) N=3; (b) N=1. Note that the objects have been drawn to scale (see methods for dimensions of objects).

3. MATERIALS AND METHODS

3.1. Subjects

We trained four *G.petersii* to discriminate between two differently shaped objects in a 2AFC setup. Our fish were obtained from a local licenced aquarist (The Goldfish Bowl, Oxford, UK)- see appendix B for more details on sourcing of fish. The total length of our individuals was between 13.9-16.2 cm (including the Schnauzenorgan) and 12.4-14.2 cm (excluding the Schnauzenorgan). We were unable to age or sex individuals in our population (see appendix B). We housed our fish in separate tanks as these fish can exhibit aggression to conspecifics (Crockett, 1983, 1986 cited in Moller, 1995; Moller, 1995; Terleph, 2004; von der Emde, *et al.,* 2012).

Each tank had approximately the same dimensions, l x w x h: 60.2 x 35.2 x 31.5 cm. We carefully controlled the water parameters that the fish were kept in, as they are highly sensitive to

impurities in the water and sudden changes to their environment- like pH (Lewis *et al.,* 1992a; see appendix B). We tried where possible to replicate an environment akin to what they would experience in the wild. Water conductivity was kept at 405 ± 25 µS/cm, temperature at 25.5 ± 0.5°C, and pH 7.3 ± 0.3. Light levels were also controlled and were between 20-30 lux (appendix B). The fish were fed a diet of frozen bloodworm and brine shrimp (Gamma, Tropical Marine Centre) supplemented with vitamins (Vitazin, Waterlife)- see appendix B for more details. During experiments, feeding was confined to training trials. Fish were fed bloodworm and we made sure that they were satiated by the end of the session. The tanks that housed the fish also served as their experiment arenas. Outside of experiments, the tanks were filled with enrichment to keep the fish engaged as they are naturally inquisitive.

This study was approved by the Department of Zoology's ethics committee and conducted in accordance with the University of Oxford's animal ethics guidelines.

3.2. Experimental setup

Each tank was divided into four compartments (Fig. 2). An individual would start a trial in the waiting area (l x w: 14.0 x 35.2 cm) and would then move into the sensing area (26.2 x 35.2 cm) once the slider door was opened. Here, they would examine the objects that were placed in A and B (with each compartment being 18.0 x 17.1 cm). The amount of space available for object exploration varied according to the placement of the barriers (Fig. 2).

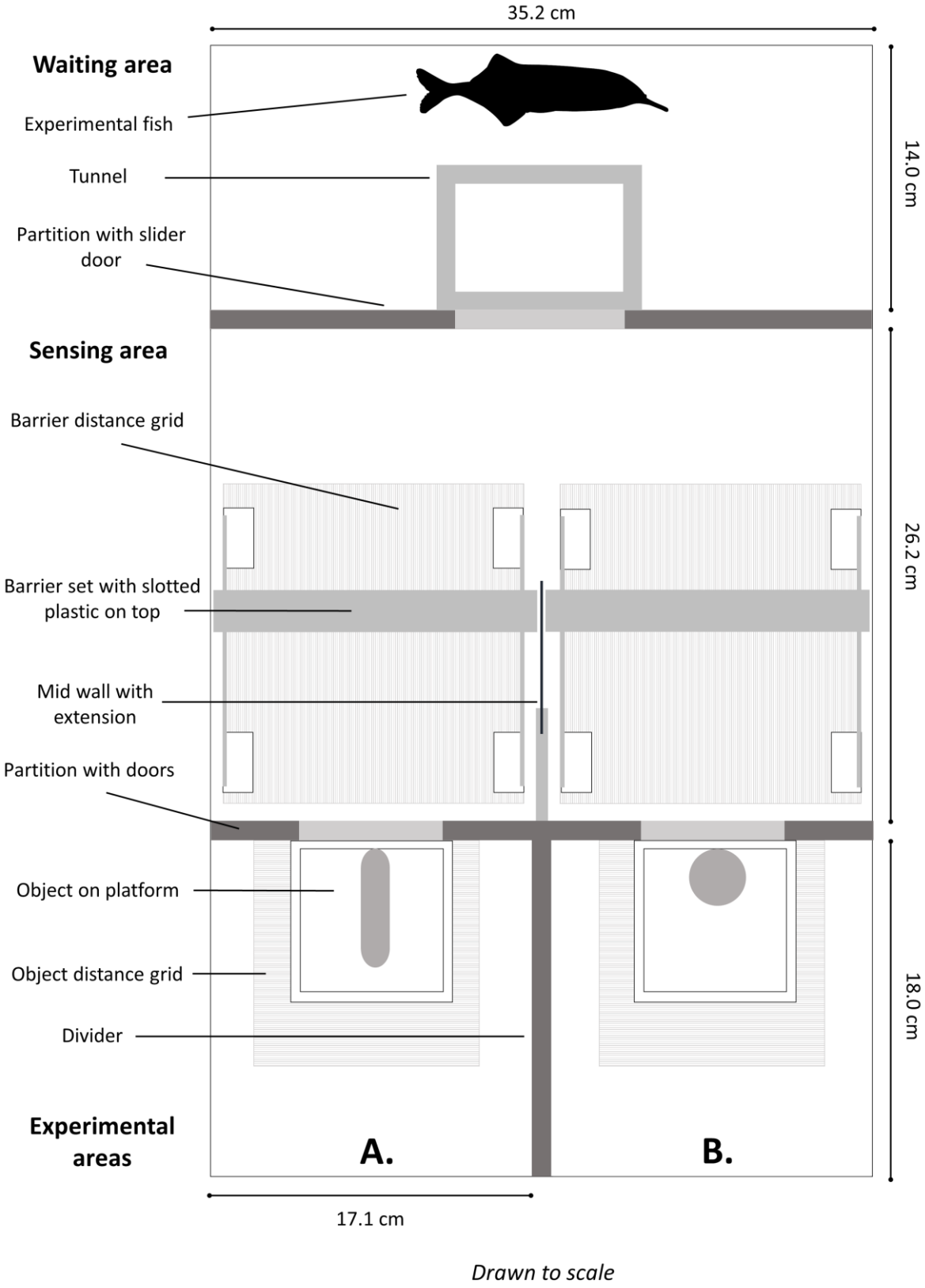


Fig. 2. Top-down view of a typical tank used in our shape discrimination study. Note that in this iteration S+ was rotated 90°.

We selected materials that interfered minimally with the electric field generated by the fish. The partitions separating off different compartments were made from a nylon mesh (mesh size: 0.71 mm per square) supported by a white, acrylic frame (thickness: 1.0 cm). Both materials are classed as electrical insulators due to their low conductance and high resistance (Rojas and Moller, 2002) and so they would have influenced the generated electric field as described by von der Emde (2006). Nonetheless, the fish were still able to use their active electric sense to locate the objects, since the mesh and gapping by the openings allowed water to flow through into the different spaces of the arena (Schumacher *et al.,* 2016b).

An additional dividing wall was placed in the sensing area (see 'mid wall with extension' on Fig. 2). This was made from a smaller piece of clear acrylic (h x w x d: 28.5 x 6.0 x 0.6 cm) and served to separate the entrances leading to A and B. This was required since fish would attempt to cut across the setup, instead of swimming and scanning in the designated spaces created by the barriers (Skeels, personal observations). The wall was extended once the barriers were placed by attaching a clear piece of Lego baseplate to it (h x w x d (excluding studs): 8.0 x 8.1 x 0.1 cm).

A slider door connected the waiting area with the sensing area, and access was controlled by pulling a handle either up or down (Fig. 2). The door was created from a thin piece of white polypropylene (h x w x d: 9.7 x 9.0 x 0.2 cm) that had its centre cut and replaced with nylon mesh (7.0 x 6.9 cm). Beside this door, a clear acrylic tunnel (28.9 x 10.9 x 7.7 cm) was placed just inside the waiting area (Fig. 2). The purpose of this tunnel was to limit the angle at which the fish left the waiting area (opening facing fish: 27.7 x 10.1 cm). This was especially important at the start of training when we were trying to override developed side preferences as the main cue for object selection.

The sensing area was dominated by four barriers that were placed in pairs- two beside the entrance to A, and another two by B (Fig. 2). A single barrier (overall dimensions, h x w x d: 18.4 x 14.4 x 0.2 cm) was made from a piece of clear acrylic with a nylon mesh insert (h x w: 14.8 x 7.9

cm)- see appendix B. We used barriers to limit the amount an individual could sample the area close to the object with their active electric sense to determine what was important about their movements for shape discrimination (Fig. 2). In other words, are fish using movement-induced modulations (MIMs) evoked by moving adjacent to the objects to extract features needed for shape recognition, or are fish taking electric snapshots close by the objects at established positions and comparing these against a reference (a memorised snapshot of each object)?

As with the previous experiment, we kept the barriers in the same position throughout training- 15.9 cm apart (D1). In testing, we randomised the position of the barriers to one of four possible positions. The barriers were placed either 15.9 (D1), 12.7 (D2), 9.5 (D3), or 6.3 cm (D4) apart (Fig. 2). We altered the positions of the barriers on a predetermined scale, so that we could test discrimination performance at distances that were not only quantifiable but repeatable. Distance grids (l x w: ~17.0 x 16.0 cm) allowed the barriers to be placed correctly. We don't believe our fish used the grid markings to make their choice, since they were identical for both grids, and due to the dominance of the active electric sense at close range, it takes effort to get individuals to use their visual sense, meaning it is unlikely that they paid attention to these markings in the first place (Landsberger *et al.,* 2008; von der Emde, *et al.,* 2010; Schumacher *et al.,* 2016a, 2017a).

We hooked a piece of clear acrylic with 11 slots (overall dimensions, l x w x d: 2.2 x 17.2 x 1.2 cm; dimensions of a single slot: 2.2 x 0.3 x 0.7 cm) over the top of each barrier pair to limit bowing, which helped us maintain a constant distance between the barriers, no matter how far apart they were (Fig. 2). We calculated the distance between two barriers from the midpoint of the slot where the first barrier was placed to the midpoint of the slot where the second barrier was placed (Fig. 3). In some setups, we used a taller piece of acrylic to ensure individuals swam in the designated space created by the barriers and not over them (see appendix A for further details).

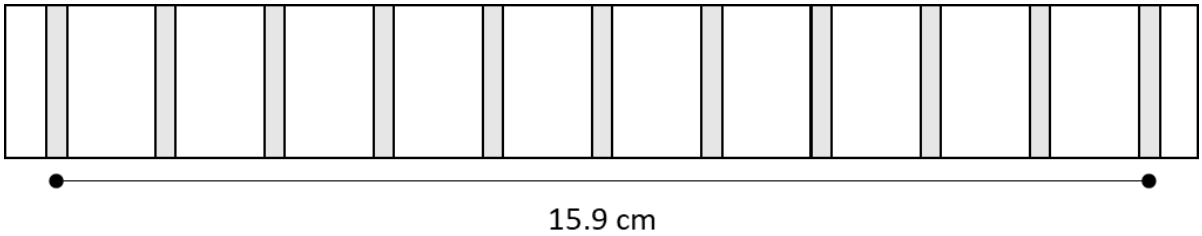


Fig. 3. Ventral view of a slotted piece of plastic which fitted over a pair of barriers and kept them at a constant distance from one another. The boxes shaded in grey represent individual slots that the barriers could fit in. The distance between barriers was measured from the midpoint of one slot to the midpoint of another (as indicated by the line).

Each object was placed on a white acrylic platform (secured with a little plasticine) that was lowered into either A or B (Fig. 2). The platforms had an overall height ca. 10.8 cm (base of platforms, l x w x d: 8.6 x 8.6 x 0.5 cm and top of platforms: 7.6 x 7.6 x 0.5 cm). The objects were positioned ca. 1.6 cm away from those trained without the doors and ca. 1.9-2.5 cm away from those trained with the doors (see appendix B). We chose these distances to ensure the objects would be within perceptual range of *G.petersii's* active electric sense. We know that *G.petersii* can recognise and discriminate objects with their active electric sense up to 4 cm away, depending on the object features being used and background conditions (von der Emde *et al.,* 2010; Fechler and von der Emde, 2013). However, it is likely that for shape, fish need to be closer (a minimum of 2.5 cm away) given it is a more complex feature to assess (Schumacher *et al.,* 2016b). In reality, our object distances are likely to be overestimates given that all fish encroached on the space via chin probing (see previous chapter for details).

To select an object, an individual had to open the door to the compartment containing that object with their Schnauzenorgan and swim into it (Fig. 2). The door was made from a combination of white polypropylene (frame Ø= 7.6 cm, total thickness of door= 0.3 cm) and nylon mesh (window Ø= 6.4 cm). The door pivoted on a screw so little force was needed to move it out of the way. The

mesh (0.71 mm per square) allowed water to flow between the various compartments which was essential for successful active electrolocation (since water is needed as a medium for signal transmission). Note that three fish were trained with the door setup (fish one, two, and three) and one without (fish five). The latter grouping allowed us to check that the doors were not inhibiting discrimination performance in a significant way.

We recorded the behaviour of fish in both training and testing trials. To do this, we placed an Apeman A70 Action Camera (30 FPS, 1920 x 1080 pixels) 31.5 cm above the water's surface which gave us a good overview of the tank. There was a slight goldfish bowl effect with the camera (image curving at the edges). To limit its effect, we positioned the camera so that the key area of interest (the sensing area) was central and so had minimal distortion.

3.3. Training regime

We initially planned to conduct this study (referred to here as the object rotation experiment) soon after the completion of the previous one (referred to here as the MIMs experiment). However, this was not possible due to unforeseen events outside our control. As such, there was a gap between experiments. This was between six and twelve months. This time gap was variable across individuals, as it depended upon when they completed their last testing session for the MIMs experiment and began training for the object rotation experiment. Since training and testing fish for both studies was time consuming, we could only work with two individuals at any one time, meaning that we only started training a new fish once we had finished testing the previous one. When we started this experiment, we based the order of training on how much an individual had retained from the previous study- i.e. we started training those that demonstrated the most advanced levels of learning first.

Individuals were trained in a 2AFC setup (Fig. 2). We started off by refreshing each fish on the associations they had learned in the previous experiment (referred to as training step one on Fig. 6; shaded in light brown). Fish were trained to select the sausage-shaped object (S+) associated with a food reward and to avoid either the sphere or cube (S-) which were both associated with a mild punishment- a tap on the glass and a gentle shoo back to waiting area (Fig. 4). Individuals were assigned to the same object pairings used in the previous experiment, and at this stage S+ kept its original orientation (Fig. 4ai).

One or two bloodworms were given to an individual when they correctly identified S+ and swam into the appropriate experimental area. Fish were hand fed with a 1 ml graduated Pasteur pipette (SciLabware Limited, but sold by Appleton Woods Ltd). The amount dispensed varied according to their level of satiation. A 30 cm plastic ruler with a metal washer attached to one end (ca. outer Ø= 2.9 cm, inner Ø= 0.9 cm, and depth= 0.5 cm) was used as a mild deterrent. If an individual mistakenly chose S-, we would use the ruler to gently tap against the glass of the tank and then direct them back to the waiting area in preparation for the next trial. Fish quickly learned of this mild deterrent and would actively avoid it, and often pre-emptively return to the waiting area when making a wrong choice. We were careful to ensure that the mild punishment was of an appropriate level- enough to induce an avoidance response but not enough to inflict stress.

The objects were positioned in either A or B (Fig. 2). We alternated which object was put in the tank first to ensure this did not influence choice. This was unlikely however as the fish started experiments well away from the objects (Fig. 2). The location of S+ was randomised albeit within predefined limits (using random.org). We wanted S+ to be in each compartment 50% of the time, so that individuals made their choice according to the learned attributes of the objects and not due to their location.

Once an individual had reached or exceeded the learning criterion of 75% in three consecutive sessions, we could be confident that they were able to distinguish S+ and S- reliably, and so we

moved them onto the second training step (shown in dark brown on Fig. 6). This was identical in structure to the first step, with the exception that S+ was rotated 90° (Fig. 4aii). An individual remained on this step until they achieved 75% or more on three consecutive sessions. They then moved onto testing once this was established, meaning we could be confident that they could recognise the newly rotated S+ as S+ and could distinguish it from S- robustly. Note that our learning criterion was based on that used by Schumacher *et al.,* (2016a and 2016b).

Fish were trained/tested once per day, with a usual session comprising of 24 trials. Towards the end of the study, when we were only training/testing fish two, we were able to increase the rate to two sessions a day (each one consisting of 24 trials, so 48 trials in total). In total, 2132 training trials were conducted in the training phase alone.

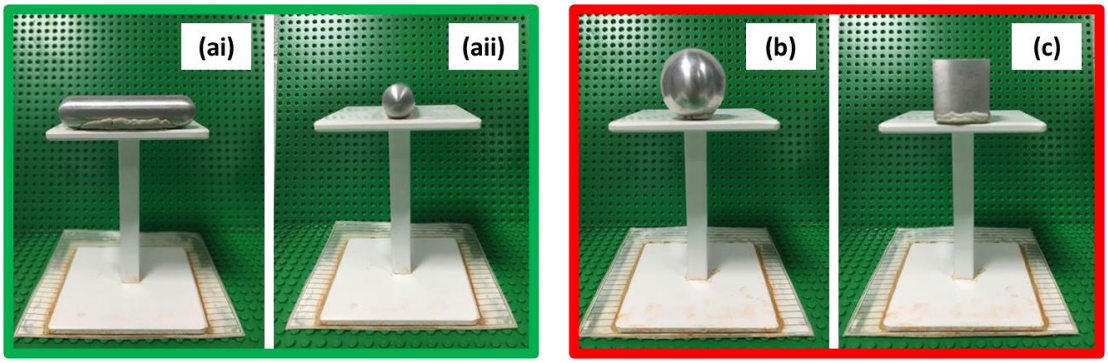


Fig. 4. Objects used in the object rotation experiment. Fish were initially trained to discriminate between two objects, S+ (bordered in green) and S- (bordered in red). All fish were offered the same S+, a sausage-shaped object (a). At the start of training, S+ was orientated so that its long side faced the fish- like in the previous experiment (ai). Once learning had been established, we rotated S+ so that its short side faced the fish (aii). Fish then had to learn to discriminate between the newly rotated S+ (aii) and S-, either a sphere (b) or cube (c).

3.4. Experimental Objects

Due to time constraints, we trained and tested four of the six fish that participated in the previous experiment. We selected fish that had retained the most information from this experiment to speed up the training process. As such, we ended up with an uneven split of individuals between the two training groups (Fig. 5). All fish were trained with the sausage-shaped object as their S+ (Figs. 4ai and 4aii). However, fish one, three, and five were trained with the sphere as their S- (Fig. 4b) and fish two was trained with the cube as their S- (Fig. 4c).

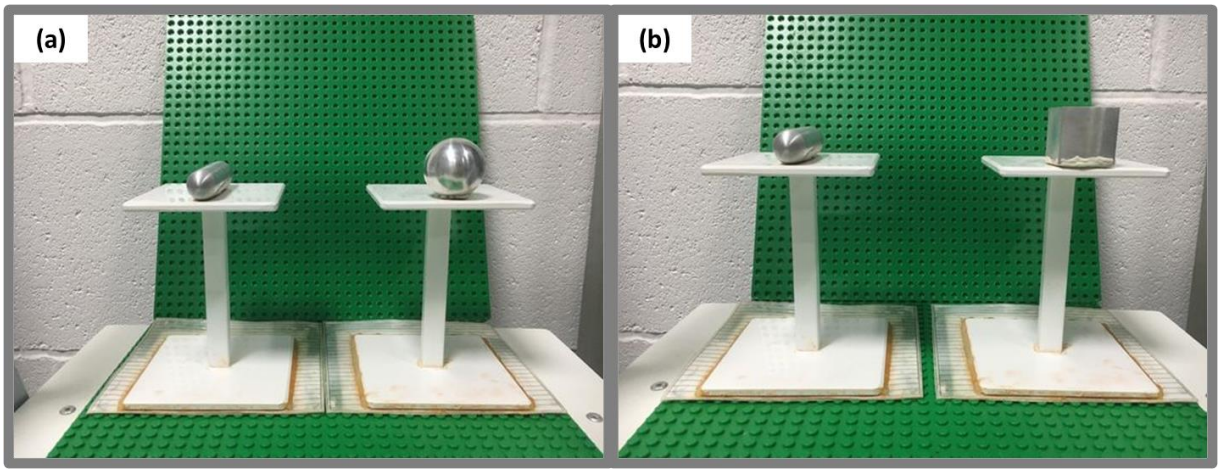


Fig. 5. Object pairings used in the object rotation experiment. Our fish were assigned to one of two object pairings. (a) Fish one, three, and five were trained to discriminate between the rotated sausage-shaped object (S+) and sphere (S-). (b) Fish two was trained to discriminate the rotated sausage-shaped object (S+) and cube (S-).

We were careful to ensure that the objects we used were consistent in all properties bar shape to avoid individuals using these other features to discriminate between the objects. All objects were made from aluminium (an electrical conductor) which meant that they stood out from the plastic apparatus (electrical insulators) and surrounding water due to their unique interaction with the self-generated electric field (Rojas and Moller, 2002; von der Emde, 2006). The dimensions of the objects were chosen to give them a similar approximate volume (Table i). The sausage-shaped

object measured, h x w x d: ca. 1.5 x 6.3 x 1.5 cm. The sphere had a diameter of 3 cm, and the cube had a side length of 2.42 cm. We calculated the sausage-shaped volume as both a cuboid ~14.18 cm^3 and capsule ~13.35 cm^3 as its shape was somewhere between the two. The volumes of the sphere and cube were ~14.14 cm^3 and ~14.17 cm^3 respectively. If we calculate the volume ratio (given here as volume of S+/volume S-) we get a value close to one for all object pairs, regardless of which volume we take for the sausage-shaped object (Table i). This means that the two objects within a pair can be considered as having very similar volumes, meaning it is unlikely this cue was used by individuals for object recognition (von der Emde and Fetz, 2007).

Table i. Volume and volume ratios of the objects fish were trained and tested with

	Object combination S+/S-	**Volume S+/S- (cm^3)**	**Volume ratio**
S+ volume- cuboid calculation	Sausage-shaped object/sphere	14.18/14.14	1.00
	Sausage-shaped object/cube	14.18/14.17	1.00
S+ volume- capsule calculation	Sausage-shaped object/sphere	13.35/14.14	0.94
	Sausage-shaped object/cube	13.35/14.17	0.94

Note that the volume ratio can also be expressed as volume of S-/volume of S+.

3.5. Testing regime

Individuals moved onto testing once we were satisfied they could discriminate between the rotated S+ and S- proficiently. This was when they reached or exceeded 75% correct choices on three consecutive sessions (shown in blue on Fig. 6; see appendix B). During testing, we mixed training with test trials (2:1). This meant that within a session fish could experience up to 16 training and eight test probe trials. Test trials differed to training trials in two key ways: the distance between the barriers was varied and the fish received no reward or mild punishment for making a choice.

We varied the space between the barriers so that we could determine whether this had an effect on an individual's ability to discriminate between the rotated S+ and S-. To do this, the position of the barriers was randomised, albeit on a pre-determined scale (Fig. 2). Barriers were placed either 15.9 (D1), 12.7 (D2), 9.5 (D3), or 6.3 cm apart (D4). In a single session, we tried to test an individual at each distance twice. For each fish, we aimed to complete approximately 30 repeats at each distance to ensure there was enough repeatability between and within individuals. We included training trials into the regime to ensure performance remained high as well as to maintain motivation and limit their frustration as much as possible (von der Emde and Fetz, 2007). During testing, we conducted 1086 training trials and 520 testing trials across the four individuals studied.

During both training and testing sessions, we made a note of the location of S+, the position of the barriers, whether the choice was correct or not, their latency, and movement patterns. This information was collected to help us assess our hypotheses. Playback of the videos allowed us to verify this data.

3.6. Hypotheses and key questions

Our key questions were:

- **How robust is MIMs as a cue for shape discrimination? (referred to as part A)**
- **What evidence is there for a MIMs-like (snapshot) strategy being utilised? (referred to as part B)**

Our hypotheses were as follows:

If an individual was using MIMs to complete the shape recognition task, we would have expected them to find the task harder than in the previous iteration (data chapter two) due to the rotation of S+. We would expect this rotation to make the two objects more similar in appearance, and so they would have likely elicited similar levels of MIMs when the individual moved along the objects. The narrowing of the barriers would have also restricted access close to the objects, thereby limiting the amount they could be scanned with the active electric sense. Together, this would presumably have made MIMs less informative for object discrimination, and so fish relying on this cue to make a choice would have been more error-prone, resulting in poorer performances. We would have expected these drops in performance to be most apparent at the shorter distances where the combined effect of distance and object rotation would have been most pronounced. In comparison to the MIMs experiment, we expected performances to generally be worse, perhaps breaking down at wider distances given the increased complexity of the task.

If fish were extracting shape information from electric snapshots instead, we might have expected to observe their head and body movements change to compensate for the object rotation. Again, we would have expected the barriers to disrupt performance by interfering with the trajectories the fish needed to take to view and recognise the objects. Performance may have been more binary, breaking down suddenly once the preferred viewpoints were unavailable.

We examined the following aspects to get a better understanding of how fish learned the task and how they responded to changes within the setup. This helped us to interrogate the hypotheses proposed above.

- **Task acquisition: how long does it take for an individual to learn the task and how well do they retain the information?**

We wanted to monitor performance during training trials in the testing phase to ensure an individual was still able to discriminate between the objects proficiently. This was important for ensuring any changes in performance observed during test probes were not due to the fish unlearning the task, but instead could be attributable to the key variables being tested (e.g. barrier distance).

Posthoc analyses

- **Latency of response during test probes: how does their response times change as the barrier distance is narrowed?**

In general, we expected fish to take less time when they were more certain of which object to select, and more time when they were less certain. If MIMs were being used for shape recognition, we expected the rotation of S+ to add a new dimension of difficulty, resulting in longer latencies- particularly at the shortest distance (where the combined effect of object rotation and narrowing space would be most evident), however not limited to these distances. We expected to see a similar trend if fish were using a MIMs-like strategy (comparing electric snapshots) since the lack of space at the narrower distances would have made compensating for the rotation of the object hard and so required fish to try other strategies instead.

- **Side switching behaviour during test probes: how do movement patterns shift with changing barrier distance?**

In general, we expected individuals to make fewer side switches when they were more certain of their choice, and more side switches when they were less certain.

If individuals were using MIMs as a cue for shape recognition, we expected them to become more uncertain of which object to pick as the space around the objects became more restrictive. This is because the lack of space would have made scanning the objects more difficult, and so it is likely that more visits would have been needed to get enough information to allow an individual to distinguish the objects properly. As such, we expected more occasions of side switching in this study than in the previous one- particularly at the shorter barrier distances, where the combination of limited space and object rotation would have made distinguishing S+ and S- extremely difficult.

If an individual was using a MIMs-like strategy (comparing electrical snapshots) we would have expected to observe a similar trend as barrier distance decreased, with the frequency of side switching increasing in two respects. Firstly, there would be more occasions of side switching, and secondly, when switching occurred, it would do so more intensely (in other words more switches per trial). Individuals would seek to optimise their swimming trajectories to match current and remembered views. However, the lack of space and rotation would force individuals to engage with the objects differently, and so they might have to make more visits to extract shape relevant information by other means.

Additionally, we were interested to determine if side switching behaviour varied between trials of different outcomes. We hypothesised that trials which resulted in a correct outcome might have few side switches reflecting a greater certainty in the task, and trials that resulted in an incorrect outcome might have more side switches reflecting less certainty in the task. Regardless, we expected individuals to vary their strategy when making their choice according to the level of certainty they were experiencing.

Note that the side switching analysis was purely qualitative due to time constraints.

3.7. Statistics

To test for the effect of distance on shape discrimination performance, we created two binomial GLMMs, as in the previous study. The models aimed to predict the chance of success at selecting S+ (our response variable) given several other variables (effects). We chose binomial models as our response variable (outcome of choice) was binary. In our first model, we had two fixed effects (barrier distance and object pair) and one random effect (fish identity). The second model was very similar to the first, but our key fixed effect (barrier distance) was removed. We then ran a likelihood (LR) test to determine which model best fitted our data. The chosen model was then examined to give us a better understanding of the most important predictor variables and their effect size (see appendix B).

We used gamma GLMMs when testing the effect of barrier distance on latency, as in the previous study. We chose this type of model due to the distribution of our data (Fig. 10). The first model contained two fixed effects (barrier distance and outcome of trial) and one random effect (fish identity). The second model omitted the key variable of interest but included everything else. A LR test compared the two models to assess which one best fitted our data. This better fitting model was investigated further to determine what was influencing response times and the strength of this influence (appendix B).

We ran the models using *R Studio, R version 3.6.2* with the *lme4* package.

4. RESULTS

4.1. Task acquisition

In general, we found that individuals quickly remembered the original task of discriminating non-rotated S+ and S- (either a sphere or cube) despite the long break between experiments (Fig. 6). The gap between experiments ranged from six to twelve months depending on the individual in

question (ca. six months for fish five, nine months for fish three, and twelve months for fish one and two). However, the relatively short time spent on training step one (five sessions for fish three, six sessions for fish five, seven sessions for fish one, and 14 sessions for fish two) indicates that individuals were able to retain previously learned information over extended periods of time without reinforcement (see light brown shaded areas on Fig. 6). Schumacher *et al.* (2017a) also found that *G.petersii* could recall information required to complete a similar discrimination task many weeks after training, but not for as long as we have recorded here.

We found that fish generally took longer to discriminate the objects when S+ was rotated. However, there was greater variability between fish in terms of how long it took them to reach the learning criterion at this training step (see dark brown areas on Fig. 6). We expected most fish to take longer to discriminate the objects since they were unfamiliar with S+ being presented in this arrangement.

During testing, we interspliced training trials with test probes to maintain motivation and discrimination performance (von der Emde and Fetz, 2007). During these training trials, fish seldom scored below 75%, so we can be confident that they had a good grasp of the task when test probes were implemented (see blue areas on Fig. 6).

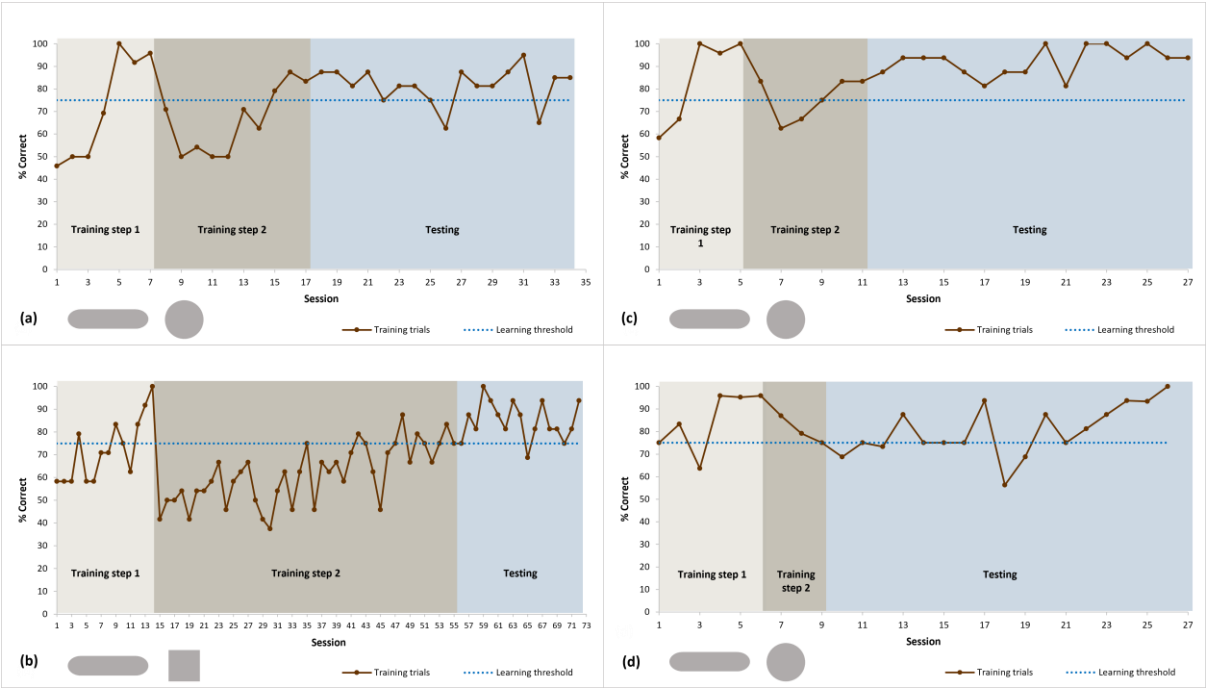


Fig. 6. Learning curves for fish one (a), fish two (b), fish three (c), and fish five (d) that participated in the object rotation experiment. Discrimination performance (calculated as % correct of training trials) was plotted for all phases of this experiment. Total number of training trials conducted across training and testing=3218 (fish one=685, fish two=1592, fish three=496, and fish five=445).

4.2. Shape discrimination performance during testing

We were interested in determining how robust MIMs was to environmental change. To investigate this, we tested whether barrier distance still had an effect on shape discrimination performance when S+ was rotated 90°. We initially created two models to predict an individual selecting the correct object. When comparing models one and two, we found that model one best fitted the data (LR test: $\chi 2=5.565$, df=1, p=0.0183). The only difference between the two models was the addition of barrier distance as a predictor of performance in model one (see appendix B). These results therefore indicate that barrier distance was a significant predictor/explanatory variable for the outcome of a test trial.

Barrier distance had a significant influence on an individual's ability to discriminate between the two objects (GLMM: Z=2.344, df=516, p=0.0191; Figs. 7, 8, and 9; Table ii), albeit seemingly to a lesser extent than that observed in the MIMs experiment (see data chapter two for corresponding plots). In general, individuals found it harder to discriminate between the two objects when the distance between the barriers was narrowed- this was most apparent for fish three (Figs. 7, 8, and 9). As with the MIMs experiment, object pairing did not have a significant influence on an individual's discriminatory performance during testing (GLMM: Z=0.136, df=516, p=0.8922).

The modelled effect of distance resulted in predicted probabilities of success which were relatively similar to what we observed during testing, and this again supports distance being a significant factor in driving performance (Figs. 8, and 9; Table ii). Our model predicted that performance would drop by a relatively steady amount with decreasing barrier distance, and the worst performance was predicted to occur at the shortest barrier distance, D4 (Table ii). Although we observed the biggest drop in performance at D4, likely relating to a discrimination threshold being met, we did not observe performance declining in a steady fashion as predicted (Table ii).

The distance model created for the MIMs experiment also predicted performance to worsen as the space between the barriers was narrowed (see data chapter two). In the MIMs model, the performance drops were predicted to get larger as you neared the shortest barrier distance, D4. This is what we observed, although our model also slightly over/underestimated some of these values. Together, the models and empirical data solidify the idea that distance had an influence on shape discrimination performance in both experiments, but the effect appears to have been greater for the MIMs experiment than the object rotation experiment. Nevertheless, success was generally lower in the object rotation experiment than in the MIMs experiment (Figs. 7, 8, and 9). The only difference between the two experiments was how S+ was presented, and so the rotation of S+ must have played a role in shaping the differing performances.

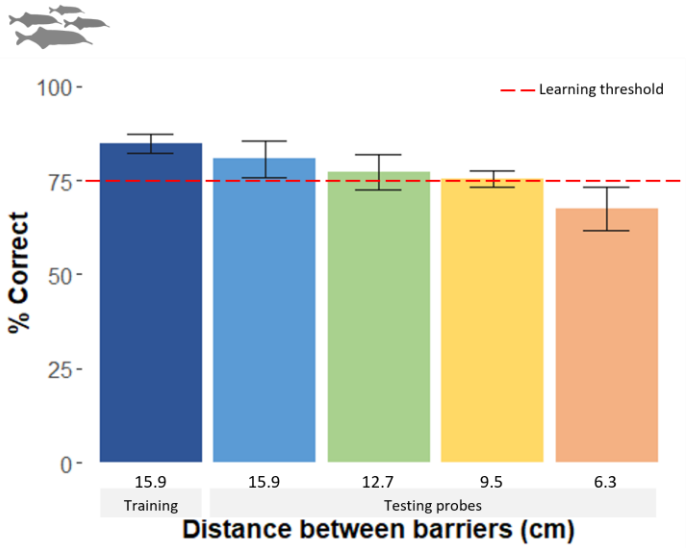


Fig. 7. Group mean performance during the testing phase of the object rotation experiment. Discrimination performance was defined as the percentage of trials in a set condition that were correct. During testing, we conducted training trials alongside test probes (2:1) to maintain motivation and shape discrimination performance. To calculate group mean performance, we pooled data from the four fish (N=4) that participated in this study (fish one, two, three, and five). The dark blue bar (far left) illustrates group mean performance during the training portion of testing and provides us with a baseline to compare other performances against. The remaining bars (right-hand side) represent group mean performance during test probes (when the barrier distance was altered, and no reward/punishment was given). Note that the black error bars denote the standard errors of the mean for each condition, and the dotted red line indicates where the 75% learning threshold is.

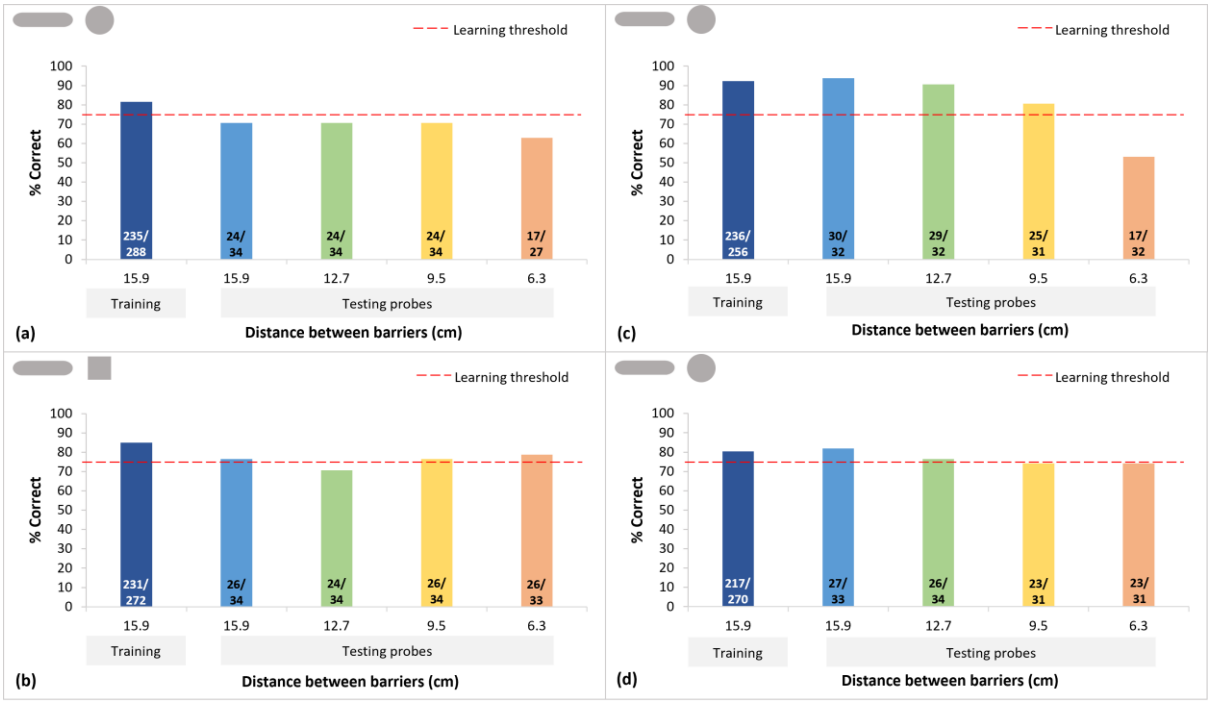


Fig. 8. Performances of fish one, two, three, and five during the testing phase of the object rotation experiment (a, b, c, and d). Both training and test probe trials were conducted in this phase (every third trial was a test). The dark blue bars show the proportion of trials that were correct during the training trials. The remaining bars show success during test probes, when barrier distance was varied, and no reward or mild punishment was given. The numbers within each bar state how many correct trials were completed under a particular condition against the total number of trials that were run for that condition. The dotted line shows the learning threshold of 75%.

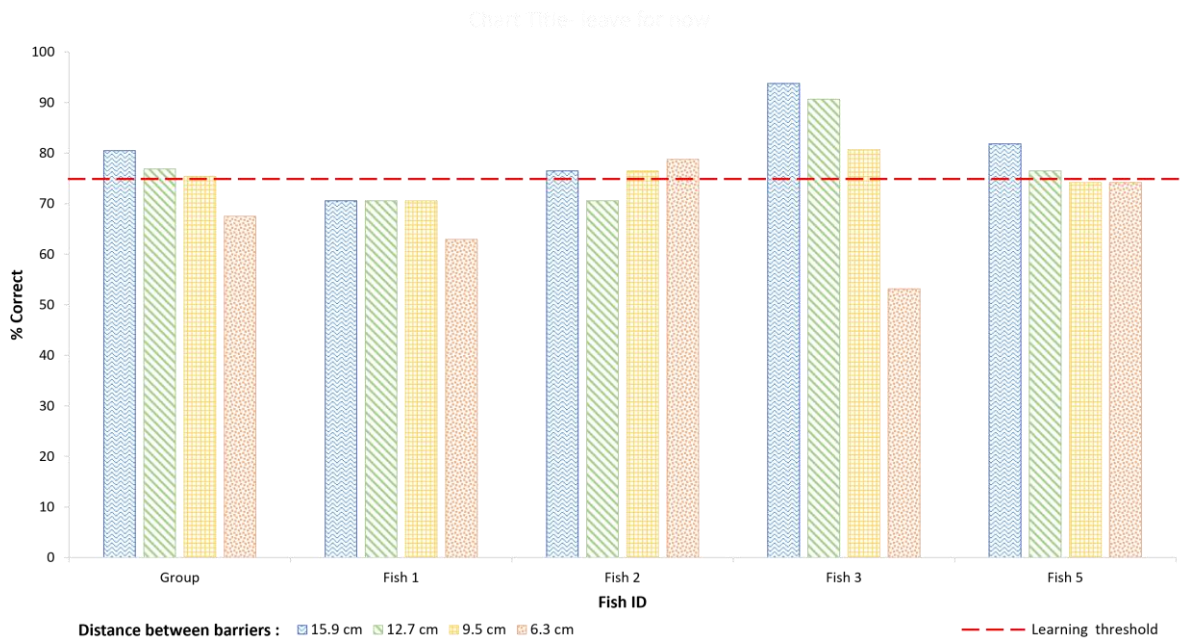


Fig. 9. Performance scores during test probe trials for the object rotation experiment, shown on both an individual and group level. Each bar represents the proportion of correct trials for a given barrier distance (colour and pattern coded). The group bars pooled data from the four fish tested. The red dotted line marks where the learning threshold is. Total N=520 trials (fish one=129, fish two=135, fish three=127, and fish five=129).

Table ii. Predicted and observed values of success at selecting S+ in a test trial given barrier distance during the object rotation study

	Predicted[1] probability of success %		**GLMM**			**Observed success[2] %**	**Predicted (observed) performance change %**
Distance	**Estimate**	**95% CI**	**Z***	**df**	**p**		
D1 (15.9 cm)	80.76	[67.51, 89.78]	2.344	516	0.0191	80.45	
D2 (12.7 cm)	77.16	[58.20, 89.44]				76.87	D1-D2= -3.60 (-3.59)
D3 (9.5 cm)	73.11	[48.27, 89.09]				75.38	D2-D3= -4.05 (-1.48)
D4 (6.3 cm)	68.63	[38.47, 88.73]				67.48	D3-D4= -4.48 (-7.90)

N=4 individuals; 520 observations (test probe trials); [1]by model one; [2]Observed success was determined when test probe data from individuals were pooled for each testing condition. Success at a given barrier distance was calculated as: (total number of trials correct/total number of trials run) x 100; *modulus of Z

4.3. Posthoc analyses

4.3.1. Latency of response during test probes

We studied response times as a way of assessing certainty in completing the shape discrimination task during test probes where the distance between the barriers was altered and S+ was rotated 90°. We hypothesised that fish would generally take less time when they were more certain of the task, and more time when they were less certain. We hypothesised that if movement related cues were being used, the task would become more difficult with the rotation of S+ and decreasing barrier distance, and as such, we expected to see response times increasing as a result. We expected this to be more pronounced than what was observed in the MIMs

experiment due to the extra layer of complexity involved here (see methods for a more detailed summary of hypotheses and rationale).

We found that latencies increased with decreasing barrier distance (Fig. 10). We created two models to predict latency. We then tested whether there was a significant difference between the two models in how well they explained our data. Model one fitted our data significantly better than model two (LR test: χ^2=187.06, df=1, p<0.001). Since the inclusion of distance was the only difference between the two models, this suggests distance was influencing response times (Fig. 10).

When we ran model one, we found that barrier distance did have a significant influence on latencies (GLMM: t=13.299 (modulus), df=507, p<0.001). Generally, we found that response times were strongly skewed to the left, meaning that most of the recorded response times were relatively short in length (Fig. 10). However, we also noticed that response times tailed off to the right, and that 'tails' lengthened as barrier distance narrowed. In other words, longer responses were rarer occurrences, but they became more frequent as barrier distance was decreased (Figs. 10di and 10dii). This is similar to what we observed in the MIMs experiment. The pattern was consistent across individuals.

Unlike with the MIMs experiment, we found that trial outcome had an influence on latencies- albeit a weaker effect than distance (GLMM: t=2.824, df=507, p=0.0048). We found that correct trials tended to be associated with frequency distributions that had longer tails than their incorrect counterparts. This was especially noticeable at the shortest distance, D4 (Fig. 10di). Although rare, there were more occasions of individuals taking a long time to select an object in trials that resulted in a correct choice than those that ended in a mistake, and this often happened when the barriers were placed at their narrowest extent- i.e. at D4 the tail for correct choice is almost twice as long as its incorrect counterpart (Fig. 10).

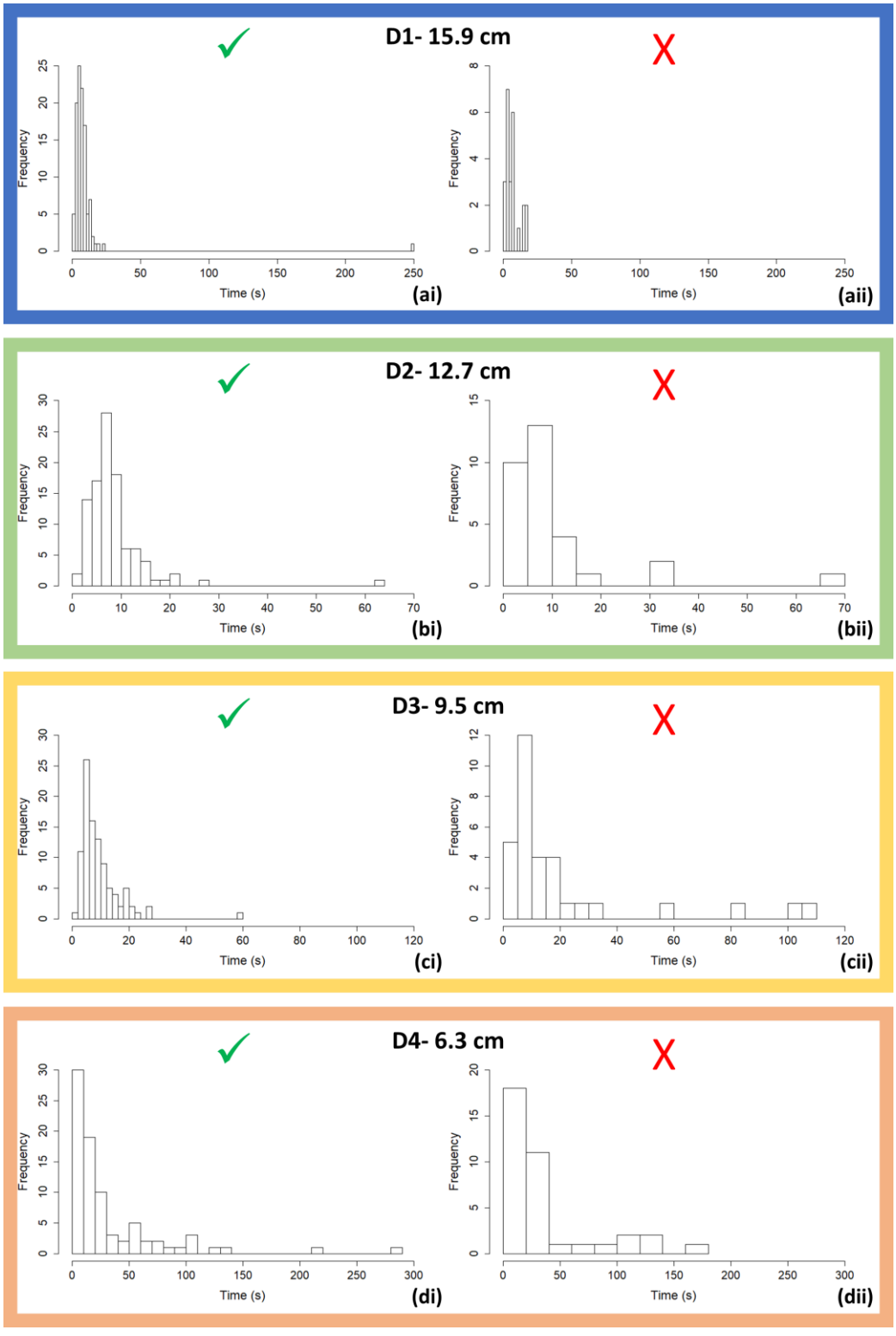


Fig. 10. Group response times for selecting an object during test probe trials in the object rotation experiment. During testing, the barriers were positioned at one of four distances (a-d). Each pair of plots shows response times for correct (indicated by a tick and i) and incorrect trials (indicated by a cross and ii) at a particular testing distance. Note that data were pooled from all individuals

tested. Responses given in seconds (s). N=512 (ai=107, aii=24, bi=101, bii=31, ci=98, cii=32, di=82, and dii=37).

4.3.2. Side switching behaviours during test probes

Multiple side switching was a more prevalent behaviour across most conditions studied than previously observed- though the change across distance was less visible (Fig. 11a).

We studied side switching as another means of assessing certainty in the task. We hypothesised that if movement related cues were being used, the amount of side switching would increase with decreasing barrier distance, and the rotation of S+ would intensify this effect. We also hypothesised that trials which resulted in the incorrect object being selected would exhibit more side switching due to an increased level of uncertainty.

As a group, we found that single side switching was the most common of the three behaviours observed, closely followed by no side switches, and finally multiple side switches (Fig. 11a). We found that no side switching fluctuated up and down with barrier distance and trial outcome, but showed an increase at the shortest barrier distance, D4 (Fig. 11a). At the same time, we found that the amount of single side switches tapered with distance, and that the proportion of trials exhibiting single side switches was either similar for correct and incorrect trials (D1 and D3), or dipped during incorrect trials (D2 and D4; Fig. 11a). Interestingly, multiple side switches remained at a relative constant proportion across distance and outcome- increasing only slightly with decreasing distance (Fig. 11a). D3I was the exception where there was marked increase in multiple side switching (Fig. 11a). Due to time constraints, we were not able to model the effect of distance or trial outcome on side switching behaviour, so we cannot quantify the effect size of these variables. As such, we can only make inferences from the plots themselves (Fig. 11).

We did observe a variety of side switching behaviours amongst our fish. Some individuals showed significant amounts of side switching (e.g. Fig. 11c) whereas others did not (Fig. 11e). Fish five showed the least amount of side switching, and this may have been in part related to the lack of doors they were trained and tested with (Fig. 11e). Despite these differences, all fish tended to change their switching behaviours when the barriers were at their narrowest extent (Fig. 11). Some of the changes we observed in behaviour seemed to relate to the outcome of a trial, however these associations were not consistent between individuals, instead being specific to an individual (Fig. 11)

We compared side switching across the MIMs and object rotation experiments on a group level. We found that fish displayed greater amounts of no side switching in the MIMs experiment (see results, data chapter two) than they did in the object rotation experiment- regardless of barrier distance (Fig. 11a). Additionally, individuals exhibited fewer multiple switches in the MIMs experiment (see results, data chapter two) than they did in the object rotation experiment for most barrier distances (Fig. 11a). In the MIMs experiment, we also found that the differences in switching behaviour between correct and incorrect trials conducted at the same barrier distance were more visible (see results, data chapter two) than in the object rotation experiment (Fig. 11a). Nevertheless, both experiments did share some similarities. We observed similar proportions of each of the three switching behaviours at conditions D1C, D4C, and D4I. The proportions were virtually identical at D4 in both experiments (see results, data chapter two and Fig. 11a). Behaviour changes also seemed to be most noticeable at the shortest barrier distance (D4) in both experiments (see results, data chapter two and Fig. 11a).

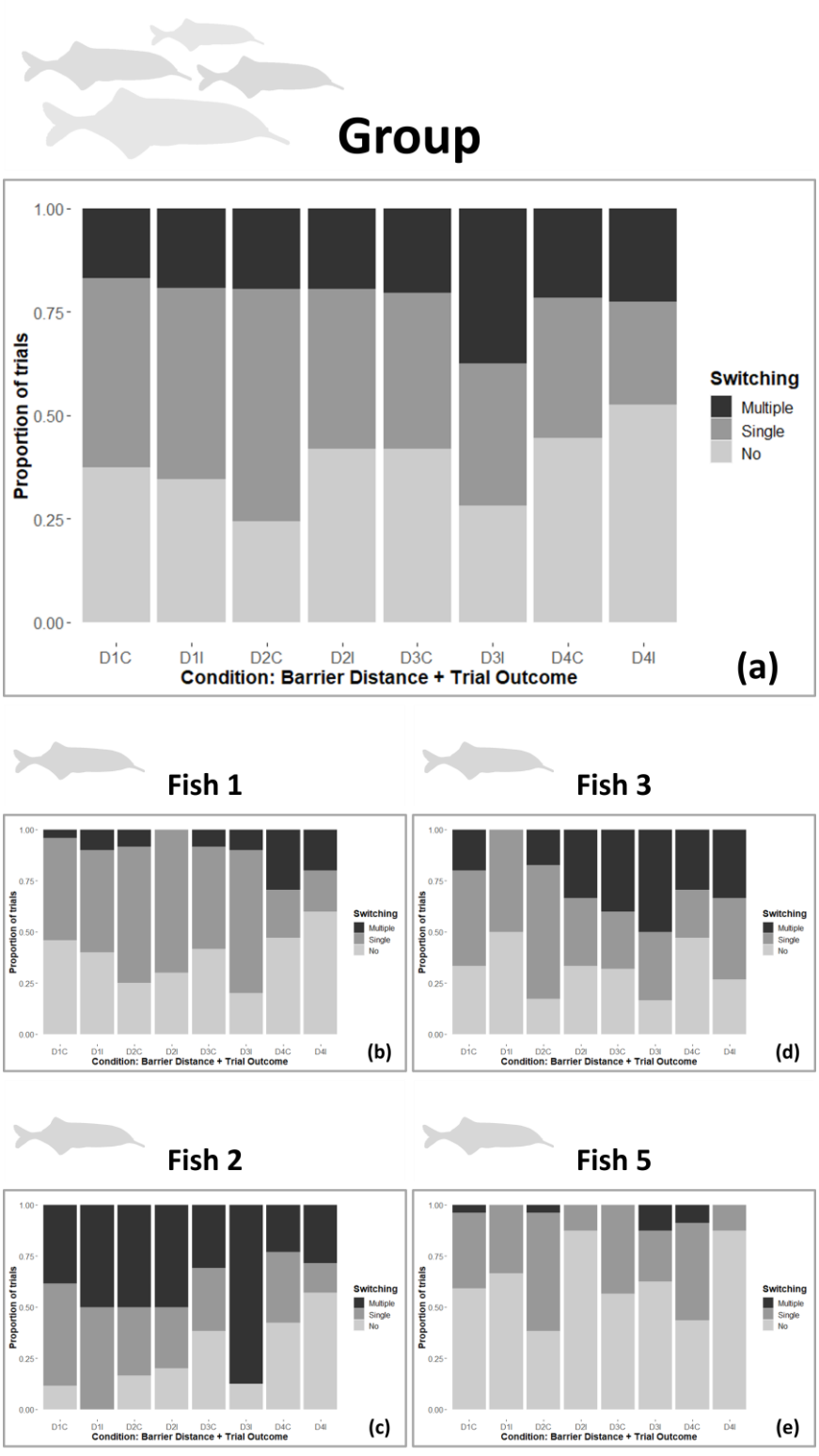


Fig. 11. Side switching behaviour of fish during test probe trials in the object rotation experiment, shown on a group (a) and individual level (b-e). Side switching was categorised as either occurring not at all, once, or multiple times in a single trial (shaded on a greyscale). Trials were assigned to different conditions which depended not only on the barrier distance (D1, D2, D3, and D4) but

also the trial outcome (C=correct and I=incorrect). Each bar represents the proportion of trials where a particular switching behaviour was observed under a set condition. Total N=520 trials (fish one=129, fish two=135, fish three=127, and fish five=129).

5. DISCUSSION

Our study set out to investigate the role of movement further in shape discrimination. Our first aim was to interrogate MIMs and determine how robust it is. Our second aim was to see what evidence there was for other movement cues. To help us test these ideas, we used the same 2AFC setup as in the previous chapter, but we also rotated S+ to change the way in which it was perceived.

G.petersii can detect and recognise three-dimensional objects with great precision at close range using their active electric sense (von der Emde and Schwarz, 2000, 2002; von der Emde and Fetz, 2007; von der Emde, *et al.,* 2010; Schumacher *et al.,* 2016a, 2016b, 2017a). Individuals can use a single image parameter or a collection of image parameters within an electric image to determine numerous object properties and enable them to quickly identify objects in their local surroundings (e.g. von der Emde and Zelick, 1995; von der Emde *et al.,* 1998; von der Emde and Schwarz, 2002; Caputi and Budelli, 2006; von der Emde, 2006; Schumacher *et al.,* 2016b). However, no combination of known image parameters within a single electric image has been found to encode shape reliably (von der Emde and Schwarz, 2000, 2002; von der Emde, 2004, 2006; Caputi and Budelli, 2006; Pusch *et al.,* 2008; Schumacher *et al.,* 2016b; Fujita and Kashimori, 2019). Instead, it has been proposed that shape information might be obtained by an individual engaging in movements close to an object, at different points of perspective, comparing the information content from successive electric images to build up a representation of that object's shape (von der Emde, 2006; von der Emde and Fetz, 2007; von der Emde *et al.,*

2010; Fechler *et al.,* 2012; Fechler and von der Emde, 2013; Schumacher *et al.,* 2016b; Fujita and Kashimori, 2019).

Schumacher *et al.* (2016b) tested and found support for movement-induced modulations (MIMs) being involved in shape recognition, but their limited sample size and inability to rule out non-movement cues mean that their conclusions are not robust. Our study described in data chapter two investigated the role of egocentric movement in shape discrimination further, and we found strong evidence that corroborates the idea that movement is a crucial component of shape perception (see data chapter two). Although MIMs seems the most plausible candidate mechanism for shape recognition, we could not rule out the possibility of other movement related mechanisms being involved, either alongside or instead of MIMs. One alternative strategy was that individuals were able to recognise an object's shape by comparing electric images from certain viewpoints, referred to here as a MIMs-like strategy, given the reliance on movement to get the body into the right position. In this study, we investigated whether MIMs was a robust cue and if there was evidence for a MIMs-like strategy being used instead. To do this, we kept the same training/testing protocol used in data chapter two, but we rotated S+ 90° (Fig. 2).

5.1. Overview of highlights

We found evidence to suggest that MIMs was less useful for shape discrimination in this study. We also found some evidence to suggest that a MIMs-like strategy (snapshots) might be used by these fish. However, it was difficult to determine which mechanism was used with certainty due to similarities in how both mechanisms responded to our manipulations.

5.2. How robust is MIMs as a cue for shape recognition?

5.2.1. Expectations

We expected to see poorer performances with decreasing barrier distance if MIMs was being used, along with longer latencies and more side switching to reflect increasing uncertainty of which object was the correct one. We also suspected that object pairing might have an effect, with individuals trained with the sphere finding it more difficult to distinguish between it and the sausage-shaped object than those trained with the cube due to the greater similarity in appearance.

We expected the effect of the manipulations to be stronger in fish who were using MIMs due to the similarity of S+ and S- having a greater effect under these circumstances.

5.3. What evidence is there for a MIMs-like (snapshot) strategy being used?

5.3.1. Expectations

If an individual was using a MIMs-like strategy, we would have expected them to move their head and body in ways that would compensate for the rotation of S+. We expected the barriers to interrupt these movements, resulting in poorer performances, particularly at the shorter distances when the fish would be most funnelled. Again, we expected to see increased latencies and more side switching as individuals tried to modify their strategies in response to environmental uncertainty.

We also expected to see an all or nothing response, with fish being able to discriminate between the objects when able to view them from set viewpoints, and the behaviour only breaking down when these became unavailable.

5.4. Task acquisition

All fish remembered how to discriminate between non-rotated S+ and S- (Fig. 6). Most fish remembered within five to seven sessions, which is remarkable given that they had not received training for six to twelve months prior to this (Fig. 6). Schumacher *et al.* (2017a) found during a similar shape discrimination task that the duration an individual was able to perform reliably depended on which senses they were trained with. Fish trained with both their active electric sense and vision were able to maintain performance at least 70% correct up to 17 weeks- ca. four months- after training. From this point, performance dropped for all fish bar one. This remaining fish was able to perform at least 75% correct throughout, even after 26 weeks- under seven months- without training (Schumacher *et al.,* 2017a). Fish trained with just vision were only able to maintain their performance (at least 70% correct) for nine or ten weeks- less than three months (Schumacher *et al.,* 2017a). Our fish had access to both their active electric sense and vision, so it makes sense that they were able to remember the task after an extended period away given what Schumacher *et al.* (2017a) observed. Even so, the amount of time passed between training periods (ca. six to twelve months) exceeds even their upper limits. Schumacher *et al.* (2017a) stopped testing one fish (trained with the active electric sense and vision) after 26 weeks, even though they were still able to perform at a high level. It is possible that this fish may have continued performing well for some time afterwards given the opportunity, and so perhaps may have exhibited task retention equivalent to what we observed in our studies. The remarkable retention of information demonstrated by our fish might also be in part a reflection of the fact that these fish have been trained on and off over an extended period of several years.

We found that fish generally took longer to learn how to discriminate between S+ and S- when S+ was rotated 90° rather than when it was left unrotated (Fig. 6). We expected individuals to take longer to learn this task, since they were not used to S+ being presented in this way, and so we expected them to need time to recognise newly rotated S+ as S+. Having said that, we know that

G.petersii exhibits rotational invariance, where they are able to recognise a three-dimensional object using their active electric sense, regardless of the position it occupies in space, but this ability can break down if the objects resemble one another too much (von der Emde *et al.,* 2010). As such, depending on how similar the two objects now appeared to our fish, they may have had a harder or easier time recognising S+. This might have contributed towards the variation we observed across our population in the time it took individuals to learn how to discriminate newly rotated S+ with S- reliably (Fig. 6).

Fish five was again trained without the doors. They completed both training steps and testing relatively quickly- particularly training step two (Fig. 6d). It is difficult to ascertain whether the lack of doors contributed towards quick task acquisition or not, as we only had one fish trained under these conditions in this study and we were not testing this directly. Certainly, the rate of progression through training step one was similar to that of fish one and three who were both trained with doors- only training step two showed a substantial difference in time between the subjects (Fig. 6). Fish five did not perform significantly better during testing compared to the other fish, and so it is unlikely that not having the doors gave them a significant advantage during active electrolocation (Fig. 6).

5.4.1. How does the MIMs hypothesis hold up when examining the task acquisition data?

Performance remained relatively stable in the training trials conducted during the testing phase, only occasionally dipping below the threshold of 75% (Fig. 6). This showed that individuals could discriminate rotated S+ with S- robustly, when the barriers were at their widest extent, doing so at a relatively high level of performance, although not performing as well as they had done before (see data chapter two). This fits in line with our expectations that, if individuals were using MIMs to extract shape information from the objects, we would expect them to find the discrimination task harder with the addition of the rotation of S+, as the two objects would now appear more

similar, eliciting similar levels of MIMs, which might not be sufficient to distinguish the two objects reliably (Figs. 1 and 5). We know from work undertaken by Schumacher *et al.* (2016b) that objects which elicit similar levels of MIMs are harder to discriminate.

5.4.2. How does the MIMs-like (snapshot) hypothesis hold up when examining the task acquisition data?

If individuals were using other movement cues, such as taking electrical snapshots at particular points on their exploratory trajectories towards the objects, then we would also expect them to find the task harder if they are unable to compensate for the rotation of S+ with their head and body movements, and consequently take longer to learn it (Stamp Dawkins and Woodington, 2000). The length of training step two for some fish and the lower performances in training trials during the testing phase provide some evidence for this (Fig. 6).

5.5. Shape discrimination performance during testing

5.5.1. Highlights

We found that barrier distance had a significant influence on whether an individual chose the right object or not (Figs. 7, 8, and 9; Table ii). Generally, we found that shape discrimination performance decreased as the barriers were brought closer together (Figs. 7, 8, and 9). This was most noticeable with fish three (Figs. 8c and 9) but could also be seen to a lesser extent with fish one (Figs. 8a and 9) and fish five (Figs. 8d and 9). In fact, compared with the MIMs experiment, the influence of barrier distance on performance was weaker here (see data chapter two). Nevertheless, success was generally lower in the object rotation experiment (Figs. 7, 8, and 9; Table ii) than in the MIMs experiment (see data chapter two). These results tell us that something different occurred in the two studies, and since the only difference between the studies was the

rotation of S+, this must have been the catalyst. The result seems to suggest that MIMs has become less informative.

5.5.2. MIMs as a putative cue

If individuals were using MIMs to extract shape information from their environment, then we expected them to find the discrimination task harder when S+ was rotated. In this orientation, a lot of the object's mass would have been rotated away from the individual, and so the electric images evoked on the skin would have been weaker, meaning that S+ would have more closely resembled a sphere- an object which was used as S- for three out of four fish trained and tested (Figs. 1 and 4b). As such, it is unlikely that MIMs would have provided sufficient information to accurately identify which object was S+, since any differences in shape between the two objects would now appear quite subtle. The barriers would have exacerbated this problem by restricting how and where the object could be scanned. Together, this would have made an individual more error-prone, particularly at short barrier distances where space by the object was most limited.

The lower performance scores observed in the object rotation experiment fits with the hypothesis that rotating S+ makes it less discernible from S-, presumably because the electric images produced from both objects were similar (von der Emde *et al.,* 2010; Schumacher *et al.,* 2016b). However, the weaker effect of barrier distance on performance when S+ was rotated was not what we expected. This might be because MIMs was less informative here and so the fish perhaps had to use a different strategy to compensate for this. As we have mentioned before, the movements of the fish might have a weaker effect on MIMs if the long side of S+ was rotated away from them. If, however, the long side was facing the fish (as in the MIMs experiment), then their movements may have induced larger changes in image amplitude, namely MIMs, and so would have been a useful cue to use for shape recognition. This explains why discrimination performance in the MIMs experiment was generally higher, but was affected strongly if an

individual was unable to generate movements beside the object, as in situations where the distance between the barriers was reduced. In the case of the object rotation experiment, movement that evoked MIMs probably didn't help as much to discriminate the objects in the first place, because of the little MIMs that were occurring due to the apparent similarity of S+ and S- from the perspective of the fish. As such, when the space for sensing was restricted, the effect of barrier distance was relatively small, since movements eliciting MIMs couldn't be used reliably for shape discrimination. Instead, an individual might have been relying on other cues for discrimination. For example, cues that either did not require movement (could be extracted from a single electric image) or were derived from movement but less interrupted by the barriers (perhaps they were still able to take electric snapshots). As we have mentioned before, an unambiguous cue(s) for shape has not been identified within an electric image (e.g. Schwarz, 2000 cited in von der Emde and Fetz, 2007; von der Emde, 2006; Fujita and Kashimori, 2019). It is possible that such a cue exists and was used by individuals in our study, but it is also feasible that they may have resorted to using another object property instead- even if it is less preferred (von der Emde, 2004; von der Emde and Fetz, 2007).

5.5.3. Size as a replacement cue

Even though our objects only varied with respect to their shape, the rotation of S+ would have made it appear smaller than S-, regardless of the object pairing (Fig. 1). We know that fish can discriminate between objects according to their size so long as there is enough degree of difference (Davis and von der Emde, 2002 cited in von der Emde, 2004). As such, perhaps an individual used object size (calculated from an electric image by combining object distance and image width) as a way of discriminating between the two objects (von der Emde, 2006).

To estimate an object's size, an accurate assessment of object distance would be needed as image width is strongly influenced by this parameter (von der Emde, 2006). This could have been

achieved by measuring the ratio between the maximum image slope and maximum image amplitude of a single electric image, termed 'SAR' (von der Emde *et al.,* 1998), or the temporal slope-amplitude ratio of successive electric images, referred to as 'tSAR' (Hofmann *et al.,* 2013a). However, as discussed previously, it can be difficult to estimate distance accurately for certain types of objects. When individuals used SAR, they tended to overestimate the distance of spheres- even when the objects were projected on the rostral region which was more stable for this kind of measurement (von der Emde *et al.,* 1998; von der Emde and Schwarz, 2000; Schwarz and von der Emde, 2001). Individuals might correct this information by taking into account the shape of the objects in front of them (von der Emde and Schwarz, 2000; Schwarz and von der Emde, 2001). However, in our study, where shape information was compromised, this would have been difficult. Individuals would have been left with an unreliable cue for estimating size, ultimately resulting in them having limited success at distinguishing S+ and S-. This would nevertheless explain the poorer performances generally seen in the object rotation experiment (Figs. 7, 8, and 9).

A similar issue might have developed if individuals had used tSAR, since the relationship between the temporal slope and object distance would have changed according to the point of the body which the object was projected onto- this would have resulted in either inaccurate or ambiguous estimates of object distance, unless an individual could have accounted for the object's position with respect to their body (Hofmann *et al.,* 2013a). This could have been addressed by an individual analysing the spatiotemporal structure of their own EODs, which would have been shaped not only by the objects in their local environment, but importantly, by their own movement through the water (Hofmann *et al.,* 2017). However, with the barriers restricting space, this might not have been possible. Size can also be considered a component of shape and volume, so with this in mind, it was probably a challenging cue to extract here- likely resulting in limited success if utilised at all (von der Emde and Fetz, 2007). It is difficult to know what other

object features could be used instead given the amount of care that went into designing the objects to minimise their differences (Figs. 4 and 5).

5.5.4. MIMs-like strategy as a putative cue

An alternative explanation for how objects might have been recognised in the absence of MIMs is that individuals used some form of template matching (e.g. Schuster and Amtsfeld, 2002; von der Emde and Fetz, 2007). This would have involved an individual attempting to match current electric snapshots of the objects with stored representations of said objects previously learned, by perceiving the objects from particular points during exploration (Schuster and Amtsfeld, 2002; von der Emde, 2004; von der Emde and Fetz, 2007). We know that this mechanism can be employed by *G.petersii* when recognising visual patterns (Schuster and Amtsfeld, 2002). However, the behaviour of *G.petersii* during active electrolocation seems to suggest that they recognise objects not by considering them as a whole, but by assessing certain features within them (von der Emde and Fetz, 2007; von der Emde *et al.,* 2010; Schumacher *et al.,* 2017a). Nevertheless, individuals might have resorted to using a more 'simplistic' mechanism of recognition during our study, when more favoured cues were inaccessible. Template matching does have a movement component which we must consider. Individuals using such a mechanism would have sought to get their heads and bodies in the best positions to optimise perception of their immediate environment. As such, we suspected that the narrowing of the barriers by the objects would have made getting into the right position more difficult, meaning that individuals would have found it hard to match their current view(s) with their memorised view(s), resulting in problems with distinguishing between the two different shapes. This would also explain why performances were generally lower in this study- with limited space seemingly compromising but not eliminating viable snapshots (Figs. 7, 8, and 9). A similar observation was made in hens when their ability to discriminate objects worsened after a large barrier was introduced that prevented them from

taking their usual routes to investigate the objects (Stamp Dawkins and Woodington, 2000). The movement behaviour of our fish supports this template matching mechanism in part. Fish tended to have a preferred trajectory they took when swimming to the objects, initially examining them from a set side, which fits with this mechanism (Skeels, personal observations). Also, when the barriers were pushed together, the fish were forced to change their routes, and funnelled into the central region of the sensing space. However, we noticed that they often performed a lot of fast, minute adjustments of their head and Schnauzenorgan when in close proximity to the objects, regardless of the barrier spacing. This behaviour fits less with the template mechanism proposed and is more in line with the feature detection mechanism we have mentioned, particularly with respect to MIMs (von der Emde and Fetz, 2007; von der Emde *et al.,* 2010; Schumacher *et al.,* 2017a). As such, their movement behaviour currently provides an inconclusive picture of which mechanism was used.

5.5.5. Object pairing

We found that object pairing had no significant influence on trial outcome in this study. This was surprising as we expected individuals trained with the rotated sausage-shaped object and sphere to have a more difficult time in distinguishing the objects than those trained with the rotated sausage-shaped object and cube. This is because the sausage-shaped object and sphere more closely resembled each other (Figs. 1 and 2). Individuals could have only used the degree of curvature to differentiate the two, whereas individuals trained with the sausage-shaped object and cube could have used the curvature of the sausage-shaped object to help distinguish the two and/or the corners of the cube. We know that individuals will pay attention to both these features of shape (von der Emde and Fetz, 2007). Fujita and Kashimori (2019) recently suggested that the *va-et-vient* movements of weakly electric fish might help them to determine whether the object has a corner, and the nature of that corner (e.g. acute, right angle, or obtuse). A possible

reason for why we did not find an effect with object pairing might be that despite the rotation of the sausage-shaped object, both the sphere and cube were still sufficiently different to be distinguished from the sausage-shaped object- perhaps by similar degrees. Since the sausage-shaped object was not a complete sphere, it is feasible that an individual was able to detect (at least in part) the rounded edges of its long side, even if there was a limited window in which to sense this (von der Emde and Fetz, 2007). The difficulty that can arise from categorising spherical objects might not have been an issue here, since the amount of training received may have given individuals time to learn how to compensate for these ambiguities (von der Emde and Schwarz, 2000; Schwarz and von der Emde, 2001). In addition, due to the time constraints of this study, we did not have an even number of individuals trained and tested with each object pairing. As such, perhaps this masked any differences between the two groups. In the MIMs experiment, we also found that object pairing had no significant influence on trial outcome.

In summary, it is likely that the lack of difference between the two object groupings in the object rotation related to the cues used by our fish, which meant that neither S- was easier nor more difficult to recognise against newly rotated S+ than the other. Although individuals can have preferences for particular features, it makes sense for them to be able to detect a broad range of features well, given the complexity of their natural environments (Moller *et al.,* 1979; von der Emde and Fetz, 2007; Olaosebikan *et al.,* 2020).

5.6. Posthoc analyses

5.6.1. Latency of response during test probes

5.6.1.1. Latency increased with declining barrier distance despite drive to optimise behaviour

Response times increased when we narrowed the space between the barriers (Fig. 10).

We expected individuals to take less time when they were certain of S+ and more time when they were uncertain. As previously expressed, we thought that the combination of the rotation of S+ and the narrowing barriers would make it more difficult for individuals to discriminate between the objects if movement was important. We expected response times to lengthen to reflect this- and more so than in the MIMs study due to extra level of detail involved.

We found that barrier distance had a significant influence on latencies. Responses increased with decreasing barrier distance- this was most noticeable at the shortest barrier distance, D4 (Fig. 10). Since individuals were taking more time to make their decision at these shorter distances, this suggests that they were experiencing an increased amount of uncertainty, presumably exacerbated by the additive effect of the rotation of S+ and the funnelling of the barriers.

Having said that, most responses were on the shorter side regardless of the condition examined (Fig. 10). As with the MIMs experiment, there seems to be an optimal way of completing the task (resulting in consistent fast times) which is somehow preserved, despite the noticeable influence of barrier distance (Pedraja *et al.,* 2020). This might be because individuals had a number of cues that they were able to rely upon, selected according to their reliability, which provided them with a robust percept (Schumacher *et al.,* 2017a).

As mentioned earlier, even though longer responses were rarer, they did become more common with decreasing distance (Fig. 10). We have attributed this to an increasing level of uncertainty in identifying S+ accurately, resulting in individuals taking more time to assess the objects, possibly by attempting to use their favoured cue (potentially MIMs) which was less reliable given our

manipulations of the components to be sensed and the space for moving and sensing. Alternatively, they might have used other cues that provided similar information but were perhaps less attuned to the situation. For example, visual cues could have informed on shape, but due to the poor spatial resolution of the visual system, it would have struggled to pick up the finer details of the objects at close range, which would have been attained through active electrolocation if uncompromised (Schuster and Amtsfeld, 2002; Landsberger *et al.,* 2008; Kreysing *et al.,* 2012; Pusch *et al.,* 2013a, 2013b, Francke *et al.,* 2014; Dangelmayer *et al.,* 2016; Schumacher *et al.,* 2017a). As such, the slight upticks we observe near the tip of the tails might relate to shifts in preferred strategy resulting from changing environmental conditions (Fig. 10).

5.6.1.2. Individual variation

Individual differences in latencies might relate to the way each fish organises their cues differently (von der Emde and Bleckmann, 1998). This makes sense considering von der Emde and Fetz (2007) found that individuals weighted object properties during object recognition either positively or negatively, and that the type of weighting not only depended on the training that they experienced, but also to their own interests- both innate and developed. However, it is difficult to know for certain, as we were not specifically testing for this.

5.6.1.3. Comparing latencies across the two studies

The trends we have described here are similar to what we observed in the MIMs experiment (see data chapter two for more details). The main difference we noticed in the object rotation experiment was, unlike in the MIMs experiment, trial outcome had an effect on latency, though this effect was weaker than that of barrier distance ($p=0.0048$; Fig. 10). We found that trials which resulted in a correct outcome tended to be longer in length- with this being most

pronounced at D4 (Fig. 10). An explanation for this is that individuals who took more time to investigate the objects were perhaps able to collect enough information to allow them to differentiate between S+ and their chosen S- sufficiently, and so were more likely to make a correct choice. This fits with observations made by von der Emde and Fetz (2007) who found that individuals who investigated the objects more thoroughly made fewer mistakes. In the object rotation experiment, the effect of trial outcome on latency was weaker than for barrier distance. This is probably because decreasing the barrier distance made recognition universally difficult, as, by limiting the space around the objects for probing motor acts, this drove increased response times across all trials (Toerring and Belbenoit, 1979; Toerring and Moller, 1984). When you look at Fig. 10, the frequency distributions of response times look remarkably similar for correct and incorrect trials at most distances- it's only really at D4 when you see that the 'tail' of the correct trials becomes substantially longer (Figs. 10di and 10dii). If a difference was to emerge between the two outcomes, it would make sense for it to happen here, where the combination of rotating S+ and the narrow gaps between the barriers would be most disruptive, possibly forcing individuals to adapt their strategies to give them a better chance of success. It is important to note that we were able to attain more response times for correct trials than incorrect trials, and that was due to our fish being more often correct than wrong. It is possible that this imbalance in samples across the two groupings contributed towards the differences that arose between them, but this is difficult to confirm as we were not testing for this specifically.

5.6.1.4. Summary

The longer latencies we observed in this experiment fit with our expectations that, if the fish were attempting to use movement cues (either MIMs or MIMs-like) for shape recognition, the narrowing of the barriers would make the task more difficult by reducing the opportunities to collect shape information, and create a level of uncertainty- possibly forcing individuals to use

other cues with limited success. The emergence of trial outcome influencing latency in the object rotation experiment, but not in the MIMs experiment, suggests that the rotation of S+ added an extra dimension of challenge, which was most marked when the barriers were at their most restrictive, and so the effect of the rotation of S+ was presumably at its most amplified. Relying on other cues might have buffered this effect, but since these cues were probably initially lower down in the hierarchy, it is likely they were not able to compensate completely. It is difficult to isolate MIMs from a MIMs-like strategy with latency data alone, as both would be impacted by limited space in similar ways. It is therefore important to consider other behaviours during decision making (more on this to follow).

5.6.2. Side switching behaviours during test probes

5.6.2.1. Fish exhibited multiple side switching more often but there was still a drive to minimise switching where possible

Multiple side switching had a greater presence in the object rotation experiment than in the MIMs experiment- though the amount of this type of side switching observed was relatively constant across distance and trial outcome (Fig. 11a; see relevant fig. in data chapter two). However, no switching was less prominent here than in the MIMs experiment (Fig. 11a).

We examined how many times the fish swam between A and B as a means of determining the level of certainty they had in knowing which of the two objects was S+ (Fig. 2). We expected individuals to make fewer switches when they were more certain of the location of S+, and more switches when they were less certain. We hypothesised that if movement cues were being used to derive information about an object's shape, then the combined effect of rotating S+ and the narrowing of the barriers would make this more difficult to accomplish. Individuals might compensate for this by visiting each object more often to make sure they had enough information

available to them before making a decision. We also hypothesised that trials that resulted in a correct outcome might display fewer switches than those resulting in a mistake because of a greater level of certainty when making a choice.

5.6.2.2. Comparing switching behaviour across the two studies suggests rotation task more complex

We found that our fish swam between the two objects more often in trials conducted during the object rotation experiment than in the MIMs experiment (Fig. 11a). This observation fits with our predictions that rotating S+ would make the task more difficult to complete, presumably by increasing the similarity between S+ and S-, and/or limiting access to the features of shape being used to characterise and categorise the objects. The increased difficulty in the task likely created an increased level of uncertainty, which meant individuals required more investigation of the objects before making a choice- hence the drive towards more side switches.

Nonetheless, we noticed that single side switching was the most common switching behaviour observed amongst our population, followed by no side switching, and finally multiple side switches (Fig. 11a). Together, these observations suggest a desire to minimise movements (and time spent) completing the trial where possible. This makes sense given the cost of investing in both active electrolocation and swimming during object exploration (MacIver *et al.,* 2010; Stamper *et al.,* 2012; Ruiz-Torres *et al.,* 2013; Salazar *et al.,* 2013; Markham *et al.,* 2016; Sukhum *et al.,* 2016).

We found that the proportion of trials that experienced no side switching fluctuated with barrier distance and trial outcome, though an increase was observed at D4 (Fig. 11a). Generally, no side switching was less prominent in this experiment than in the MIMs experiment (see data chapter two). Again, this is likely the result of the task increasing in difficulty across distance due to the

addition of rotated S+. Trials with limited investigation (fewer side switches) gave way to trials with more investigation (greater side switches). The uptick at D4 is interesting however, as it is at this point that the space for scanning the objects was at its most limited, and, as such, perhaps fish resorted to old rules when the usual sources of shape information became unreliable. For example, choosing an object based on their preferred side to visit.

Occasions of single side switching were less abundant as the distance between the barriers shortened- but there was no obvious difference between correct and incorrect trials at the same distance (Fig. 11a). It seems that at the shortest distance, single side switching behaviours were replaced largely by occasions where no side switching occurred. The change in behaviour coinciding with the shortest barrier distance suggests that a sensing threshold was met, where movement cues (presumed to be the preferred way of recognising shape) were no longer viable, and so other cues that were less attuned to the task had to be used instead. We would have expected the fish to have compensated for this with more inspections of the objects, but it seems that this was not always the case (Fig. 11a). It is important to note that there may have been occasions where side switching was not necessary, for example when S+ was encountered and recognised straight away. To identify these occasions, one must look at other data collected during the trial, such as the position of S+.

Even though multiple switching was more prominent in this study, it still accounted for a small proportion of the total behaviours observed across all conditions examined (Fig. 11a). What's interesting however, is that its proportion remains relatively constant across conditions- perhaps slightly increasing with decreasing barrier distance (Fig. 11a). A possible explanation for this behaviour is that it is a direct consequence of the rotation of S+, which likely made the task hard even when the barriers were at their widest extent- there was little restriction on the space for scanning the objects here. Perhaps the task was so difficult that multiple side switches were not enough to enhance the animal's chance of success, and so they were not invested in as much as

we expected. D3I was an exception however, where there was a marked increase in multiple switching (Fig. 11a). This might have been an indication of a level of uncertainty with the task- particularly as this condition represents one of the shorter barrier distances and an incorrect choice being made. It might have also been driven by individual behaviour (Figs. 11c and 11d) or sampling imbalances- as described in the latency section. It is difficult to determine either way though as we did not have time to model the switching data and account for variables such as fish identity. This is something that could be studied as an extension to this current work.

5.6.2.3. Individual differences

As we have discussed, individuals showed variation in their switching behaviour in this study- with some favouring switching more than others (Fig. 11). Interestingly, some individuals were relatively consistent with their behaviour across the two studies (fish three and five) whereas others were not (fish one and two). Perhaps this was due to individual preferences or them employing different strategies in completing the shape recognition task (von der Emde and Bleckmann, 1998; von der Emde and Fetz, 2007; Schumacher *et al.,* 2017a, 2017b). Maybe some strategies could be preserved, whereas others had to be adapted. For example, maybe a MIMs-like strategy resulted in fewer switches if the viewpoint(s) were preserved, and more when they were disrupted. It is difficult to be sure as the switching data is ambiguous, as different strategies can culminate in the same result. Context is key and is why we must consider these posthoc analyses together.

5.6.2.4. Summary

We found that, in the object rotation experiment, our fish exhibited more bouts of multiple side switching and fewer bouts of no side switching compared to the MIMs experiment (Fig. 11a; see figure in data chapter two). This likely related to the increased complexity of the task (the addition of rotating S+) which we presumed would make using movement cues difficult, due to the objects eliciting similar modulations of the electric field due to their resemblance, or the inability of individuals to match electric snapshots to their remembered views successfully. In both situations, individuals might have tried to compensate for this by performing more manoeuvres to get the same amount of information as they would have unconstrained.

In the object rotation experiment, there seems to be less of a difference in the level of side switching between trials differing in outcome for a set distance. This contrasts with the latency information which indicated that latencies were longer during correct trials (Fig. 10). This disparity might relate to sampling limitations, but it also suggests that side switching, and latency do not always increase proportionally to one another- as we had hypothesised. For example, there might be occasions where an individual spent a long time investigating one object or investigated both objects multiple times in a short span of time. The result likely was dependent on the cues being used for the task and the prevailing environmental conditions. It is possible that the head and Schnauzenorgan movements were more important than whole body movements for some individuals, but this would not be picked up by the side switching data. Detailed tracking of the head during trials would be required to make an assessment on this.

At this stage, it is difficult to distinguish between MIMs and a MIMs-like strategy- even when considering the latency and side switching behaviour together. Both seem plausible with our current data, and it is possible that these strategies were not mutually exclusive. What we can say, however, is that D4 seems to represent a threshold for both experiments, where big changes in behaviour occur- fitting with our hypothesis that this distance alone brings challenge.

5.7. Concluding remarks

Our study has provided further evidence for the importance of egocentric movement during shape recognition. We found that barrier distance had a significant influence on an individual's performance during shape discrimination tasks. Individuals were more likely to make a mistake when the barriers were positioned close together. However, the effect was weaker than that observed in the MIMs experiment, which suggests that MIMs was used to a lesser extent here, possibly because the rotation of S+ in combination with the narrowing barrier distance meant that S+ and S- resembled one another more, eliciting similar levels of MIMs. As such, this cue was less helpful for discriminating the two objects. Interestingly, object pair had no effect on performance, which was unexpected, as we thought the sausage-shaped object would be easier to differentiate from the cube than the sphere. This might have been because MIMs was not being used so much in this study. Instead, an individual might have been using other cues which did not rely on picking out particular features of the objects. For example, individuals might have used a MIMs-like strategy, where they recognised objects by their position within a scene, by comparing electric snapshots of current views with remembered views- so long as the space allowed for it. As with MIMs, this strategy would have been of limited help if the viewpoints were interrupted by the repositioning of the barriers.

Shape discrimination performance in this experiment was generally lower than previously recorded, which fits with our expectations that rotating S+ added an extra layer of complexity to the shape discrimination task. Despite this, they were still able to perform around the learning threshold, which shows resilience, probably resulting from their abilities to dynamically order strategies according to their current levels of reliability and use of multiple senses to obtain similar kinds of information from their environments. Response times lengthened with narrowing barrier distance, which suggested an increased level of uncertainty, presumably in part because the increased difficulty in the task made completion harder and more time consuming.

Interestingly, trials that resulted in a correct outcome tended to be lengthier- though this was most noticeable at the shortest barrier distance, D4. This might be because investing more time into investigating the objects resulted in fewer mistakes. We found in this study that fish displayed more bouts of multiple side switching and less bouts where no side switches were recorded than in the MIMs experiment. Again, this suggests an increased level of uncertainty which meant more inspections of the objects were needed to resolve the task. The variety of latencies recorded suggest a range of strategies that were employed- most likely implemented to current environmental conditions and individual preferences. Nonetheless, there seems to have been a drive to optimise behaviour and minimise costs where possible. What unites both studies is that D4 seems to represent a threshold where behaviour shifts, presumably because movement becomes less reliable, perhaps to a point where it is unusable.

It was difficult to detangle MIMs from a MIMs-like strategy, but it seems that MIMs was probably less utilised here thanks to the rotation of S+. It is possible that feature extraction was favoured when conditions were optimal, but fish may have resorted to template matching when the former became unreliable. In the future, further analyses of the data collected might help to shed more light on which strategy is favoured and the conditions required for its implementation. Head and Schnauzenorgan movements were difficult to quantify in this experiment specifically, but they most likely played a prominent role in the object recognition process. Despite these limitations, this study has further cemented the importance of self-driven movements to resolve complex recognition tasks, and that these movements may provide the same information by different mechanisms. These fish show great resourcefulness, through their use of movement to modulate their active sense, their multisensing abilities, and dynamic weighting of sensory inputs. This most likely gives them a competitive advantage compared to similar animals on their trophic energy level, and probably allows them to occupy niches unavailable to others. It also enables them to cope with the varied habitats that they reside in, which can change on both a daily and seasonal basis.

GENERAL DISCUSSION

1. Thesis overview

In this thesis, we have provided substantial evidence for the involvement of egocentric movement in sensory acquisition in *G.petersii,* particularly its role in object recognition and discrimination. This corroborates the growing body of work supporting the hypothesis that movement is vital for shaping the sensory experience of these animals (Hofmann *et al.,* 2013a, 2013b, 2014, 2017; Schumacher *et al.,* 2016b; Pedraja *et al.,* 2018, 2020; Engelmann *et al.,* 2021).

2. The active electric sense has allowed weakly electric fish to thrive despite its energetic costs

G.petersii occupy a broad geographic range, being found in freshwater habitats across Central and Western Africa (Olaosebikan *et al.,* 2020). This species has been able to compete and successfully occupy these niches thanks to the variety of senses it has at its disposal, particularly its active electric sense (Nilsson, 1996; Lavoué *et al.,* 2000; Markham *et al.,* 2016; Schumacher *et al.,* 2017a). The active electric sense is a unique sensory modality, since it requires the animal to invest energy in producing the signal, in this case EODs (Caputi and Budelli, 2006; Nelson and MacIver, 2006; von der Emde, 2006). Although weakly electric fishes have adapted to minimise the costs of EOD production and detection, there is still a considerable energetic demand put on these animals (Salazar and Stoddard, 2008; Reardon *et al.,* 2011; Stoddard and Salazar, 2011). For instance, Salazar *et al.* (2013) found that in some gymnotiforms the costs of EOD generation can be as much as 30% of routine mass-adjusted oxygen consumed. In the mormyridae, extreme encephalisation characterises many of its species and is thought to relate to their electrosensory abilities (Nilsson, 1996; Sukhum *et al.,* 2016, 2018). However, Sukhum *et al.* (2016) found that encephalisation increased the metabolic cost on the animal, and in doing so reduced their ability to withstand low oxygen environments. These pressures have helped shape the evolution of

active electrosensing and they explain in part why it is a rare phenomenon, found in only two lineages of teleost fish, and why some adaptations for electrosensing are confined to certain taxonomic groups (Baker *et al.,* 2013; Markham *et al.,* 2016; Sukhum *et al.,* 2016). Nevertheless, it is clear that this sensory modality has many advantages that mean the heavy investment is worthwhile. These fish tend to live in dark, sediment-heavy environments where visual cues are more limited (Kreysing *et al.,* 2012). As such, active electrolocation (e.g. von der Emde *et al.,* 2010) and electrocommunication (e.g. Gebhardt *et al.,* 2012) allow weakly electric fishes to engage with their environment when other senses would otherwise struggle. Active electrolocation also has high spatial resolution, meaning that it can provide highly detailed information about an object if it is at close range (von der Emde, 2006; von der Emde *et al.,* 2010; Schumacher *et al.,* 2017a). It can also provide information about the properties of an object that would otherwise be inaccessible, such as its complex impedance (von der Emde, 1990; Budelli and Caputi, 2000; von der Emde and Schwarz, 2002). Indeed, MacIver *et al.* (2010) found that *A.albifrons* swam inefficiently as it improved their detection of prey during active electrolocation, and that this more than made up for the extra locomotion costs they incurred.

3. Movement actively shapes perception

As we have emphasised before, movement seems to be important for sculpting the sensory landscape of weakly electric fishes, and likewise, the stimuli around them will influence their movements (Hofmann *et al.,* 2013b). We have discussed some of the active sensing strategies that have been identified in these animals (particularly *G.petersii*) and how they might modify the sensory information available to an individual, and how the resulting information can enable them to complete certain perceptual tasks (e.g. Hofmann *et al.,* 2013a, 2013b, 2014, 2017; Pedraja *et al.,* 2018, 2020; Schumacher *et al.,* 2016b; Engelmann *et al.,* 2021). Our work has also

demonstrated the interdependency between their active electric sense and locomotor behaviour in this species of weakly electric fish.

4. Key findings of thesis

4.1. Key findings from data chapter one

4.1.1. G.petersii collect electrical information bidirectionally

In data chapter one, we studied the sensory and motor patterns of *G.petersii* as they freely explored a tank. We know weakly electric fish are highly proficient at swimming both backwards and forwards- with the behaviour thought to improve prey capture by getting the animal in the right place for lunging at prey as well as enhancing their electrosensory perception by bringing the target closer to their electric foveae (e.g. Lissmann, 1951, 1958; Lissmann, 1974 cited in Lannoo and Lannoo, 1993; Lannoo and Lannoo, 1993; Nanjappa *et al.,* 2000; MacIver *et al.,* 2001; Snyder *et al.,* 2007; Ruiz-Torres *et al.,* 2013; Fotowat *et al.,* 2019; Engelmann *et al.,* 2021). We investigated the latter by determining whether there were significant differences in the electrical information obtained by *G.petersii* during different forms of swimming. We confirmed that they produce EODs during forwards and backwards swimming, with both swimming modes seemingly involved in sensory acquisition. From this, we can infer that *G.petersii* have the neuronal circuitry to collect and process electrical information from both directions. Here we aim to expand and discuss the putative mechanisms underpinning the encoding of this information.

4.1.1.1. Possible neurons and circuits involved in processing electrical information collected during forwards and backwards swimming

There is evidence in other weakly electric fish, particularly gymnotiforms, that there are neurons in the brain that can respond to bidirectional movement. Khosravi-Hashemi *et al.* (2011) showed

that electrosensory neurons in the midbrain of the gymnotiform *Apteronotus leptorhynchus* can encode the direction of a moving object by bursts of spiking activity. Later, Khosravi-Hashemi and Chacron (2012) found that spike bursts and isolated spikes from some of these neurons could encode opposing directional information. This would likely help with discriminating objects moving in different directions within a neuron's receptive field (Khoshravi-Hashemi and Chacron, 2012). In the same species, Clarke and Maler (2017) demonstrated a midbrain feedback loop which produces a neural code for motion reversal in the on and off type electrosensory pyramidal cells found in the hindbrain, providing an accurate encoding of where an object is, no matter the direction of travel. An indirect feedback loop is also activated, and the interaction of the two loops strongly enhances the response to sensory input resulting from body movements (Clarke *et al.,* 2014, 2015; Engelmann *et al.,* 2021). Fujita and Kashimori (2019) hypothesised that the same neurons identified by Clarke *et al.* (2014), which are involved in contrast coding motion reversal, might also be useful in encoding object shape information, since both tasks involve handling signals that vary with respect to both space and time. Recently, Wallach *et al.* (2018) made neural recordings from the preglomerular complex (a thalamic region in the brain seemingly involved in spatial learning) in *A.leptorhynchus.* Neurons within this region respond to motion onset, offset, and reversal, and as such, they are well suited to responding to the self-driven movements generated by these fish (Engelmann *et al.,* 2021). Wallach *et al.* (2018) found that they were able to encode the time between object encounters and hypothesised that this could contribute to spatial learning and navigation with the active electric sense (Engelmann *et al.,* 2021). Finally, Fotowat *et al.* (2019) wirelessly recorded the neuronal activity from the pallium of *Gymnotus sp.* whilst they swam freely within a tank. They found that activity was elicited only during swimming, with many neuronal units responding to backwards swimming, and this seemed to be associated with enhanced electrosensory sampling of the environment. As such, these neurons seem well attuned to encoding sensory input deriving from self-motion, and again, they seem to be an important component of spatial learning (Fotowat *et al.,* 2019; Engelmann *et al.,* 2021). Together,

these examples show that there are neurons within the brain that are responsive to motion (be it object or self-generated motion) and that egocentric movement might modify the sensory information to match the characteristics of their nervous systems (Stamper *et al.,* 2012).

Having said that, the studies described here focussed on describing how gymnotiform fish might respond and process motion- with particular attention towards bidirectional movement (e.g. Khosravi-Hashemi *et al.,* 2011; Khosravi-Hashemi and Chacron, 2012; Clarke *et al.,* 2014; Clarke and Maler, 2017; Wallach *et al.,* 2018; Fotowat *et al.,* 2019). Since gymnotiformes and mormyriformes can differ in the way in which they process electric signals, then perhaps mormyrids, like *G.petersii,* handle motion information differently (Sawtell *et al.,* 2005). However, a recent review looking at the electrosensory and motor processes used for spatial navigation by gymnotiform and mormyrid fish highlighted similarities amongst the two lineages, not only in terms of behavioural adaptations to promote sensory acquisition, but in some of the hindbrain circuitry involved in processing the electrosensory information (Engelmann *et al.,* 2021). As such, perhaps *G.petersii* do process motion information in some of the ways described above. A goal of future work in this area should focus on confirming the neuronal pathways activated during forwards and backwards swimming (Engelmann *et al.,* 2021).

4.1.2. Sensory activity differed between swimming modes during object exploration only

We found that there were highly significant differences in the duration of IDIs in the two swimming modes- however this was only apparent when the animals were actively exploring the objects and not when they were exploring an empty tank. Again, this indicates that the body movements we observed directly related to sensory acquisition. We generally found that IDIs were shorter during bouts of forwards swimming, meaning more EODs were being produced in a set amount of time. Since we know that mormyrids like *G.petersii* will increase the amount of information they are able to extract from the environment by increasing the rate at which they

produce EODs, this suggests that forwards swimming facilitates increased sampling (Toerring and Belbenoit, 1979; von der Emde, 1992; Schwarz and von der Emde, 2001). Functionally, it makes sense for *G.petersii* to want to sample their environment more during this mode of swimming, since they are front loaded with electroreceptors. Most importantly, two electric foveae have been identified in *G.petersii*: the nasal region and the Schnauzenorgan, both of which lie at the cranial end of the animal (Bacelo *et al.,* 2008; Hollmann *et al.,* 2008; Pusch *et al.,* 2008; von der Emde *et al.,* 2008; Amey-Özel *et al.,* 2012). These areas fulfil the four conditions required to be considered electric foveae (von der Emde, *et al.,* 2008). They contain the highest densities of electroreceptors on the body, the brain regions devoted to processing information from both areas are overly large, receptors in both areas show morphological specialisations for electrosensing, and lastly, *G.petersii* exhibit behaviours that concentrate objects onto these areas (von der Emde and Schwarz, 2002; Bacelo *et al.,* 2008; Engelmann *et al.,* 2008; Hollmann *et al.,* 2008; Pusch *et al.,* 2008; von der Emde *et al.,* 2008). For example, when *G.petersii* are looking for prey, they will adjust their body pitch to optimise and maximise the field of view for the nasal region (von der Emde *et al.,* 2008). *G.petersii* will also move their Schnauzenorgan from side-to-side rhythmically, reminiscent of a metal detector, to detect invertebrates hidden below the substrate (von der Emde *et al.,* 2008; Engelmann *et al.,* 2009). When a target is detected, the fish will abruptly change the movements of the Schnauzenorgan in a way which allows them to get a good sense of the overall composition of that target (von der Emde *et al.,* 2008). With this in mind, it makes sense that we observed more sampling during forward swimming, given the bias of *G.petersii* using their head region for information extraction since it is well adapted for this task, the limited number of sensors towards their caudal end, and the costs of electrosensing which we have already discussed in some depth (Engelmann *et al.,* 2008; Salazar *et al.,* 2013; Sukhum *et al.,* 2016; Pedraja *et al.,* 2020).

4.1.3. EOD sampling declined with repeated encounters of the same object

We also found that sampling intensity declined over the course of a session. Fish produced more EODs when they were encountering an object for the first time than when they had visited it multiple times previously. This makes sense given what we know about these electric fish. We know that mormyrids can regulate their EOD rate during acts of exploration (von der Emde, 1992). Post and von der Emde (1999) demonstrated that *G.petersii* will produce a 'novelty response' when they encounter something unfamiliar, which is characterised by a short increase in EOD rate, and will eventually exhibit habituation towards it. How quickly they will show signs of habituation depends on the strength of the stimulus and the time between exposures (Post and von der Emde, 1999). Being able to reduce their action to a known stimulus is important in allowing them to attend to new or more pressing stimuli in the environment. It is also important for *G.petersii* to reduce their sampling rate and associated behaviours when they have enough detail on an object to know how to respond to it appropriately, given the significant costs of electrosensing (e.g. Nilsson, 1996; MacIver *et al.,* 2010; Salazar *et al.,* 2013; Sukhum *et al.,* 2016). Nevertheless, these changes in behavioural strategy do not just apply to *G.petersii,* but could apply to any animal wanting to maximise their chances of detecting a target whilst minimising their costs- especially those that exhibit a form of active sensing (Nelson and MacIver, 2006).

4.1.4. Summary of data chapter one

This chapter illustrated that self-driven movement plays a notable role in object exploration in *G.petersii,* but it did not demonstrate what information the movements might help this species to extract from their environment. As we have discussed in other chapters, some active sensory strategies have been attributed to the extraction of certain types of information. For example, tail bending in weakly electric fishes seem to provide information about the lateral distance of objects (Sim and Kim, 2011), and *va-et-viens* create electric flow, facilitating motion parallax, and

thus enabling these fishes to perceive depth- even in the murkiest of environments (Pedraja *et al.,* 2018). It is likely that these active sensing strategies have more than one role however, varying according to the problem needing to be solved. For instance, *va-et-viens* motion might be necessary or improve the extraction of shape information from the environment, and therefore be invaluable for successful object recognition and discrimination (Schumacher *et al.,* 2016b; Fujita and Kashimori, 2019).

4.2. Key findings from data chapter two

4.2.1. Body movements play a significant role in shape recognition

In data chapter two, we investigated whether body movements could be used to help *G.petersii* to distinguish between two objects that differed only in their shape, with the leading hypothesis being that fish might use the change in voltage patterns observed over a series of electric images when swimming alongside an object, referred to as 'movement-induced modulations' (MIMs), to encode shape (Schumacher *et al.,* 2016b). We found fish were highly successful at discriminating between the objects when the distance between the barriers was large. However, when the space between the barriers was reduced, discrimination performance decreased- with the decline being most substantial when the barriers were closest together. These results indicate that the amount of space beside the objects was important for determining how successful an individual was at distinguishing between the two objects, presumably because the greater the space, the more they were able to perform scanning movements such as those described by Toerring and Belbenoit (1979) and Toerring and Moller (1984). This supports the empirical work of Schumacher *et al.* (2016b) who found that individuals were able to discriminate objects from further away when they were more dissimilar in their level of MIMs.

4.2.1.1. Possible mechanism for encoding shape information

Recently, Fujita and Kashimori (2019) demonstrated through their modelling that weakly electric fish might be able to integrate the peak amplitude and half-maximum width from multiple electric images to get an unambiguous measure of an object's shape, with these electric images being generated as the fish swims past an object. Fujita and Kashimori (2019) then went on to propose that neurons within the hindbrain's ELL might encode the various features of shape by their spike bursting activity, the same ones known to encode stimulus motion reversal (Clarke *et al.,* 2014). These might then be integrated in a higher brain centre, like the torus. This has yet to be verified by additional modelling or by experimental work- but might work on some of the principles used in other models made for estimating the size and distance of objects (Fujita and Kashimori, 2006 cited in Fujita and Kashimori, 2019; Fujita *et al.,* 2007; Fujita and Kashimori, 2019). Having said that, Fujita and Kashimori's work has focussed on wave-type gymnotiform fish, so it remains to be seen how it might relate to a pulse-type mormyrid fish. Further investigation of how movement might facilitate extraction of shape information, and how this is encoded in the brains of gymnotiform and mormyrid fish, will not only help us understand the mechanism of shape recognition in *G.petersii* better, but will help us determine whether the strategies employed are fundamental in nature (Engelmann *et al.,* 2021).

4.2.2. Latencies and level of side switching gave some indication of the amount of certainty individuals had in the task

We found that response times and the level of side switching observed in trials seemed to indicate that individuals felt more uncertain during trials where space was most limited. This makes sense if they were typically relying on movement cues to complete the task, and under these conditions these would have been compromised. However, there was some ambiguity in these results, especially if one parameter was studied in isolation. A future goal will be to run a

model (like the one used for the latency data) to quantify the effect of distance and other noteworthy variables on the level of side switching observed during trials, since we did not have the time to complete this. We also plan to investigate the interactions between latency and side switching. Together, we hope this will help us to resolve the ambiguities when explaining the behaviours.

4.2.3. Summary of data chapter two: MIMs likely cue used in shape recognition task but cannot discount other movement cues

In data chapter two, we provided substantial evidence to support the hypothesis that movement was an important component of shape recognition and discrimination in these animals. However, our results were inconclusive in terms of whether we could attribute this to MIMs or not. We hypothesised that *G.petersii* might encode shape through MIMs, with the idea being that objects of differing characteristics will elicit dissimilar levels of MIMs (Schumacher *et al.,* 2016b). Alternatively, individuals might not rely on using certain features of an object for recognition, but instead they might compare snapshots taken with their active sense at known locations with remembered views to determine whether the object facing them is S+ (template matching)- which we describe here as a MIMs-like strategy given that movement is involved (von der Emde; 2004; von der Emde and Fetz, 2007). As such, the purpose of data chapter three was to determine how robust MIMs was as a cue for shape discrimination (part A) and to determine if snapshots could be utilised for this task (part B).

4.3. Key findings from data chapter three

4.3.1. How robust is MIMs as a cue for shape recognition and discrimination? (Part A)

4.3.1.1. MIMs was a less reliable cue for determining shape

We found that barrier distance was a significant predictor of shape discrimination, with performance declining when the space between the barriers was narrowed (as in data chapter two). However, the effect was weaker than previously observed. This suggests that MIMs was used to a lesser extent here. This is because we rotated S+ in this iteration of the study, which caused S+ to appear more similar in shape to S-. As a result, these objects likely produced similar levels of MIMs, meaning that MIMs would not have been a suitable cue for discriminating between the two objects (Schumacher *et al.,* 2016b). Nevertheless, these findings do not discount the role of MIMs in the previous study. In fact, our results here suggest that MIMs likely played a role in the former study, given that the objects would have produced more dissimilar levels of MIMs, and that performances were generally higher (at least at the most unconstrained distances), but dropped more at the most constrained distances. This fits with the growing body of evidence that suggests more complex object recognition in *G.petersii* involves the assessment of an array of local features within an object (von der Emde and Fetz, 2007; von der Emde *et al.,* 2010; Schumacher *et al.,* 2017a).

Having said that, object pairing did not have a significant effect on discrimination. This was surprising, given that the sausage-shaped object (S+) was curved like the sphere (S- for training group one) and so contrasted with the right-angle corners of the cube (S- for training group two). With such obvious differences, we would have expected individuals to have had an easier time discriminating between the two objects when S- was a cube. Our results however suggest that the sphere and cube were just as easy (or hard) to discriminate from the sausage-shaped object as each other. Shape is a property that represents an amalgamation of several smaller features (von der Emde and Fetz, 2007) and so perhaps there was enough differing both object sets, even

with the rotation of S+ making some features potentially harder to extract. Alternatively, since MIMs seem less useful in this study, perhaps the mechanism which they used was less reliant on the extraction of features (Srinivasan, 1994; von der Emde and Fetz, 2007).

4.3.2. What evidence is there for other movement cues being used? (Part B)

4.3.2.1. Some evidence for the use of a MIMs-like (snapshot) strategy during shape discrimination

In part B, we considered an alternative mechanism by which an individual might recognise S+ (and therefore its shape). We proposed that an individual might take snapshots of their local environment with their active sense, and then compare these to known, stored representations of the object of interest. This method of template matching requires an individual to take their snapshots from set positions, and the overall appearance of the object and how it is situated in the environment are more important than the individual features themselves (Schuster and Amtsfeld, 2002; von der Emde and Fetz, 2007). Our results indicate that it is certainly possible that these fish used this MIMs-like strategy, especially considering the limited evidence for MIMs, which seem to have been the favoured method of recognition in the previous study. Template matching is certainly a simpler mechanism of object recognition, as it requires an individual to only remember an object from a select number of views, so long as they take the same vantage points when examining the object each time (Stamp-Dawkins and Woodington, 2000; Schuster and Amtsfeld, 2002). Of course, it would only have been of use if the views required were undisrupted (Stamp-Dawkins and Woodington, 2000). We did not observe any definitive behaviour to confirm that individuals moved to certain locations to take snapshots of the objects, however we did notice that individuals usually took similar trajectories out of the waiting area and examined the objects in a set order in the sensing area. Those with doors tended to open them the same way each time as well, so long as space was not too compromised. As such, these

movements might have been the result of individuals trying to get themselves in the right position to take snapshots of the objects.

4.3.2.2. Alternative roles for movement behaviour

We know from Pedraja *et al*. (2020) that *G.petersii* will alter their routes during an object detection task, and will reduce the variability of these trajectories with sufficient training. This seems to result in individuals using the space more efficiently and being more optimally aligned to the object. This, along with other sensory-motor behaviour changes, seem to enhance the information that they can obtain from their active sense, and potentially extends their detection range (Pedraja *et al.,* 2020). As such, the behaviours we have just described might be adjustments to improve their perception generally. We therefore cannot conclusively confirm the use of a MIMs-like strategy in this study. Tracking key points on the body might help to address this issue, especially the head region, which they seem to adjust quickly and finely- presumably because of its role as the site of two electric foveae (von der Emde *et al.,* 2008).

4.4. Latency and side switching reflected an increased amount of uncertainty

Response times and the level of side switching both increased in the study, suggesting that fish were experiencing more uncertainty in the task. Again, this supported the idea that this iteration of the task was harder, presumably because it reduced the viability of movement cues- like MIMs. However, as with the study described in data chapter two, these measures cannot tell us any more about the mechanism(s) at play. Given *G.petersii's* use of multiple senses and their ability to dynamically weight their own sensory inputs, it is likely that these fish possess different strategies to complete this task, which emerge depending on the prevailing environmental conditions (Rojas and Moller, 2002; Schumacher *et al.,* 2016a, 2017a, 2017b). For instance, individuals likely tried to

complete the task using the most optimal strategy known to them (potentially MIMs). MIMs would likely have been a better strategy than the MIMs-like one, as it is viewpoint invariant, and so would be more resilient to shifts in perspective (Schumacher *et al.,* 2016b). However, if this was unsuccessful or uninformative in the first place, they would have likely switched to other strategies that they had learned which were more reliable at that time (possibly our MIMs-like strategy if their preferred viewpoints were not interrupted by the barriers too much). These strategies are not necessarily mutually exclusive, but likely exist on a continuum, with the chosen strategy depending on both the task and the intrinsic nature of the stimulus (Vanrie *et al.,* 2002).

4.5. Summary of data chapter three

Although we cannot confirm with certainty which movement cues were used, data chapter three (parts A and B) has managed to provide further evidence for the importance of egocentric movement during object recognition, as well as evidence that suggests different movement cues might provide equivalent information. Despite the task increasing in complexity, and individuals struggling more as a result, most remained relatively competent at it. This indicates a robustness in the percept, which arises from *G.petersii* having multiple senses that they can use complementarily, synergistically, or even redundantly depending on their level of reliability (Moller, 2002; Rojas and Moller, 2002; Schumacher *et al.,* 2016a, 2017a, 2017b). This most likely has allowed them to occupy and thrive in an ecological niche few others would. Indeed, there are more than 200 species belonging to mormyridae (Fricke *et al.,* 2021). This lineage has expanded and diversified thanks to its unique electrosensory system (Carlson *et al.,* 2011; Carlson and Arnegard, 2011).

5. Individual variation

One thing that is worth discussing briefly is the individual variation we observed in the performances of our fish in the shape discrimination experiments (see data chapters two, three A, and three B). We found that the trends we observed were generally consistent within and between individuals. Nonetheless, there were some differences between them. The differences in how long it took individuals to learn the two shape discrimination tasks might have related to the absence or presence of the doors, the object pairings they were trained and tested with (von der Emde and Fetz, 2007), individual approaches to learning (von der Emde and Fetz, 2007), and even age. During test probes, the differences in discrimination performance might have related to the size/length of fish (Knudsen, 1975; Caputi and Budelli, 1995; Caputi *et al.,* 1998; Pedraja *et al.,* 2014), their level of motivation (von der Emde, 1992), and individual prioritisation of cues (von der Emde and Bleckmann, 1998; von der Emde and Fetz, 2007; Schumacher *et al.,* 2016a, 2017a). Differences observed in response times and level of side switching across individuals might have related to which cues they used for the task, their level of motivation, the presence/absence of doors, their ultimate destination, and even personality (Kareklas *et al.,* 2016). Swimming speed may have contributed to response times as well. We can theorise why these differences exist, however since our work was not designed to interrogate these theories, we can only speculate. Nonetheless, these putative causes of individual variation are worth studying in the future, especially since few have been studied in the context of a cognitive task.

6. G.petersii can remember task details for an extended period of time

This thesis has also provided us with a unique opportunity to study *G.petersii* in the lab over an extended period of multiple years- even though this was not its purpose (relates to the work covered in data chapters two and three). Remarkably, we found that fish quickly remembered the basics of a discrimination task within a small number of sessions, despite a gap in training of

between six to twelve months (depending on the individual). This challenges the upper limit of 26 weeks reported by Schumacher *et al.* (2017a) for an individual who had access to both the visual and active sense cues (like our fish). It is possible that this individual could have reached a comparable level to ours if testing had continued. Nevertheless, our results suggest that *G.petersii* are able to retain information for much longer periods of time than previously thought. Future work should focus on verifying this retention period since our project was not designed to investigate this explicitly. Determining how retention length varies according to the type of task being learned and recalled would also be important, along with understanding the neural circuitry that would consolidate this information to long-term memory.

7. Take home message of thesis

Overall, this thesis represents a substantial amount of work investigating sensory-motor interactions in the weakly electric fish, *G.petersii.* This species displays motor behaviours which are tightly coupled with their ability to actively electrolocate. We have shown that both forwards and backwards swimming are important components of sensory acquisition, and that egocentric movements play a key role in shape discrimination. We have proposed two potential mechanisms by which shape might be extracted from movement- MIMs, and a MIMs-like (snapshot) strategy. Both seem feasible, but their employment likely depends on which one is more reliable in a given situation. Although *G.petersii's* active electric sense was most dominant at the working range we were interested in, individuals might have chosen to use non-movement cues if they found the electric sense to be compromised. *G.petersii* have access to an array of senses, and order cues according to their reliability. This flexibility in sensing enables them to get a robust view of their environment, even when it goes through dramatic changes which occur with the changing of the seasons. Mormyrids have spread and diversified across the African continent thanks to their active electric sense, which has revolutionised the way they sense the world and communicate

with conspecifics. Future work should focus on determining the function of other active sensing strategies long described in these fish, with the best approaches marrying theoretical with empirical work. Insight from this thesis can also be used to interpret other active sensing strategies in animals using different modalities, since some of the fundamental principles are the same. The study of sensory-motor interaction in these fish is an exciting area of current research, with new insights emerging regularly. This is owed in part to advancements in technology, but it is also due to how remarkably well suited these animals are for this type of investigation.

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APPENDIX A: for data chapter two

A1. Additional information for methods section

A1.1. Water level

Water level did not reach the top of each tank, instead it reached 25.2 cm.

A1.2. Partitions

The partitions that separated the various compartments were made from two components: the surround was made from thicker pieces of white acrylic and the walls from a nylon mesh (mesh size: 0.71 mm per square). These materials were electrical insulators due to their high resistivity and low conductivity (Rojas and Moller, 2002). As such, they did have some impact on the field (von der Emde, 2006). Nevertheless, the gapping in the mesh meant that water could flow through between the compartments, facilitating electrolocation (Schumacher *et al.,* 2016b).

A1.3. Mid wall

The wall was further extended by clipping a small, clear piece of Lego baseplate to it (h x w x d (excluding studs): 8.0 x 8.1 x 0.1 cm). The wall could not be this length from the outset as we needed the space to place the barriers properly.

A1.4. Barriers

A1.4.1. Appearance

The mesh started ca. 3.6 cm from the top of each barrier and extended to the bottom. The top edges were rounded to make them fish safe. At the base of each barrier were two 'feet'. Each foot comprised of one 2 x 4 Lego brick at its base, followed by two 2 x 2 Lego bricks, and a single semi-opaque blue marble on top (this provided weight to each barrier, preventing it from floating upwards). Care was taken to ensure the materials used in the setup had as minimal an effect as possible on the electric fields produced by the fish. The materials used were insulators however, so they did modify the field to some extent, and as such were detectable by the fish's mormyromasts used in active electrolocation (Rojas and Moller, 2002). Acrylic is not a source of DC potentials (the other materials used are likely to be as well) meaning that they were probably not detectable by the fish's ampullary receptors during passive electrolocation (Rojas and Moller, 2002).

A1.4.2. Barriers moved using distance grids as an aid

The distance between the barriers was easy to vary since the feet of the barriers were comprised of Lego blocks. This meant that the barriers could be readily attached to the barrier distance grid in any configuration that was required as the top layer of the grid was also made of Lego (Fig. 2c). The barrier distance grid had markings that allowed us to align two barriers by each door correctly. We were not concerned that the markings could be used as a cue to discriminate the

objects, because the markings were the same by each opening, and research suggests that this species does not spontaneously attend to two-dimensional visual stimuli (Landsberger *et al.* 2008). Each barrier distance grid comprised of three layers. The top layer was made from a clear Lego baseplate (l x w: 16.9 x 16.0 cm). This was where the barriers attached to. The middle layer consisted of a laminated sheet of paper (17.0 x 16.0 cm) with lines that were 1.6 cm spaced apart. This was visible through the top layer and used to space the barriers out correctly for each distance of interest. Finally, the last layer consisted of a piece of glass (17.0 x 16.0 cm) which provide some weight to the grid, preventing it from floating upwards. The three layers were adhered together using aquatic-safe sealant (CT1, Bearing Station). The total thickness of the grid (combining all three layers) was 0.8 cm- including the studs on the Lego baseplate.

A1.4.3. Slotted plastic piece kept barriers in place

A piece of clear acrylic (l x w x d: 2.2 x 17.2 x 1.2 cm) with slots was placed between each barrier pair to ensure that the space between the two barriers was constant and that any bowing was kept to a minimum (Fig. 2a). This piece of plastic resembled a comb. Multiple slots (11 in total) were made in the plastic to accommodate for the barriers being placed at different distances. Each slot was 2.2 x 0.3 x 0.7 cm. The distance between the barriers was measured from the midpoint of the slot that the first barrier was placed into to the midpoint of the slot that the second barrier was placed into.

Some fish would swim over the barriers and not in the channels created by the two barriers. This was undesirable as we wanted to constrain the movements of an individual to a set space to see if this had an impact on their ability to discriminate the object shapes correctly. To prevent these individuals (fish four and six) from swimming over the barriers, we created a taller version of the slotted plastic arrangement described above (Fig. 2b).

A different piece of acrylic was used as a base (h x w x d: 3.8 x 17.1 x 1.2 cm). This had 11 slots like the other piece which the barriers could slide into (slot size, l x w x d: 1.2 x 0.3 x 0.6 cm). An additional groove was placed on top which ran the length of the piece which allowed a second piece of clear acrylic to be attached (h x w x d: 5.7 x 17.1 x 0.3 cm). This gave the pieces a combined height of 8.8 cm (area of overlap subtracted) which meant that those fish inclined to swim over the barriers could not. To keep these taller pieces (combs) in position, an accompanying clear acrylic wall (h x w x d: 8.6 x 24.5 x 0.3 cm) was placed in the sensing area. It attached to the mid wall with a plastic peg and extended across to where the slider door was (fitted just above it). In addition to providing stability to the taller pieces connecting barrier pairs, the accompanying wall served another purpose. It limited an individual's ability to cut across and reach an opening without going through the channels created by the barrier pairs. As with the mid wall, we did this to control the space the fish could occupy by the objects.

A1.5. Doors

The doors hooked onto the partition with the openings, so only a small amount of force was needed for them to be moved out of the way. Again, we were careful with the materials we chose for the doors to ensure that they did not impair active electrolocation too much. Although both materials were electrical insulators, and so had some effect on the field, namely decreasing

stimulus amplitude, the structure of the mesh meant that water could flow through facilitating perception (Rojas and Moller, 2002; von der Emde and Fetz, 2007; Schumacher *et al.,* 2016b).

A1.6. Object distance

A1.6.1. For those trained without the doors

Object distance= distance between top and bottom of platform + partition depth + depth of opening rim

A1.6.2. For those trained with the doors

Object distance= distance between top and bottom of platform + partition depth + depth of opening rim + depth of door + small gap to allow door to be pried open

A1.7. Camera

Distance from camera lens to bottom of tank was 56.7 cm.

A1.8. Training and testing notes

A1.8.1. Mild punishment

The washer was added to the ruler to increase its aversiveness, as without training, fish tend to avoid metal objects (von der Emde and Fetz, 2007). This is likely due to their unfamiliarity with these objects (they are absent in nature) and the fact that they will cause very strong firing of electroreceptors due to their very low electrical resistance (Bell, 1990 cited in von der Emde and Fetz, 2007; von der Emde and Fetz, 2007). As such, they may appear quite intense (like something very bright or hot) and be perceived as something to be wary of and actively avoid if possible (von der Emde and Fetz, 2007).

A1.8.2. Criteria for responding to test trials when there was a performance dip

If fish scored less in one session, we made a note of this, but continued testing, since it was to be expected that there may be occasions where an individual may have a 'blip' in performance- e.g. due to lack of motivation. However, if an individual scored less than 75% correct in two consecutive sessions, and the second session scored worse than the first, then both sessions were omitted, and the individual went back to training. These testing sessions were omitted from our analysis, since we could not be certain of how well the task was remembered, and we did not want this to conflate our results. This also happened if there had been a significant time gap and the fish performed poorly (well below 75%) in their first session after this break. Note that on occasion individual training and test trials were omitted from our analysis if there were incidents which made them non-comparable with similar trials- e.g. if we accidentally rewarded a fish in a test trial.

A1.8.3. Test trials

We ran 1508 test trials, but 48 trials of these were removed from the test analyses as they weren't comparable to the other trials.

A2. Additional binomial GLMM information- main analysis

A2.1. R code for mixed models without notes

```
#From UPDATED MAY 2021 MIMs Logistic Regression Code doc

#Ran code a line at a time

getwd()

setwd ("C:/Users/sarah/Documents/MIMs Logistic Regression/")

list.files()

data <- read.csv("Group_dataset_290421.csv")

data

head(data)

unique(data$Fish)

unique(data$Distance)

unique(data$Correct)

unique (data$pair)

require(lme4)

str(data$Fish)

str(data$Distance)

str((data$pair))

str(data$Correct)

data$Distance <-as.numeric(data$Distance)

str(data$Distance)

data$Correct <-as.numeric(data$Correct)

str(data$Correct)

data$pair <-as.factor(data$pair)

str(data$pair)

model1 <- glmer(data$Correct ~ data$Distance + data$pair + (1|data$Fish), family = "binomial")

model2 <- glmer(data$Correct ~  1 + data$pair + (1|data$Fish), family = "binomial")
```

```
anova(model1, model2)
summary(model1)
cc <- confint(model1,parm="beta_")  ## slow (~ 11 seconds)
ctab <- cbind(est=fixef(model1),cc)
oddsd0<-exp (ctab[1,1])
D0<-(oddsd0/(oddsd0+1))*100
logoddsd1<-ctab[1,1]+ctab[2,1]
oddsd1<-exp(logoddsd1)
D1<-(oddsd1/(oddsd1+1))*100
difference1<-D0-D1
logoddsd2<-ctab[1,1]+(ctab[2,1]*2)
oddsd2<-exp(logoddsd2)
D2<-(oddsd2/(oddsd2+1))*100
difference2<-D1-D2
logoddsd3<-ctab[1,1]+(ctab[2,1]*3)
oddsd3<-exp(logoddsd3)
D3<-(oddsd3/(oddsd3+1))*100
difference3<-D2-D3
logoddsd4<-ctab[1,1]+(ctab[2,1]*4)
oddsd4<-exp(logoddsd4)
D4<-(oddsd4/(oddsd4+1))*100
difference4<-D3-D4
logoddsd0ll<-ctab[1,2]
oddsd0ll<-exp(ctab[1,2])
lld0<- (oddsd0ll/(oddsd0ll+1))*100
logoddsd0ul<-ctab[1,3]
oddsd0ul<-exp(ctab[1,3])
uld0<- (oddsd0ul/(oddsd0ul+1))*100
logoddsd1ll<-ctab[1,2]+ctab[2,2]
oddsd1ll<-exp(logoddsd1ll)
lld1<-(oddsd1ll/( oddsd1ll+1))*100
```

```
logoddsd1ul<-(ctab[1,3]+ctab[2,3])
oddsd1ul<-exp(logoddsd1ul)
uld1<-(oddsd1ul/ (oddsd1ul+1))*100
logoddsd2ll<-ctab[1,2]+(ctab[2,2]*2)
oddsd2ll<-exp(logoddsd2ll)
lld2<-(oddsd2ll/(oddsd2ll+1))*100
logoddsd2ul<-ctab[1,3]+(ctab[2,3]*2)
oddsd2ul<-exp(logoddsd2ul)
uld2<-(oddsd2ul/(oddsd2ul+1))*100
logoddsd3ll<-ctab[1,2]+(ctab[2,2]*3)
oddsd3ll<-exp(logoddsd3ll)
lld3<-(oddsd3ll/(oddsd3ll+1))*100
logoddsd3ul<-ctab[1,3]+(ctab[2,3]*3)
oddsd3ul<-exp(logoddsd3ul)
uld3<-(oddsd3ul/(oddsd3ul+1))*100
logoddsd4ll<-ctab[1,2]+(ctab[2,2]*4)
oddsd4ll<-exp(logoddsd4ll)
lld4<-(oddsd4ll/(oddsd4ll+1))*100
logoddsd4ul<-ctab[1,3]+(ctab[2,3]*4)
oddsd4ul<-exp(logoddsd4ul)
uld4<-(oddsd4ul/(oddsd4ul+1))*100
```

A2.2.1. Table of values for the estimate derived from model one

Distance	**Log odds ratio**	**Odds ratio**	**% likely to get correct**	**Performance step change (%)**
D0(intercept)	3.4334	30.9831	96.87	
D1: 15.9 cm	2.7738	16.0196	94.12	D0-D1: -2.75%
D2: 12.7 cm	2.1142	8.2828	89.23	D1-D2: -4.89%
D3: 9.5 cm	1.4546	4.2826	81.07	D2-D3: -8.16%
D4: 6.3 cm	0.7949	2.2143	68.89	D3-D4: -12.18%

A2.2.2. Table of values for the lower and upper limits derived from model one

Distance	Log odds ratio (for lower limit)	Odds ratio (for lower limit)	Lower limit % likely to get correct	Log odds ratio (for upper limit)	Odds ratio (for upper limit)	Upper limit % likely to get correct
D0(intercept)	2.5550	12.8712	92.79	4.3991	81.3744	98.79
D1: 15.9 cm	1.6794	5.3625	84.28	3.9442	51.6339	98.10
D2: 12.7 cm	0.8039	2.2341	69.08	3.4893	32.7629	97.04
D3: 9.5 cm	-0.0717	0.9308	48.21	3.0344	20.7888	95.41
D4: 6.3 cm	-0.9473	0.3878	27.94	2.5795	13.1910	92.95

A2.3. Random effect

We left our random effect (fish ID) in the model, as although it does not seem to absorb much of the variation seen in discrimination performance (σ^2=0.2118, σ=0.4603), if we were to remove it, our degrees of freedom would be inflated leading to an overestimation in the significance of what we are modelling. Certainly, we can observe from the plots that most fish show a decline in performance as the space between the barrier decreases incrementally- even if there is some variation in the extent (Fig. 6).

A3. Additional gamma GLMM information- latency analysis

A3.1. R code for mixed models without notes

```
#Ran a line at a time

getwd()

setwd ("C:/Users/sarah/Documents/")

list.files()

MIMsdata<- read.csv("MIMs_test_latencies.csv")

unique(MIMsdata$Individual)

unique(MIMsdata$Barrier_Distance)

unique(MIMsdata$Correct)

unique(MIMsdata$Latency_s)

require(lme4)

MIMsdata$Barrier_Distance<-as.numeric (MIMsdata$Barrier_Distance)
```

```
str(MIMsdata$Barrier_Distance)

MIMsdata$Latency_s<-as.numeric (MIMsdata$Latency_s)

str(MIMsdata$Latency_s)

MIMsdata$Correct<-as.factor (MIMsdata$Correct)

str(MIMsdata$Correct)

modelone <- glmer (MIMsdata$Latency_s ~ MIMsdata$Barrier_Distance + MIMsdata$Correct + (1
|MIMsdata$Individual), family= Gamma)

modeltwo <- glmer (MIMsdata$Latency_s ~ 1 + MIMsdata$Correct + (1|MIMsdata$Individual),
family= Gamma)

anova(modelone, modeltwo)

summary(modelone)
```

A3.2. Random effect

Our random effect (fish identity) had σ^2= 0.0006 and σ=0.0239.

Residual σ^2= 0.7026 and residual σ= 0.8382.

A4. R codes for producing some of the plots

A4.1. Group mean performance plot (with standard error bars) code

```
library(ggplot2)

library(dplyr)

getwd()

list.files()

data7 <- read.csv("MIMs_group_performance_results_16_02_2022_mark6.csv")

unique(data7$distance)

unique(data7$performance)

data7$distance<-as.factor(data7$distance)

data7

latestdatasummary <- data7 %>%

 group_by(distance) %>%

 summarise(
```

```
  n=n(),
  mean=mean(performance),
  sd=sd(performance)
 ) %>%
 mutate( se=sd/sqrt(n))  %>%
 mutate( ic=se * qt((1-0.05)/2 + .5, n-1))
ggplot(latestdatasummary) +
 geom_bar( aes(x=distance, y=mean), stat="identity", fill="grey", alpha=0.5) +
 geom_errorbar( aes(x=distance, ymin=mean-se, ymax=mean+se), width=0.4, colour="black",
alpha=0.9, size=0.7) + xlab("Distance between barriers (cm)") + ylab("% Correct") +
theme(panel.background = element_blank()) + theme(axis.text = element_text(size = 12)) +
theme(axis.title = element_text(face= 2, size = 14))
```

-Plot recoloured using *Paint app (Microsoft Windows, Version 21H1)*

-Note this plot was created using *R Studio, R version 4.1.2*

A4.2. Latency histogram code- an example

-Used to generate a group latency plot for condition D1C

```
library(readxl)
Group_MIMs_D1C <- read_excel("Group_MIMs_D1C.xlsx")
View(Group_MIMs_D1C)
Group_MIMs_D1C<-(data.matrix(Group_MIMs_D1C))
hist(Group_MIMs_D1C, breaks="FD", xlab="Time (s)", xlim=c(0,40), ylim=c(0,50), main=NULL)
```

A4.3. Stacking plot code for side switching- an example

-Used to generate plot for fish three (Margie)

```
library(readxl)
Ma_MIMs_switching <- read_excel("Ma_MIMs_switching.xlsx")
View(Ma_MIMs_switching)
library(ggplot2)
Ma_MIMs_switching1<-Ma_MIMs_switching
Ma_MIMs_switching1$Switching<-factor(Ma_MIMs_switching1$Switching, levels= c("Multiple",
"Single", "No"))
```

```
ggplot(Ma_MIMs_switching1, aes(fill=Switching, y=TotalFrequency, x=Condition)) +

geom_bar(position="fill", stat="identity") + xlab("Condition: Barrier Distance + Trial Outcome") +
ylab("Proportion of trials") + scale_fill_grey() + theme(panel.background = element_blank()) +
theme(axis.text = element_text(size = 12)) + theme(axis.title = element_text(face= 2, size = 14)) +
theme(legend.text= element_text(size = 12)) + theme(legend.title= element_text(face= 2, size=
14))
```

A5. Other notable behaviours during the testing phase

During training and test probe trials, *G.petersii* displayed particular behaviours whilst investigating the objects behind doors A and B. When leaving the waiting area, individuals tended to head to one side first, often the side that they had a pre-existing preference for. This preference seemed to be specific to an individual and likely developed over time. During their approach, the head, Schnauzenorgan, and body were held out relatively straight and level in the water column. Once beside the objects, they adjusted their body and head positions frequently and were often more angled.

The fish would approach the object very directly and quickly, stopping swiftly when reaching one of the doors. This practice is described as stationary/head probing, a type of probing motor act, and we often observed it when the fish was examining one side before moving onto the other (Toerring and Belbenoit, 1979; Toerring and Moller, 1984; von der Emde and Fetz, 2007). This behaviour was usually followed by extensive 'chin probing' where the Schnauzenorgan was poked into the gap left between the door and opening to allow for easy movement of the door. The Schnauzenorgan was moved very flexibly during this period. The fish made lots of small adjustments to their head and body whilst interacting with the doors and examining the objects. Some individuals on occasion would touch or tap very briefly the object with their Schnauzenorgan (Toerring and Belbenoit, 1979; Toerring and Moller, 1984; von der Emde and Fetz, 2007). Each fish had their own way of interacting with the objects, for example, fish four would tap them with some force. We also noticed other probing behaviours during object exploration. Sometimes individuals would approach an object tail first by backwards swimming towards it. This behaviour was often followed by 'tail probing' where the tail was moved side-to-side close to the object (von der Emde and Fetz, 2007). This behaviour was often exhibited when an individual was unfamiliar or wary of the object (von de Emde and Fetz, 2007). Tail probing is often associated with radial *va-et-vient* (Toerring and Belbenoit, 1979; Toerring and Moller, 1984). *'Va-et-vient'* describes the movement of an individual as it swims forwards and backwards along/around an object, usually keeping a fixed distance from said object (Toerring and Belbenoit, 1979; Toerring and Moller, 1984; von der Emde and Fetz, 2007). We sometimes observed lateral *va-et-vient* (movements occurred to the side of an object). However, this was mostly observed when the space between the barriers was wide, or when the fish had entered the compartment with the object and so could inspect it more closely. Tangential probing, where the fish heads directly for the object, and then rapidly changes its direction, seems to have been displayed by these fish as well (Toerring and Moller, 1979; Toerring and Belbenoit, 1984). These probing behaviours were difficult to define at times however, due to the nature of the space which meant individuals had to perform these actions at an angle by one face of the object. As such, not all probing motor acts were possible due to the constraints of the setup (Fig. 1). Indeed, some behaviours were more apparent once the fish had chosen a side and had full access to the object on that side. For instance, after entering A or B, individuals would swim forwards and backwards

over the object (superior *va-et-viens*) or around the object and its platform (lateral probing; Toerring and Belbenoit, 1979; Toerring and Moller, 1984).

Decreasing barrier distance had an impact on these movements. Fish could not always complete their movements. They tended to approach and inspect the door and the object behind it from more directions and angles when at the shortest barrier distances compared to longer distances. This behaviour was also reflected in their door opening technique which tended to change at the shortest distances regardless of the technique previously employed. We saw changes in how the space was used even in fish who were not trained and tested with the doors. This suggests these changes came about as a result of modifying the distance between the barriers and not simply because the fish could not access the doors adequately.

In summary, the occurrence of each behaviour seemed to be determined by several factors, including individuality, the level of familiarity an individual had with the task, what part of the trial the individual was participating in, what behaviours had occurred previously, and most interesting to us, where the barriers were positioned (Toerring and Belbenoit, 1979; Toerring and Moller, 1984; von der Emde and Bleckmann, 1998; von der Emde and Fetz, 2007; Kareklas *et al.,* 2016, 2018).

APPENDIX B: for data chapter three (parts A and B)

B1. Additional material for methods section

B1.1. Sourcing of fish

We are uncertain of the exact locations where these fish were caught in the wild. However, it is likely that they were collected in Nigeria (Edo, Delta, or Lagos state). We could not use captive bred specimens as these fish are notoriously difficult to breed in a laboratory setting. This is due to changes in their physiology that they undergo when they are brought into a controlled environment (Landsman, 1991, 1993). We have tried where possible to limit the numbers of individuals used in our study for both ethical and environmental reasons (Animal Behaviour, 2018). This species is listed as 'Least Concern' on the IUCN Red list, since they have a wide geographic range across central and western Africa and are not known to have any major threats across this range- despite population data lacking in parts (Olaosebikan, *et al.,* 2020). As such, it is a more viable species to study in the lab from a conservation perspective.

B1.2. Sensitivity to environmental conditions

Many studies have demonstrated that water impurities (both organic and inorganic) can affect the EOD rate of *G.petersii* (e.g. Geller, 1984; Lewis *et al.,* 1990, 1992b)- sometimes even changing the shape of the EOD waveform as well (Prabhakar and Landsman, 1994 cited in Olaosebikan *et al.,* 2020). These toxins can go on to have a harmful effect on the gills of *G.petersii.* Alazemi *et al.* (1996) studied the effect of cadmium, chromium, copper, cyanide, and atrazine on gill tissue. They found that each pollutant was associated with a different type of gill damage which seem to be related to the concentration of that given pollutant and its chemical activity (Alazemi *et al.,* 1996). It was therefore important for us to keep water conditions stable and impurity free to ensure our fish were in good health and could electrolocate properly. To do this, we used reverse osmosis water treated with a remineralisation formula (Tropic Marin Re-Mineral Tropic, Tropical Marine Centre, UK) which provided our fish with the essential minerals they needed, and it raised and stabilised the carbonate hardness of the water to a more suitable level.

B1.3. Lighting

The lights were set to a 12:12h light-dark cycle, with light levels slowly ramping up and down at the start and end of the day. Fish completed experiments during the light part of the cycle. Light levels were measured above the tanks with a light meter (LX1330B, Dr.Meter). We did not measure light levels during the dark part of the cycle, but we assumed this would be close to 0 lux given that there were no obvious light sources in the room at this time (Landsberger *et al.,* 2008).

B1.4. Diet

Fish were primarily fed bloodworms (twice a day, six times a week). They were given brine shrimp (twice a day once a week) and vitamins (once a week) to enrich their diet.

B1.5. Barriers

The mesh started ca. 3.6 cm from the top of the barrier and extended right down to the bottom. Each barrier was supported by two 'feet' made from Lego- a single foot comprised of one 2 x 4 block with two 2 x 2 blocks on top. A semi-opaque blue marble was secured to the top of each foot with aquarium sealant. This helped to keep the barrier weighted to the base of the tank. Again, we tried to be considerate with our selection of materials. Most materials used were insulators, which meant that they did alter the self-generated electric field surrounding the fish, and these deviations in the field would have been detected by an individual's mormyromasts (Rojas and Moller, 2002; von der Emde, 2006).

B1.6. Object distance

B1.6.1. Without doors

Distance between top and bottom of platform + partition depth + depth of opening rim= object distance

B1.6.2. With doors

Distance between top and bottom of platform + partition depth + depth of opening rim + door depth + door gap= object distance

B1.7. Monitoring task retention

We interspliced training trials with test probe trials to ensure discrimination performance was maintained at a high level (von der Emde and Fetz, 2007). We assumed that the task had been learned and retained once the fish was consistently performing at 75% correct or more. If an individual dipped below 75% in a single session, we assumed this was simply a natural blip relating to changes in motivation and/or attention. As such, these sessions were retained in the testing analysis and testing sessions continued to be conducted. If a fish performed significantly below 75% across multiple successive sessions, we stopped testing and returned to the last training step to shore up understanding. These testing sessions were removed from our analysis, as we could not rule out the possibility that the fish had forgotten the task, meaning that any changes in performance observed in test probes may have related to this, and not to what was happening in these probes. When fish scored below 75% in two consecutive sessions, things were handled in one of two ways, either sessions were retained or omitted, and this decision was made according to the performance of the second session. We omitted the testing sessions if the fish performed worse in the second session, as this suggested that an individual was on a downward trend in terms of their retention of the task, and so they most likely needed to return to training to refresh their understanding. If their performance in the second session equalled or exceeded the first session, it was assumed that the dip was a natural blip, and so testing could remain in progress. Sometimes we had to omit individual trials from our analysis. We only did this when the trials were not comparable with like trials- e.g. when we accidentally punished an individual in a test probe.

B2. Additional binomial GLMM information- main analysis

-Code unnecessary since practically identical to code used in data chapter two (see appendix A).

B2.1.1. Table of values for the estimate derived from model one

Distance	Log odds ratio	Odds ratio	% likely to get correct	Performance step change (%)
D0(intercept)	1.6515	5.2150	83.91	
D1: 15.9 cm	1.4344	4.1973	80.76	D0-D1: -3.15
D2: 12.7 cm	1.2173	3.3781	77.16	D1-D2: -3.60
D3: 9.5 cm	1.0002	2.7189	73.11	D2-D3: -4.05
D4: 6.3 cm	0.7831	2.1882	68.63	D3-D4: -4.48

B2.1.2. Table of values for the lower and upper limits derived from model one

Distance	Log odds ratio (for lower limit)	Odds ratio (for lower limit)	Lower limit % likely to get correct	Log odds ratio (for upper limit)	Odds ratio (for upper limit)	Upper limit % likely to get correct
D0(intercept)	1.1316	3.1005	75.61	2.2096	9.1123	90.11
D1: 15.9 cm	0.7313	2.0777	67.51	2.1730	8.7849	89.78
D2: 12.7 cm	0.3310	1.3923	58.20	2.1364	8.4693	89.44
D3: 9.5 cm	-0.0693	0.9330	48.27	2.0999	8.1650	89.09
D4: 6.3 cm	-0.4696	0.6253	38.47	2.0633	7.8717	88.73

B2.2. Random effect

Like with the previous binomial GLMM, we left our random effect (fish identity) in the model, even though it does not absorb much of the variation (σ^2=0.0036, σ=0.0602) seen in discrimination performance. This is because, if we were to remove it, our degrees of freedom would be inflated resulting in an overestimation in the significance of what we are modelling.

B3. Additional gamma GLMM information- latency analysis

-Codes unnecessary since practically identical to those used in data chapter two (appendix A).

B3.1. Random effect

The random effect was σ^2=0.0018, σ=0.0426. Residual values were σ^2=2.6995, σ=1.6430.