Electrosensory-based search strategies in weakly electric fish

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Abstract

Effective exploration of the environment is a critical aspect of adaptive behaviour, enabling animals to identify food sources, potential mates, refuge locations, and other important resources. The particular strategies used during exploratory behaviours depend on a variety of factors including context, personality traits and natural ecology. Weakly electric fish rely specifically on a short-range electric sense to search and locate objects in their environment in low-light conditions. However, little is known about the exploratory strategies used. We characterized the exploratory movements of two species of weakly electric fish, *Apteronotus leptorhynchus* and *Apteronotus albifrons*, in a laboratory setting. Our results suggest that there are behavioural differences between species in their exploratory strategies. *Apteronotus albifrons* spent more time in the open, travelled at a slower speed when out in the open, and had a higher total feeding time. Interestingly, *Apteronotus leptorhynchus* had a higher total displacement and preference for wall-following. A subsequent study on the behavioural function of wall-following in the two species suggested that wall-following is used for exploration in weakly electric fish, rather than for protection, and is not an artifact of restricted movement and tank shape.

Résumé

L'exploration efficace de l'environnement est un aspect critique des comportements adaptifs, qui permet aux organismes d'identifier des sources de nourriture, des partenaires potentiels pour la reproduction, des refuges ainsi que d'autres ressources nécessaires. Les stratégies particulières utilisées l'exploration dépendent d'une variété de facteurs incluant le contexte, et l'écologie naturelle. Les poissons faiblement électriques comptent surtout sur leur sens électrique à courte portée afin de chercher et localiser des objets dans leurs environnements en condition de faible luminosité. Par contre, nous connaissons peu sur les stratégies qu'ils utilisent. Nous avons caractérisé les mouvements explorateurs de deux espèces de poissons faiblement électriques, *Apteronotus leptorhynchus* et *Apteronotus albifrons*, en laboratoire. Nos résultats supportent qu'il y a des différences entre les mouvements explorateurs danse *Apteronotus albifrons* et *leptorhynchus*. *Apteronotus albifrons* passaient plus de temps en espaces ouverts, voyageaient à une vitesse plus lente dans les espaces ouverts et passaient plus de temps à manger. De façon intéressante, *Apteronotus leptorhynchus* montraient une plus grande préférence à suivre les parois du bassin, ce qui nous a poussés à étudier la fonction comportementale de ces observations dans les deux espèces. Nos résultats supportent l'hypothèse selon laquelle suivre les parois du bassin agit en tant que comportement explorateur dans les poissons faiblement électriques, plutôt que d'agir comme une forme de protection ou un artefact des mouvements restreints ou de la forme du bassin.

Acknowledgements

Chapter 1. General Introduction

1.1 Searching behaviour

Searching is an important element of behaviour because resources (e.g. food, mate, refuge) are essential for the growth and maintenance of an organism and ensuring the success of future generations (Bell 1990). However, searching is not restricted to the active movement of non-human animals seeking resources in the wild. For example, daily human activities involve a great number of visually guided actions in order to determine the presence and location of objects every day (Ehinger et al. 2009). Human information seeking behaviour is another example of searching where the purpose of seeking information to satisfy a goal. During the course of seeking, an individual can interact with manual information systems (e.g. newspaper), or with a computer-based system (e.g. news website) (Ehinger et al. 2009).

Being a product of natural selection, searching behaviour has presumably evolved to produce efficient strategies that maximize the intake of some currency, such as energy, and minimize its output (Heinrich 1979). Costs associated with searching include the risk of predation while engaging in searching behaviour, as well as time taken away from other necessary activities such as protection of territory or courting (Bell 1990). The particular search strategy that is best will depend on the combination of three factors: (1) the biological characteristics and abilities of the searcher, including locomotion and perception of sensory information, (2) external environmental factors such as resource availability and predation risk; and (3) internal factors, such as individual needs at a particular time (Bell 1990). For example, many species of ants use their well-developed olfactory sense to follow chemical trails that have been laid out by other

foragers of the same colony. Since these trails provide a continuous map to the foraging areas and back (Schultheiss & Cheng 2011), a local search strategy is sufficient. Diurnal desert ants, however, like the Australian desert ant (*Melophorus bagoti*), cannot rely on elaborate trail systems because chemical trails are ineffective in their harsh habitats. Instead, these ants have developed sophisticated navigation tools, involving visual terrestrial cues and path integration, which involves integrating distances and directions taken throughout the trip (Merkle et al. 2006).

When first introduced into a novel environment, searching is often characterized by exploration (Bell 1990). Exploration of an environment provides individuals with the necessary information regarding the locations of important resources for future use. In many cases, animals use environmental boundaries and edges to guide their movement (Merkle et al. 2006). This wall-following behaviour is useful for forming spatial maps of a new environment and its landscape features (Sharma et al. 2009). Animals may also choose to search out in the open. Special movements in the open characterized by random walks are often analyzed in order to determine the most efficient searching strategy (Humphries et al. 2012).

1.1.1 Exploration

A key aspect of animal behaviour research is to understand an animal's exploratory behaviour (Bell 1990). Exploration represents an important element of searching behaviour because it allows an organism to observe characteristics of a new environment and search for resources efficiently. During exploration, an animal gathers general information about the structural properties of its surroundings and specific features such as food location or potential mates

(Tebbich et al. 2009). Depending on the needs at a particular time, different methods of exploration are used. Exploration that occurs simultaneously with other searching behaviours is defined as extrinsic exploration, for example searching for potential refuge or foraging. In contrast, intrinsic exploration is exploratory behaviour that is conducted in order to gain information that will be used at a later time (Tebbich et al. 2009). Increased exploratory behaviour is often characterized by increased total displacement throughout the test arena, reduced latency to exit the shelter at the start of the trial, increased time spent out in the open, and increased time spent exploring novel stimuli (von Merten & Siemers 2012).

Costs and benefits

The costs and benefits of exploration are influenced by many ecological factors such as diet and habitat characteristics, which may possibly account for the variation in exploratory behaviours found among species as well as in populations within species (Mettke-Hofmann et al. 2002). An example of diet influencing behaviour is in birds that feed on concealed food (e.g. insect under moss) or that have diverse diets (Tebbich et al. 2009) compared to species with easily accessible food or more specific diets. Concealed food and diverse diets require a higher investment in learning about different environments and different foraging locations, and therefore animals must spend more time exploring (von Merten & Siemers 2012). For example, food that is difficult to extract from a substrate (e.g. nuts under bark) requires more handling time and time spent in the open than easily accessible food and should favor more exploratory activity (Tebbich et al. 2009). Living in these complex habitat involve higher exploration rates suggesting that extracting information is more time-consuming than in a simple, stable environment (von Merten & Siemers 2012).

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The costs associated with exploration are usually related to energetic costs and predation risk (Morse 1980). The absence of predators or a low risk environment reduces the cost of exploring and can enhance the prospect of experimenting with new strategies and target goals such as searching for refuge or finding a mate. A common example used is the famous fearlessness and curiosity of island species (Grant 1986). Island species tend to have fewer competing species and therefore broader niches than species that live on the mainland. Island species also are less threatened by predation risks, which reduce the costs of exploration (Tebbich et al. 2009). This highly exploratory behaviour is especially advantageous for species living on small islands where resources are limited and the discovery of new food types and niches is crucial for survival (Pulliam 1986).

Darwin's finches of the Galápagos Islands are examples of island species with a unique evolution of exploratory behaviours. The finches show inter-specific differences in ecology based on diet and food types and have been divided into two groups: the ground finches that are mainly seed-eaters and the tree finches that are mainly insectivorous (Tebbich et al. 2009). Also, because of the changes in climate which vary along altitudinal gradients, availability and abundance of food resources vary. As a consequence, there are also variations within species depending on their geographical distribution.

Inter-individual differences in exploratory behaviours are receiving increased attention (Sih et al. 2004). It is suggested that within a species different individuals can behave differently in the same context (e.g. foraging, conspecific interactions), but that the same individuals will demonstrate similar reactions across different contexts (von Merten & Siemers 2012). Typically, some individuals behave more boldly, aggressively, and are more active while others behave

more shy, docile, and are less active regardless of the situation (Ehinger et al. 2009). These suites of correlated behavioural reactions are referred to as animal personality traits or behavioural syndromes (Sih et al. 2004). In the context of behavioural syndromes, it is suggested that individuals within a population can have varying behaviours based on their life history strategies (Biro & Stamps 2008). Previous work by Biro and Stamps (2010) investigated the relationship between exploratory behaviour and basic metabolic rate (BMR). This study confirmed that individuals in a variety of species with higher BMR tend to explore more actively and spend less time exploring than individuals with lower BMRs. These differences have led to the introduction of two exploratory strategies; thorough and superficial exploration. This study and other previous work focused on differences in life history among individuals, however differences in behavioural strategies can also be found between species (Careau et al. 2009). One of the first comparative multispecies studies on the relationship between exploratory behaviour and life history was done by von Merten and Siemers in 2012. They tested species of shrews (Soricidae) with different life histories, fast-lived animals with high metabolic rate and short life span with those that are slow-lived ones. Shrews of the fast-lived genus *Sorex* were quicker to start exploration and locate a food patch. They also moved faster than the slow-lived genus *Crocidura*. The authors demonstrated that *Sorex* performed more frequent but shorter exploration bouts, supporting the hypothesis that exploratory behaviour is related to life history. These two species also vary in habitat preferences which may play a role in differences in exploratory behaviour. *Sorex* often inhabit more cold and humid environments than *Crocidura*, and these humid habitats, such as swampy forests, are likely to be more complex than dryer ones, such as savannahs (von Merten & Siemers 2012). The species in these genera differ in the climatic

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zones in which they inhabit and therefore experience different selection pressures which diversified the life histories and exploratory strategies for their specific habitats.

1.1.2 Wall-following behaviour

When introduced into a novel environment, a variety of species including cockroaches (Jeanson et al. 2003; Daltorio et al. 2013), fish (Patton et al. 2010), and rodents (Simon et al. 1994) tend to move along and/or maintain contact with physical boundaries or edges in the environment. This behaviour is called wall-following, and is a form of thigmotaxis, the tendency for an animal to orient itself in space by physical contact (Patton et al. 2010).

Wall-following is a typical behavioural reaction to the risk of predation, whereby animals exhibit an innate aversion to open spaces. Remaining close to larger structures decreases exposure and can reduce anxiety (Lamprea et al. 2008). In a study on wall-following in rats, individuals preferred to spend time in open spaces as long as there was at least one close wall; further, the amount of time spent in the open was a function of the number of nearby walls present (Lamprea et al. 2008). This supports the hypothesis that wall-following serves a protective function. Grossen and Kelley (1972) demonstrated that wall-following was significantly enhanced in rodents with the administration of a fearful stimulus and would avoid shock by going to a platform near a wall rather than a platform in the center of an arena. These experiments suggest that the wall-following behaviour may be a protection-seeking or anxiety-relieving function (Sharma et al. 2009).

Wall-following behaviours may also function as an aid in exploration independent of predation risk used to locate potential resources such as refuges, food, and mates in a new environment

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(Patton et al. 2010). Travelling along boundaries of an environment would allow an animal to move throughout and build spatial knowledge based on any distinctive features encountered during the traverse. The presence of physical heterogeneities in the environment is important in guiding the movement of many animals. For example, it is known that ants and termites will orient themselves along structural guidelines created by rocks and grooves (Klotz et al. 2000). At a larger scale, Desrochers and Fortin (2000) found that despite the fact that the home ranges of chickadee flocks were found in all forested parts of the study area in Alberta, chickadee flocks strongly responded to forest boundaries. These boundaries acted as movement conduits and influenced daily movements more than home range locations (Desrochers & Fortin 2000).

Interestingly, wall-following behaviours are also associated with exploration in species that are active under visually-restricted or deprived circumstances (Sharma et al. 2009). In these cases, animals are unable to use long-range senses (e.g. vision) and cannot sense distant resources from a single vantage point. Continuous movement throughout the environment is therefore necessary to allow for short range senses to create spatial knowledge of the landmark features. Examples include cockroaches (Creed & Miller 1990; Jeanson et al. 2003), and crayfish (McMahon et al. 2005) which use mechanoreceptors on the antennae to sense and explore wall surfaces. Also, Sharma et al (2009) demonstrated that Mexican blind cavefish exhibit wall-following behaviour when introduced into a darkened novel environment using their 'touch-at-a-distance' lateral line system for sensing stationary objects. They demonstrated that the sighted morphs of the cavefish exhibited wall-following behaviour in a darkened novel environment but not when light was provided.

1.1.3 Random Walks

In theory there are 'optimal search strategies' and 'rules' of movement whereby an animal might increase the encounter rate with a resource (Heinrich 1979). Since these resources are crucial in determining the fitness of an individual, search strategies should be efficient in terms of energy expended (Viswanathan et al. 1999). When searching, an animal must decide what prey to consider, the best patch choice, when to leave the patch, and the optimal movements between resource areas (Pyke et al. 1977). However, a specific search strategy is not necessarily optimal in every context. There are many interrelated variables such as landscape features, food abundance, predator-prey interactions, and reproduction that are involved in determining an efficient strategy (Ferreira et al. 2012). In other words, certain search strategies may be optimal under specific environmental conditions but not in others, and there may be a range of efficient foraging strategies within a single system.

Successful searching by animals depends largely on the spatial and temporal distribution of resources in the environment. When the environment is unchanging and predictable, movement can be guided by external cues (e.g. vision) and cognitive processing (e.g. memory). For visually-guided search, the simplest form of movement is a straight line to the nearest target site at a particular distance. However in most cases, resource availability and target locations are unknown and searching becomes non-directed (Viswanathan et al. 2002). When there is no target in sight, an optimal forager should choose a random direction and a random distance l_j at which to travel. If a target is not detected along this step distance l_j, the searcher then chooses a new direction and new step distance l_{j+1} from a probability distribution P(l_j) (Reynolds & Rhodes 2009). A Brownian motion-based search, where step-length is fixed and direction is drawn from

a Gaussian distribution (power-law exponent, $\mu \geq 3$), has been found to be an efficient strategy when prey is abundant in the environment (Reynolds & Rhodes 2009). Interestingly, the optimal search pattern in the context of low prey abundance is known as a Lévy-flight, which follows a specialized random walk involving particular combinations of step lengths (Humphries et al. 2012).

More specifically, Lévy flights describe a movement pattern composed of many small-steps interspersed by longer relocations and are drawn from a probability distribution with a power-law tail (a Pareto-Levy distribution) (Humphries et al. 2012). This pattern is observed across all scales, and is characterized by the probability of l_j (a given flight or step length) $P(l_j) \sim l_j^{-\mu}$, with the power-law exponent $1 < \mu \leq 3$. Through experiments and analysis on the Levy-flight model, an exponent of $\mu \approx 2$ has been found to be optimal for scarce or patchy prey distributions. Foraging patterns approaching this optimal value have been identified in many organisms ranging from micro-zooplankton (Bartumeus et al. 2003), bees (Viswanathan et al. 1999), large terrestrial herbivores (Marell et al. 2002), albatrosses (Humphries et al. 2012), sharks (Humphries et al. 2010), and even humans (Brockmann et al. 2006). The Lévy-flight strategy is considered more efficient than other random walks for scarcely distributed prey because the probability of returning to a previously visited site is much smaller than for a strategy where step length follows a Gaussian distribution with directions distributed uniformly (Viswanathan et al. 1999). It is also argued that following a Lévy-flight distribution increases the number of new target sites encountered due to the longer relocation movements (Berkolaiko et al. 1996).

1.2 Weakly electric fish

We find very complex sensory and motor adaptations in fish involving one or a combination of all sensory modalities to assist in spatial orientation, homing, migration, foraging, feeding, and social communication. Representing only a small percentage of all fish species (approximately 1.3%) are species endowed with the ability to generate electricity through specialized electric organs, known as electric fish (Moller 1995). Electric fish have played an important role in the discovery of animal electricity, the analysis of electrosensory systems, and in the study of the molecular biology of neurotransmission (Moller 1995).

1.2.1 Diversity

The electric organs, which are the critical components of the electrosensory system, are thought to have evolved independently six times and four major orders have developed: the Rajiformes, the Mormyriformes, the Siluriformes and the Gymnotiformes (Moller 1995). To distinguish between the orders, it is customary to consider the voltage generated by their electric organs, in other words to differentiate between strongly and weakly electric discharging fish. It is thought today that weakly electric organs evolved first and strong electric organs after independently in cartilaginous and bony fishes (Albert & Crampton 2005). Strongly discharging species rely on their electric sense predominantly as a predatory and defense mechanism, whereas the weakly electric fish rely on their electric sense for exploration, communication, prey localization and capture (Moller 1995).

Siluriformes (catfish) and the Rajiformes (skates and rays) generate strong electric discharges (up to hundreds of volts) and are found in both marine and freshwater systems (Moller 1995).

Mormyriformes and Gymnotiformes are weakly electric fish (discharges in the millivolt range) found in the freshwaters of African and South America respectively (Moller 1995; MacIver et al. 2001). Feeding habits of the Mormyriformes can be divided into two groups: some species are highly piscivorous predators that also feed on shrimp and large aquatic insects and their larvae, and others that are bottom feeders that depend on the abundance of invertebrate food items. The food of Gymnotiforme fish comprises mainly insect larvae, but occasionally feed on smaller weakly electric fish relatives (MacIver et al. 2001).

Weakly electric fish can be further categorized by the type of electric signal that is emitted, pulse-type and wave-type (Moller 1995). Pulse and wave-type species of weakly electric fish are distinguished based on the temporal relation between the EOD and the inter-EOD interval. Pulse-type species have inter-EOD intervals that last much longer than the EOD itself and have discharge frequencies that range from less than 1 Hz to 150 Hz. Wave-type species, on the other hand, have EODs that last about as long as the interval between two consecutive EODs, and rates are as high as 2000 Hz (Turner & Maler 1999).

The EOD is associated with the presence of an electric field, and this combined electrosensory system enables fish to exploit habitats that are not easily accessible to those organisms that predominantly rely on visual cues (Moller 1995). In wave-type weakly electric fish, the EOD creates a quasi-sinusoidal electric field with a fundamental frequency ranging from 200-2000Hz and field strength of approximately 1 mVcm^{-1} near the fish (MacIver et al. 2001). These fish are able to sense the self-generated electric field as well as extrinsic electric fields using two sub modalities of the electrosense, each with separate receptor populations. The high-frequency electrosense which allows the fish to sense fields similar to its own is mediated by tuberous

electroreceptors. On the other hand, the low-frequency electrosense is sensitive to oscillating fields under 40 Hz, and is mediated by ampullary electroreceptors (Nelson & Maciver 1999).

Natural habitat of Gymnotiform fishes

Gymnotiformes make up a substantial portion of the biomass and numbers of fish communities in Amazonian várzeas floodplains, and the seasonally flooded grassland habitats of the Venezuelan Ilanos (Moller 1995). Gymnotiformes occur in most conceivable lowland aquatic habitats of the Amazon including river channels, flooded forests and grasslands, forest streams, deep subterranean caverns, swamps, coastal creeks, and estuarine reaches (Ladich & Myrberg 2006). There are distinct correlations between habitat selection, species diversity and electric signal diversity. Regions can be classified into three paleo environmental Neotropical aquatic systems based on their physical structure: streams composed of terra firme streams and small rivers, floodplains including lakes and flooded forests, and rivers composed of deep, swiftly flowing river channels. The ecological distribution of species with pulse-type EODs is more dependent on water conductivity than wave-type species. Terra firme forest and savannah streams of the equatorial regions of South America host specialized communities of small gymnotiform species that create refuges in submerged vegetation, root mats and leaf litter. Larger species will often hide in holes or caverns in undercut banks (Ladich & Myrberg 2006). In the Tefé region, rainforest streams host a variety of genera including the *Sternopygus* and *Eigenmania*. Most wave-type species present in these streams tend to forage in the most open areas and outside dense mats of leaf litter and root tangles, while pulse-type species forage mostly in dense substrates (Ladich & Myrberg 2006).

The nutrient-rich waters of várzea lakes support growth of dense stands of grasses and free-floating macrophytes. These meadows support very diverse communities of fishes, with weakly electric Gymnotiformes forming a dominant component of the overall biomass (Albert & Crampton 2005). Floating meadows are rich sources of food but are exposed to large temperature fluctuations and prolonged seasonal anoxia. These variables may be decisive factors in excluding most wave-type species from these areas. The wave-type species that are present, *Eigenmannia sp.*, *Sternopygus macrurus*, *Parapteronotus hasemani*, and *Adontosternarchus sp.*, are found in lakes close to the edge of the várzea where there is some ingress of oxygenated water from adjacent rivers (Ladich & Myrberg 2006).

The benthos of large, swiftly flowing river channels of South America host diverse and specialized communities of gymnotiforms with 81% of species endemic only to this ecosystem. Apteronotids such as *Apteronotus leptorhynchus* (brown ghost knife fish) and *Apteronotus albifrons* (black ghost knife fish) dominate the Amazonian whitewater, clearwater, and blackwater rivers. Only a few species of pulse-type are found in the deep river channels, but they can be abundant in terms of numbers and biomass (Moller 1995). The physical aspects of deep river channels have resulted in the evolution of unique morphological and physiological adaptations in the Gymnotiformes. The absence of light at the bottom of deep river channels has led to a number of taxa with highly reduced eyes and pigmentation, and a greater dependence on the electric sense. Elongated snouts with small terminal mouths have also evolved in some species, most likely used to extract larvae and other insects from small burrows in the substrates of river beds (Moller 1995). Surveys with electrodes indicate that most electric fish are found in one of three microhabitats: within or just downstream of submerged structures like fallen trees,

in pockets of slack water or slowly swirling eddies, or in the troughs of sand or mud waves that form the river bed.

Apteronotus leptorhynchus and *Apteronotus albifrons* are two widely-studied species of South-American wave-type weakly electric fish. The two species have different habitat preferences (Moller 1995). Both are found in the turbid freshwater systems of South America. However *A. albifrons* are found in all three of the different ecological zones, and are able to survive and reproduce in streams (terra firme streams, small rivers), floodplains (lakes, flooded forests), and rivers (river channels), whereas *A. leptorhynchus* are found only in river channels. Like *A. leptorhynchus*, the great majority (81%) of Gymnotiforme wave-type species occur in just one of these three ecosystems, with only two species (*Sternopygus macrurus* and *Apteronotus albifrons*) representing 1% of Gymnotiformes occur in all three ecosystems (Albert & Crampton 2005).

Personal observations have suggested that *A. albifrons* are more active and more aggressive than *A. leptorhynchus* in a variety of laboratory-based contexts. *A. albifrons* cannot be housed in tanks along with other conspecifics due to their aggressive behaviours which include biting of the tail and ribbon fin. On the other hand, *A. leptorhynchus* can be housed in small groups (4-6 individuals, which is what has been observed in the wild (Fortune et al. 2003)) with aggressive acts exhibited only occasionally. Further, *A. albifrons* will exit their shelter and readily approach a plastic pipette dispensing food and will begin eating right away, whereas *A. leptorhynchus* remain in their shelters until the food has settled at the bottom of the tank.

1.2.2 Active electrolocation

Some animals, including weakly electric fish, do not have to rely solely on passive external signals in order to successfully gain information about their environments. For example, bats obtain information from their environment by monitoring reflections in their self-generated acoustic pulses through echolocation (Fenton 1999). Similarly, blind cave fish orientate by reflections of self-generated water waves, known as the active flow-sensing lateral line system (Patton et al. 2010). Weakly electric fish, respond to specific modulations of their self-generated signals, known as active electrolocation (Moller 1995; Snyder et al. 2007).

During active electrolocation, perturbations in the self-generated electric field change the patterns of intensity and distribution of electroreceptor stimulation. Objects in the environment can cause modulations in the amplitude and/or phase of the EOD, resulting in 'images' on the fish's body, if their impedances are different from the impedance of the surrounding water (Moller 1995). An interesting characteristic of active sensing is the potential control over certain properties of the signal such as intensity, timing, and direction (Nelson & MacIver 2006). For active sensing organisms, characteristics such as the spatial volume and intensity of the emitted signal influence the volume of space in which objects can be detected. The volume of the sensing range of the electrosensory system in weakly electric fish has a unique omnidirectional shape, and allows for detection of objects from all directions along the length of their entire body.

There are however, limitations to active sensing that can affect its efficiency. Although the volume of the emitted energy spans the length of the body, the sensory range is limited. In *A. albifrons* (black ghost knife fish), small prey (~3-5mm) can be detected at a distance of approximately 30 mm (MacIver et al. 2001). This distance is less than a full body length of the

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animal (typically greater than 10cm) and therefore the animal requires more movement in order to gain information about the environment. Echolocating bats, on the other hand, have a sensing range that spans several meters for small prey such as mosquitoes, and therefore are able to detect more prey objects from a single distance (Nelson & MacIver 2006). Dolphins and whales also use ultrasonic pulses for prey detection, and can detect prey-sized objects at distances on the order to 100 m (Snyder et al. 2007).

1.2.3 Locomotion

In addition to having unique sensory capabilities, *A. leptorhynchus* and *A. albifrons*, two closely related species of wave-type weakly electric fish, have an unusual locomotor system which enables them to hover and swim forwards, backwards, and sideways (Lannoo & Lannoo 1993; Nelson & MacIver 1999). These movements are possible because of undulations of a long ribbon fin that runs most of the length of the ventral body surface (Postlethwaite et al. 2009). When in motion, the trunk of the body remains fairly rigid allowing the fish to easily slice through the water like a knife, hence the name "knife fish". The control of the ribbon fin is independent of the trunk and therefore the fish has great flexibility in its movement and orientation of the body, and consequently its electric organ and electroreceptor array (Nanjappa et al. 2000). This flexibility and ease of movement is thought to provide Gymnotiformes with a large amount of manoeuvrability and is thus very advantageous when searching in complex environments (Nanjappa et al. 2000).

1.3 Thesis objectives

Weakly electric fish are popular model organisms for neurobiological and ethological studies. Many studies focus on providing links between neural processes, motor outputs and behaviour; despite this, there is a lack of work focusing on the proximate and ultimate behaviours of these species.

1.3.1 Specific Objectives

Objective 1

The first objective of my thesis was to characterize the exploratory movements of both species when introduced into a novel environment in which food was *abundant* or *scarce*. I predicted that *A. albifrons* which exhibit more active tendencies in the laboratory than *A. leptorhynchus* (pers. obs.), should exit the shelter more readily, spend more time exploring out in the open, have a higher displacement and spend more time feeding. I also predicted that in a novel environment with no knowledge of resource location and distribution, individuals should exhibit Lévy flight search patterns when food is *scarce*, and should exhibit Brownian motion search patterns when food is *abundant*.

Objective 2

The second objective of my thesis was to document the active wall-following of weakly electric fish when introduced into a novel environment, using two closely related Gymnotiforme species; *A. leptorhynchus* and *A. albifrons*. I tested the hypothesis that wall-following functions as an exploratory behaviour in these species, similar to the function in other organisms which rely on

short-range senses. Thus, when introduced into a novel environment, individuals should use wall-following for the localization of important resources such as food and refuge. I predicted that if the exploratory hypothesis was valid, then wall-following behaviours would be exhibited by active individuals of both species, and less in reactive individuals since activity levels and explorations are often correlated with boldness and aggression in the context of behavioural syndromes. More active individuals are expected to be more aggressive, take more perceived risks and have higher rates if exploration. Conversely, if the predator-avoidance hypothesis was valid, then wall-following behaviours would be exhibited by reactive individuals of both species and less in active individuals.

Chapter 2. Exploratory behaviour in weakly electric fish: *Apteronotus leptorhynchus* and *Apteronotus albifrons*

2.1 Introduction

In a changing environment, it is beneficial for animals to assess the state and variability of resources such as shelter, food, and predators (Tebbich et al. 2009). One way to learn about the environment is through exploratory behaviour. In most cases, behaviours are motivated by direct needs, such as foraging to find food or courtship to find a suitable mate. Exploration, on the other hand, can be performed without a specific need, and used to gain insight into the structural properties of an animal's environment (von Merten & Siemers 2012).

The exploratory behaviour of species and/or populations varies depending on different aspects of their ecology. One example of this is habitat complexity, where investigation of complex and variable environments is more crucial for survival than in more simple, stable environments (von Merten & Siemers 2012). Therefore, complex habitats have been shown to favor high exploration rates (Greenberg 1990). Variation in the behaviours within and between species may also be due to differences in behavioural traits (Sharma et al. 2009). Individual differences in terms of behavioural syndromes can have effects on exploration in a wide variety of groups including fish (Bell 2005a). Behavioural syndromes are often characterized based on particular types of behaviours in different contexts (Sih 2004). Exploration and activity levels have been shown to form a syndrome with aggression and/or boldness (Bell 2005a). Individuals who tend

to be more proactive will take more perceived risks, and show higher levels of aggression than individuals who tend to be more reactive (i.e. lower levels of aggression, lower levels of dispersal in novel environments). Therefore, in novel environments, it is expected that proactive individuals would demonstrate more exploratory behaviours such as spending more time in the open. Consistent behavioural differences between individuals could lead to differences in exploratory behaviour between populations and even species depending on what selection pressures are present (Wray et al. 2011). External factors such as predation pressure and food availability also play a role in shaping the behaviours in a population (Dingemanse et al. 2007). From an evolutionary perspective, populations with consistent behavioural differences may eventually diverge into different species, given that other factors such as reproductive barriers are present (Dingemanse et al. 2007).

Landscape heterogeneities can affect the spatial distribution of organisms during exploration of the environment, in particular affecting their movement patterns (Jeanson et al. 2003). Theoretically, in situations where animals are exploring a novel environment with no information about the whereabouts of resources, a specialized random walk known as a Lévy flight can yield high encounter rates with sparsely and randomly distributed targets. This is in contrast to random walks such as Brownian motion, which are efficient when resources are abundant and more predictable (Humphries et al. 2012). Lévy flights are comprised of random sequences of movement-segments (e.g. flying, swimming) with lengths, l_j drawn from a probability distribution function with a power-law tail, $P(l_j) \sim l_j^{-\mu}$ where μ is the power-law exponent and has a range of $1 < \mu \leq 3$ (Viswanathan et al. 1999). This distribution is said to have a "heavy" tail because frequently occurring but relatively short move lengths are punctuated by

rarely occurring longer lengths (Reynolds & Frye 2007). Viswanathan et al. (1999) demonstrated that $\mu \approx 2$ constitutes an optimal Lévy flight search strategy that minimizes the mean distance travelled and the mean energy expended before encountering a target. Lévy flight is presumed to be optimal if the searcher is engaged in exploration, has no prior knowledge of target locations, and if the mean spacing between successive targets exceeds the searcher' sensing range (Reynolds & Frye 2007). Lévy flights with $\mu \approx 2$ have been found to characterize the movement patterns of a diverse number of species including albatrosses (Humphries et al. 2012), bumblebees (Reynolds et al. 2007), micro-zooplankton (Bartumeus et al. 2003), fish (Sims et al. 2008) and even humans (Brockmann et al. 2006).

In this study, we provide a detailed description of the exploratory behaviours of weakly electric fish in a novel environment. Two species, *Apteronotus leptorhynchus* and *Apteronotus albifrons*, are found in the turbid freshwater systems of South America (Moller 1995). Although these species are found in the same geographical area, *A. albifrons* are adapted to a wider range of aquatic systems. This species is able to tolerate conditions that are more variable than *A. leptorhynchus*, including small streams and river, floodplains, and deep river channels (Albert & Crampton 2005). Along with differences in habitat preference, there are possible differences in behavioural traits. When wild-caught individuals are housed in captivity, *A. leptorhynchus* demonstrate reactive behaviours (pers. obs.): individuals will tend to remain hidden in their refuges rather than swim out in the open; when food is presented, *A. leptorhynchus* do not readily approach until the food has settled at the bottom. On the other hand, *A. albifrons* demonstrate active behaviours: individuals must be housed alone rather than in groups due to high levels of

aggression towards conspecifics; they also actively seek out food in the open and will often approach the plastic pipette used to dispense food.

We analysed exploration of a novel environment in both species in a laboratory setting when food is *abundant* or *scarce*. We predicted *A. albifrons* to exhibit higher levels of activity and different movement patterns than *A. leptorhynchus*, such as more time spent in the open. We also predicted the movements in the open when the food is *abundant* to be characterized by Brownian motion and the movements when the food is *scarce* to be characterized by Lévy flight.

2.2 Materials and Methods

2.2.1 Experimental animals

Wild caught *A. leptorhynchus* (brown ghost knife fish) and *A. albifrons* (black ghost knife fish) were purchased from tropical fish suppliers (AQUAlity, Mississauga, ON; Mirdo, QC). *A. leptorhynchus* individuals ranged from 7 to 16 cm in length and the *A. albifrons* ranged from 6 to 15 cm in length (Table 2.1). *A. leptorhynchus* were placed in house tanks in groups of 4-6 individuals, whereas the *A. albifrons* were housed individually because they are more aggressive towards conspecifics. Tank conditions were maintained at temperatures of 27 to 28 °C and conductivity of approximately 150 µS under 12:12 (dark/light) photoperiod. Fish were held in the house tanks for one week prior to the experimental procedures. Regular feeding regiments consisted of frozen bloodworms given three times a week. Protocols for the maintenance, care, and experimental use of fish followed the guidelines approved by the University of Ottawa Animal Care Committee (BL-229).

2.2.2 Experimental setup

The experimental tank consisted of a circular plastic arena 100cm in diameter. The test arena was filled with water with similar conditions as the home tanks (e.g. water temperature ~27-28 °C, conductivity ~150 µS) to minimize stress factors associated with differences in water properties. The water depth of the test arena was kept shallow (~5 cm) to minimize vertical excursions. A video camera (D-Link DCS 2210) with integrated infrared LED illuminator was mounted approximately 200cm above the arena to record the swimming behaviour. Two additional infrared spotlights were used to aid in illumination of the arena. Properties of the electric organ discharge were not determined, however EOD frequency and length were recorded (Table 2.1).

2.2.3 Behavioural procedures

Following two days of no food, the behaviours of *A. leptorhynchus* and *A. albifrons* were observed when introduced into a novel arena in a dark environment during the dark period of the photoperiod. Using a plastic-lined mesh net, fish were transferred from their home tanks into the centre of a small refuge (10cm x 10cm square) located inside the test arena. After a 10 minute acclimation period, the door to the refuge was opened, allowing individuals to exit and move throughout the test arena for 20 minutes. The trial consisted of either *abundant* food conditions (15 prey items placed randomly throughout the test arena) or *scarce* food conditions (3 prey items). Each prey item consisted of approximately 10 bloodworms placed at the bottom of the tank. For both the *abundant* and *scarce* food conditions, one of the prey items was placed just outside of the refuge. After the behavioural observations, fish were then transferred into tanks with mesh tank dividers until their next experiment in order to properly identify experimental

individuals over non-experimental fish. During the trial, individuals were allowed access to the refuge at all times to minimize stress due to classic open field tests (Archer 1973), and thus increase the likelihood of natural exploratory strategies.

2.2.4 Analysis of movements

Video capture software (D-link DCS 2210, 25fps) was used to record the 20 minute behavioural trials. Head positions of the fish were tracked manually using Videopoint software (Lenox, MA, US), at a resolution of 5fps (200ms). Custom MATLAB scripts were used to extract various behavioural parameters (listed below).

The test arena was divided into four zones of interest (Figure 2.1): (1) 'refuge zone', defined as a 10cm by 10cm square around the refuge; (2) 'feeding zone', defined as a 5cm radius around any food spot; (3) 'wall-following zone', defined as a distance of 12cm from any wall, including the outer wall of the tank and the walls of the refuge and (4) 'open zone', defined as any space not within the 12cm of a wall and includes the feeding zone. Using custom Matlab scripts, we calculated the following parameters:

- (1) total displacement
- (2) mean speed
- (3) total fractions of tank visited
- (4) proportion of exploration time spent wall-following
- (5) time spent in the open
- (6) latency to leave the shelter for the first time
- (7) total feeding time

- (8) total number of feeding events

Swimming speed was expressed as body length (BL) per second in order to take into account differences in body size. Latency to exit the shelter was defined when an individual was a full body length outside of the refuge coordinated for a minimum of 1s. The time and body length requirements were to prevent mistaking latency to exit the shelter with in and out scanning motions that are typical of these two species. Feeding was defined as being within the 5cm radius of a food spot within a minimum speed of 0.05 BL/s. Feeding events were defined as when an individual entered the radius and met the speed criteria and then ended when the individual exited the radius or was above the speed criteria. An individual was said to be in the open if it was outside of the 12cm distance from a wall for a minimum of 0.5s. This was to reduce noise associated with very small excursions between two close wall-following events. The minimum value for speed of 0.05 BL/s was chosen for feeding events as a conservative value based on the overall mean speed during for the trials.

To evaluate movement strategy and determine if Lévy flight or Brownian motion strategies were present, distributions of open time displacements were constructed. Open times were used for the calculations based on the assumption that movements around the periphery of the tank (i.e. wall-following) represented linear displacements with a constant probability to leave the wall per unit time. Open displacements were calculated using the criteria that an individual must not be within the 12cm distance from a wall for a minimum of 0.5s. From these distributions, maximum likelihood estimates of the power law exponent were used in order to test for Lévy-flight patterns, following the equation Eq 1 (Edwards 2008):

$$\hat{\mu} = 1 - \frac{n}{n\log a - \sum_{j=1}^{n}\log x_j} \tag{1}$$

where μ represents the power-law exponent, x_i are the step lengths, n is the number of steps, and a is the start of the tail of the step length distribution. These parameters were compared between species and food conditions (both within and between species) to test the exploratory behaviour in a novel environment.

2.2.5 Statistical analysis

We tested to see if there were differences in exploratory behaviour between the two species as well as food conditions. As data for some parameters were not normally distributed or showed equal variances, nonparametric Mann-Whitney tests were used throughout. Statistical tests were performed using Graphpad Prism® software. A parametric two-way ANOVA was performed to test statistical differences and interactions between species as well as mean power-law exponents extracted from the distributions.

2.3 Results

2.3.1 Food abundance

Apteronotus albifrons spent more time feeding and had a higher number of feeding events; however, there were no significant differences in majority of behaviours related to food abundance for either species. The amount of food present in the novel environment did not impact the behaviours. For example, the mean proportion of the test arena visited by *A. albifrons* for the *abundant* condition was 0.24±0.03 and 0.26±0.05 for the *scarce* condition (Mann-Whitney, P=0.8629). The mean amount of time spent out in the open in *A. albifrons* for the
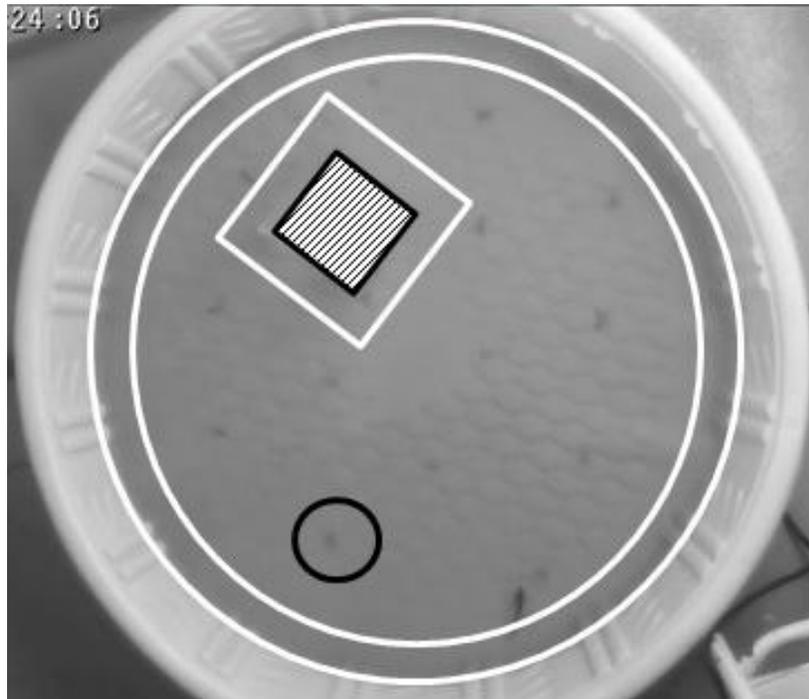
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Figure 2.1 Schematic representation of the test arena (diameter 100cm) with the shelter zone (black outline with striped interior), the feeding zone (black circle), the wall-following zone (white outline around periphery of tank and around refuge), and the open zone (the rest of the arena including the feeding zone).

Table 2.1 Summary of individual *A. leptorhynchus* and *A. albifrons* (n=36) with respective EOD (Hz) and length (cm) recorded. Sex was not determined due to the variation in the degree of sexual dimorphism of the electric communication signals. The magnitude and direction of the sex differences in behaviour vary across closely-related species and therefore make it difficult to confidently identify not post mortem (Zhou & Smith 2006).

Species	EOD (Hz)	Length (cm)
A. leptorhynchus 1	889	9.3
A. leptorhynchus 2	894	9.05
A. leptorhynchus 3	754	11.2
A. leptorhynchus 4	875	9.1
A. leptorhynchus 5	795	10.5
A. leptorhynchus 6	826	7.8
A. leptorhynchus 7	896	14.9
A. leptorhynchus 8	764	11.4
A. leptorhynchus 9	725	8.75
A. leptorhynchus 10	891	10.3
A. leptorhynchus 11	912	10.7
A. leptorhynchus 12	761	11.6
A. leptorhynchus 13	870	9.8
A. leptorhynchus 14	843	15.75
A. leptorhynchus 15	791	12.1
A. leptorhynchus 16	825	8.4
A. leptorhynchus 17	763	7.46
A. leptorhynchus 18	901	10.3
A. albifrons 1	943	14
A. albifrons 2	898	14.9
A. albifrons 3	1036	14
A. albifrons 4	1209	15.8
A. albifrons 5	962	7.5
A. albifrons 6	886	6.5
A. albifrons 7	799	6.07
A. albifrons 8	986	6.59
A. albifrons 9	1070	7.88
A. albifrons 10	896	9
A. albifrons 11	978	6.58

A. albifrons 12	870	8.75
A. albifrons 13	812	7.45
A. albifrons 14	799	9.5
A. albifrons 15	795	7.5
A. albifrons 16	1170	10
A. albifrons 17	825	8.2
A. albifrons 18	1125	9.7

abundant and *scarce* conditions were not significantly different from *A. leptorhynchus* (Mann-Whitney, P=0.6242) (Figure 2.2a, b). The total amount of time spent feeding in *A. albifrons* (Mann-Whitney, P=0.3347) (Figure 2.2c), was not significantly different than in *A. leptorhynchus* (Mann-Whitney, P=0.9758) (Figure 2.2d).

2.3.2 Overall movement on first day

Food conditions were combined in this section because no significant differences were reported between *abundant* and *scarce* conditions (see section 2.3.1 for details). The mean total displacement of *A. leptorhynchus* was 166.78±40.18m (mean ±SD) and was significantly higher than that of *A. albifrons* with a displacement of 90.07±20.12m (Mann-Whitney, P<0.0001) (Figure 2.3a). The mean speed in *A. albifrons* was 0.89±0.29 BL/s and was also significantly higher than that of *A. leptorhynchus* at 1.36±0.4 BL/s (Mann-Whitney, P=0.0002). There was no significant difference between species in the proportion of the test arena visited (Mann-Whitney, P=0.0731) or in the amount of time spent in the open (Mann-Whitney, P=0.1201). Although not significant, there is a slight trend suggesting that *A. albifrons* do spend more time in the open than *A. leptorhynchus* (218.01±83.59s vs. 177.4±56.36s respectively) (Figure 2.3c). *A. leptorhynchus* spent significantly more time wall-following the outer periphery and refuge than *A. albifrons* (Mann-Whitney, P<0.0001) (Figure 2.3b). There was no significant difference between species in the latency to exit the shelter at the beginning of the trial (Mann-Whitney, P=0.2231), however once in the open *A. albifrons* spent significantly more time feeding (115.65±62.01s vs. 29.78±16.25s respectively) (Figure 2.3d) than *A. leptorhynchus* (Mann-Whitney, P<0.0001) and had a higher number of feeding events (Mann-Whitney, P<0.0001).

Characterization of Lévy flight and Brownian motion

The general patterns of exploratory movements of the two species were different, with *A. albifrons* spending slightly more time in the open (although not significant) and more time feeding than *A. leptorhynchus*. On the other hand, *A. leptorhynchus* had a greater displacement throughout the test arena and a higher preference for active wall-following. In order to describe the patterns of searching strategies further, we tested whether Lévy flight search patterns were present in the extrinsic exploratory behaviour of *A. albifrons* and *A. leptorhynchus* when food was *abundant* and if Brownian motion search patterns were present when food was *scarce* while moving in the open. We calculated movement steps as the distance between consecutive excursions into the open area of the tank greater than 5s for each individual *A. albifrons* and *A. leptorhynchus* , known just as excursions from here on, and calculated whether the Pareto-Lévy or Brownian distributions best fit the observed data.

We chose to use excursions rather than traditional movement steps which use turning angles as their definition because of the unique swimming patterns in these species. The ribbon fin allows for smooth transitions between directions. This along with the unique ability to swim backwards and sideways made characterizing movement steps using turning angles very difficult. Therefore we chose a method similar to that of Humphries et al (2012), which used distances between consecutive landings on the sea surface for individual albatrosses as the movement steps. Using Maximum Likelihood Estimation (MLE), we found support for individual excursions approximating Lévy flight (power-law) search patterns in both *A. albifrons* and *A. leptorhynchus* regardless of the food condition (Figure 2.4) during the trial.
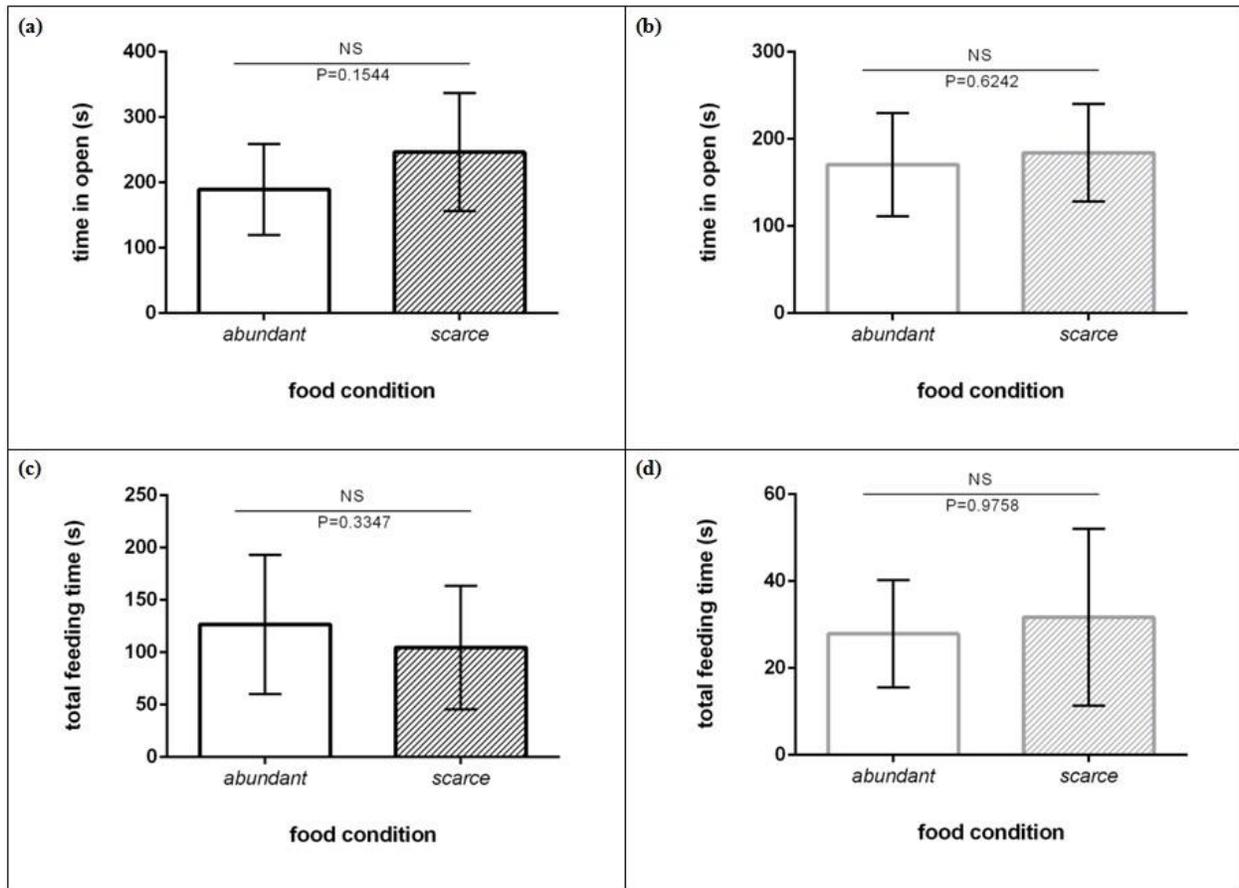
Figure 2.2 Bar graphs representing time in open (a, b) and total feeding time (c, d) as a function of food condition in *A. albifrons* (left column) and *A. leptorhynchus* (right column). Mean±SD presented with P-values above bars, n=18 for each food condition for each species
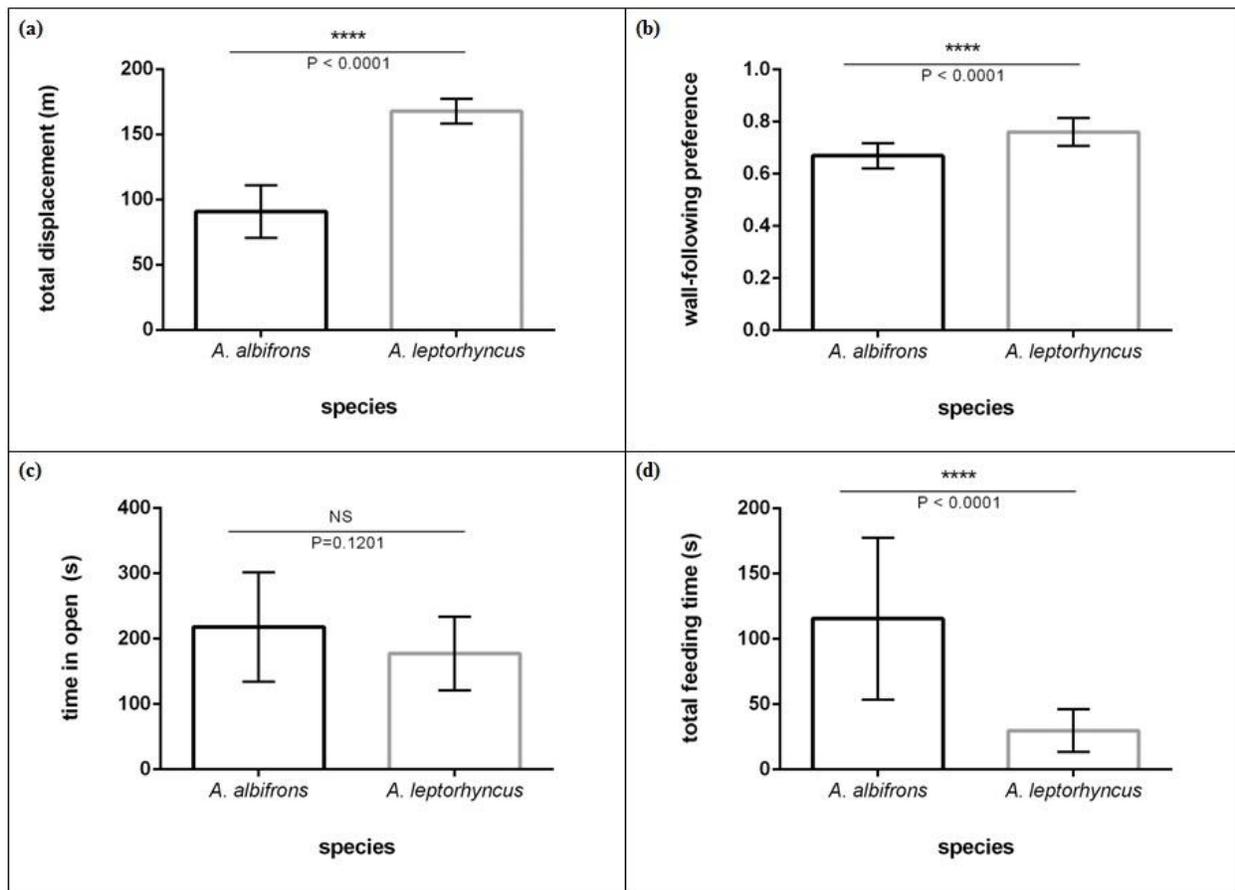
Figure 2.3 Bar graphs representing total displacement (a), wall-following preference (b), total time in open (c), and total feeding time (d) in *A. albifrons* (black bars) and *A. leptorhynchus* (grey bars) during the trial (20 min). Mean±SD presented with P-values over bars, n=18 for each species.

The μ values of truncated power-law fits were within range of values consistent with the hypothesized $1 < \mu \leq 3$. We calculated mean exponent values of 1.54 ± 0.076 and 1.59 ± 0.072 for *A. albifrons* and *A. leptorhynchus*, respectively in *abundant* food conditions, and 1.49 ± 0.088 and 1.60 ± 0.075 respectively in *scarce* food conditions; therefore no effect of food condition was found in the strategies performed by either species. However, there is a significant difference in the mean exponent during the trial in the *scarce* food condition between species (two-way ANOVA, $P < 0.05$) (Figure 2.4).

2.4 Discussion

2.4.1 Overall movements

Overall movements during the trial were characterized in *A. leptorhynchus* and *A. albifrons*. Although no significant difference was found between species in the amount of time taken to start exploration, *A. albifrons* had a lower mean speed and spent slightly more time in the open than in the open than *A. leptorhynchus*.

When exploring the open zone, *A. albifrons* also had a higher number of feeding events and a larger total feeding time. This is demonstrating that although *A. albifrons* moved slower while exploring the environment, a thorough examination rather than a superficial examination was performed. However, *A. leptorhynchus* had a greater total displacement and no significant difference from *A. albifrons* in the latency to exit the shelter at the start of the trial. *A. albifrons* demonstrated less of a preference for wall-following than *A. leptorhynchus*, and had no significant difference in the total fractions of the tank visited. It was expected *A. albifrons* would demonstrate higher levels of activity throughout the test arena, including both a higher total
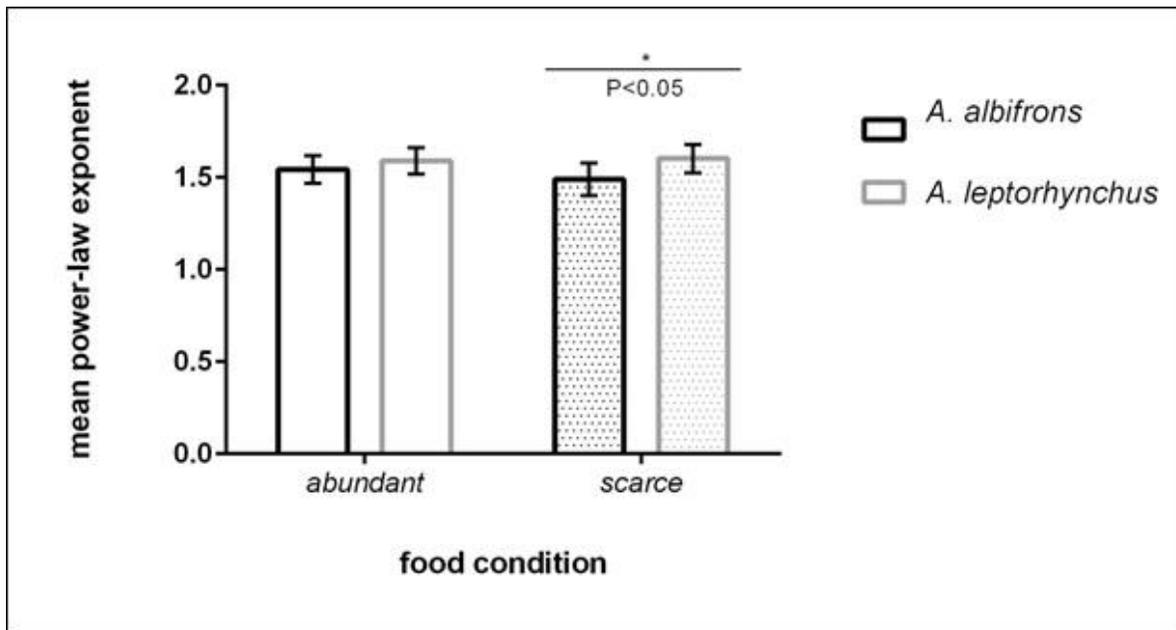
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Figure 2.4 Bar graph representing mean power-law exponent as a function of food condition in *A. albifrons* (black bars) and *A. leptorhynchus* (grey bars). Mean±SD are presented with P-value on top of the bar, n=18 for each species in each food condition.

displacement throughout the tank and a higher preference for wall-following and that *A. leptorhynchus* would have a slower speed than *A. albifrons* while exploring.

This study showed that species differences were in fact present in exploratory behaviours. The results cannot strongly suggest that *A. albifrons* are more active than *A. leptorhynchus*. It is possible that *A. albifrons* assess a higher risk of predation by moving at a slower speed (von Merten & Siemers 2012); however this is unlikely due to the greater time spent in the open and total feeding time. Although *A. leptorhynchus* had a higher number of excursions into the open, the speed was much higher than *A. albifrons* suggesting a stronger predatory response to exit the open zone. Differences in behavioural traits may also be due to habitat. Greenberg (1990) showed that sparrows from similar species living in different habitats showed consistently different latencies for feeding while exploring novel objects. The song sparrow, *Melospiza melodia*, is a habitat generalist and a good colonizing species, while the swamp sparrow, *M. georgiana*, specializes on marsh habitats. Experimental results suggested that inhibition to feed at objects was greater in swamp then song sparrows consistently over a variety of objects, suggesting that swamp sparrows respond differently during extrinsic exploration to novel stimuli than do song sparrows. In terms of feeding, *A. albifrons* live in many types of environments and could potentially adapt to feed on many small organisms. *A. leptorhynchus*, however, specialize in a small geographical area and are less likely to feed often if in a novel environment where the food is unknown.

The lower displacement observed in *A. albifrons* could be due to the total time spent feeding, which would require individuals to remain relatively stationary. Spending more time stationary would result in a lower displacement throughout the test arena. We expect however that if *A.*

albifrons were given a larger amount of time to explore the tank that a better representation of their behaviours would follow. It was also not expected that *A. albifrons* have a lower preference for wall-following. This is interesting to note because wall-following is thought to be involved in exploration in animals, especially in situations in which only short-range senses are available (Sharma et al. 2009). It is therefore expected that *A. albifrons* should demonstrate a higher preference for wall-following behaviour since more active individuals are said to have higher levels of exploration. Further investigation of the behavioural relevance of wall-following behaviour is necessary to answer this question. More active individuals are also expected to exit the shelter and initiate exploration faster than reactive individuals. In our study, there was no significant difference in the mean amount of time taken to exit between species. In the data, however, there was a large amount of inter-individual variation in the latencies. For example, there was an individual *A. leptorhynchus* who exited the refuge as soon as the door was lifted, and one individual who didn't exit the refuge for 127s. There were less inter-individual differences in *A. albifrons* with the minimum latency at 0.48s and the maximum at 60s.

2.4.2 Food abundance

This study shows that no differences were observed between the two different food conditions in any of the parameters measured. Both *A. leptorhynchus* and *A. albifrons* were consistent whether there were 3 prey items or 15 prey items. The movement of both species suggested a Lévy flight strategy in both the *abundant* and *scarce* conditions. One possible reason for the lack of difference between food conditions could be due to the limitations in the sensory capabilities of the two species. In the experimental set up, the mean distance between each of the prey items in the abundant trials was approximately 10cm. Therefore it is assumed that with an average body

length of 10cm that an individual in either species would be within detection range of food regardless of the direction of travel. However, the sensory ranges of these species are quite low (approximately half a body length (Snyder et al. 2007)) and it is possible that the *abundant* condition was not sufficiently abundant to result in different behaviours. Possible future experiments in this area could be to saturate the experimental test arena with food in order to pull out any small differences in the movements. Another explanation for the lack of differences observed could be that Lévy flight movement patterns are used as a 'default' strategy in organisms that rely on short-range senses. In these organisms that cannot sense long distance, it is possible that all novel environments are scarce and Lévy flight patterns are the most efficient strategy to find resources with minimal energy expenditure. Further experiments in this area could be to alter the sensing range in both species by changing the conductivity of the water. A study by Snyder et al in 2007 demonstrated that the detection distance in *A. albifrons* increased as the conductivity decreased. If the conductivity were to decrease in the *abundant* food condition, it would be expected that individuals would have a better detection rate and the movement patterns would demonstrate Brownian motion.

2.3 Conclusions

In the present study, we characterized differences in the exploratory behaviours exhibited by *A. albifrons* and *A. leptorhynchus.* However, firm conclusions on the proximate causes of these differences cannot be made. *A. albifrons* demonstrated some behaviours that may be considered more active, however some of the levels of activity were not as expected. Longer exploratory trials should be performed in order to pull out more differences. Also, an individual characterization of boldness and aggression in both species would be useful in making more

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concrete conclusions on behavioural traits. This study also showed that extrinsic motivation in the form of food abundance did not influence the exploratory behaviours in either species, possibly due to their limitations in sensing capabilities. It will be interesting to test this further in future studies. More research could also be done to see how changes in environmental complexity elicit changes in exploratory behaviours in these species. Future experiments could be performed by the addition of different sized objects to the test arena on different days and examining similar exploratory behavioural parameters.

Chapter 3. Wall-following behaviours in weakly electric fish: *Apteronotus leptorhynchus* and *Apteronotus albifrons*

3.1 Introduction

Wall-following behaviours represent an unconditioned preference for maintaining contact with a wall or boundary of an environment. These behaviours are often prominent when an animal encounters a novel environment (Sharma et al. 2009). Wall-following behaviour is observed in a diverse array of species, including cockroaches (Jeanson et al. 2003; Daltorio et al. 2013), rodents (Simon et al. 1994), and blind cavefish (Sharma et al. 2009) both in the wild and under laboratory conditions. Despite the large number of taxa in which wall-following behaviours are observed, the functions of these behaviours remain somewhat unclear (Patton et al. 2010).

One possible function of wall-following behaviours could be to serve as a protective function in unfamiliar environments when searching for food, shelter, or escape routes (Sharma et al. 2009). A study by Kallai et al (2007) looked at the cognitive and emotional aspects of human thigmotaxis (a wall-following spatial strategy) during exploration of virtual and physical spaces. Participants were assessed with learning-memory tasks and with fear and anxiety questionnaires. Their results demonstrated a positive correlation between thigmotaxis and general phobic avoidance of public places (Kallai et al. 2007). Furthermore, participants who underperformed in the memory tests and in spatial construction tasks exhibited greater thigmotaxis and a higher potential for fear response. Rodents have also shown experimentally to wall-follow as a fear-

response. Anti-anxiety drugs reduced the proclivity of the animals to stay near the walls and increased the amount of time spent in the center of the test arena (Simon et al. 1994). For this reason, wall-seeking behaviours have been described as 'centrophobic' and serve as 'anxiety-relieving' function (Sharma et al. 2009).

Wall-following behaviours could also serve as an exploratory function in many species. These behaviours have the potential to serve as exploratory strategies especially in animals whose spatially-acute senses are short ranged (Patton et al. 2010). For example, wall-following behaviours are associated with species that are under visually-deprived conditions such as the Mexican blind cavefish (*Astyanax sp.*). In the absence of vision, these cavefish are unable to sense distant landmarks and therefore must move around their environment and bring their short-range senses (lateral line) within close range of environmental features (Sharma et al. 2009). Cockroaches (*Blattella germanica*) also exhibit wall-following behaviours when introduced into a novel environment. While walking close to the edge of an arena, a cockroach holds its antennae forward in a slightly lateral position and maintains its relative position to the wall with the mechanoreceptors associated with the antennae (Jeanson et al. 2003).

A third hypothesis related to the function of wall-following is that it is simply an artifact of confining animals in relatively small concave enclosure. Creed and Miller (1990) hypothesized this obstacle-avoidance rule: move in a straight line until you hit an obstacle, and then turn just enough to avoid it. This obstacle-avoidance hypothesis of wall-following predicts that the behaviour is a consequence of forward motion being blocked rather than as a tool used to gather knowledge through exploration of a new environment (Creed & Miller 1990).

As stated in Chapter 2, both *A. leptorhyncus* and *A. albifrons* demonstrate active wall-following behaviour when introduced into a novel environment. These species of weakly electric fish rely almost exclusively on their active electrosense. In *A. leptorhynchus* and *A. albifrons*, the sensory volume of the self-generated electric fish has a unique omnidirectional shape (Snyder et al. 2007). Despite the advantage of being able to detect objects in all directions, the distance at which prey can be detected is approximately only half a body length (MacIver et al. 2001). Therefore knowledge of the spatial organization of the environment for these species with a short-range electrosense can only be achieved through sequential sampling of the space, and temporally linking information across moments in time (MacIver et al. 2001).

In this study we document the active wall-following of *A. leptorhynchus* and *A. albifrons* when introduced into a novel environment. We describe species differences in the wall-following behaviours between species. We test the exploratory hypothesis of wall-following function, a strategy used to bring the short-ranged sense of weakly electric fish within range of the new environmental boundaries. We suggest that if the exploratory hypothesis is valid, then wall-following behaviours should be exhibited by active individuals of both species, and less in reactive individuals since activity levels and explorations are often correlated with boldness and aggression in the context of behavioural syndromes. More active individuals are expected to be more aggressive, take more perceived risks and have higher rates if exploration. Conversely, if the predator-avoidance hypothesis is valid, then wall-following behaviours should be exhibited by reactive individuals of both species and less in active individuals.

3.2 Materials and Methods

Please note that experimental animals and experimental set-up methods used for this study are similar to those stated in Chapter 2.

3.2.1 Experimental animals

Briefly, wild caught *Apteronotus leptorhynchus* (brown ghost knife fish, n=18) and *Apteronotus albifrons* (blacks ghost knife fish, n=18) were purchased from tropical fish suppliers (AQUAlity, Mississauga, ON; Mirdo, QC). *A. leptorhynchus* were placed in house tanks in mixed sex groups of 4-6 individuals, whereas the *A. albifrons* were housed individually because they are more aggressive towards conspecifics. Tank conditions were maintained at temperatures of 27 to 28 °C and conductivity of approximately 150 µS under 12:12 (dark/light) photoperiod. Protocols for the maintenance, care, and experimental use of fish followed the guidelines approved by the University of Ottawa Animal Care Committee (BL-229).

3.2.2 Experimental setup

The experimental tank consisted of a circular plastic arena 100cm in diameter. A video camera (D-Link DCS 2210) with integrated infrared LED illuminator was mounted approximately 200cm above the arena to record the swimming behaviour. Two additional infrared spotlights were used to aid in illumination of the arena.

3.2.3 Behavioural procedures

The behaviours of *A. leptorhynchus* and *A. albifrons* were observed following the introduction into a novel arena in a dark environment during the dark period of the photoperiod with either

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abundant or *scarce* food conditions. After a 10 minute acclimation period, the door to the refuge was opened, allowing individuals to exit and move freely throughout the test area for 20 minutes. Trial 2 occurred two days later, and the individuals were given the same food conditions as trial 1 with a slight change in placement of prey items. Trial 3 occurred two days after trial 2 and consisted of an empty test arena. Food was eliminated in Trial 3 to determine if prior experiences influence exploratory behaviour across days. A schematic outline of the trials is presented in Figure 3.1.

3.2.4 Analysis of movements

Video capture software (D-link DCS 2210, 25fps) was used to record the 20 minute behavioural trials. Head position of the fish was tracked manually using Videopoint software (Lenox, MA, US), at a resolution of 5fps (200ms). Custom MATLAB scripts were used to extract various behavioural parameters. The test arena was divided into two zones of interest: (1) 'wall-following zone', and (2) 'open zone'. The following parameters were analyzed for each trial:

- (1) wall-following preference
- (2) number of wall-following events
- (3) total displacement
- (4) mean speed of wall-following
- (5) mean speed of movement in the open
- (6) number of excursions into the open
- (7) preference for being frozen near a wall
- (8) time spent in refuge

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Wall following behaviour was designated as when an individual travelled within 12cm of either the peripheral wall or the outside walls of the refuge with a minimum speed of 0.05 BL/s. Swimming speed was expressed as body length (BL) per second in order to take into account differences in body size. A wall-following event started when an individual entered the minimum wall-following distance with the speed requirement and terminated when an individual either went below the minimum speed or left the designated wall-following area. The time and body length requirements were to prevent mistaking latency to exit the shelter with in and out scanning motions that are typical of these two species An individual had to be a full body length outside of the shelter for a minimum of 1s in order to considered to have left the shelter. An individual was said to be in the open if it was outside of the 12cm distance from a wall for a minimum of 0.5s.

2.2.5 Statistical analysis

We tested to see if there were differences in exploratory behaviour between the two species as well as between food conditions. As data for some parameters were not normally distributed and did not show equal variances, nonparametric Mann-Whitney tests were used throughout. In order to test for differences across the three days, nonparametric one-way Friedman tests with repeated measures were used along with Dunn's multiple comparisons. These statistical tests were performed using Graphpad Prism® software. Parameters of activity (total displacement, latency to exit shelter, and speed in open) were combined in order to get an overall measure for each individual on the active-reactive continuum. To reduce the dimensionality in this way without bias, principal component analyses (PCA) were performed on the data sets (Bell 2005b). Composite scores were generated from the first principal component. Therefore, an
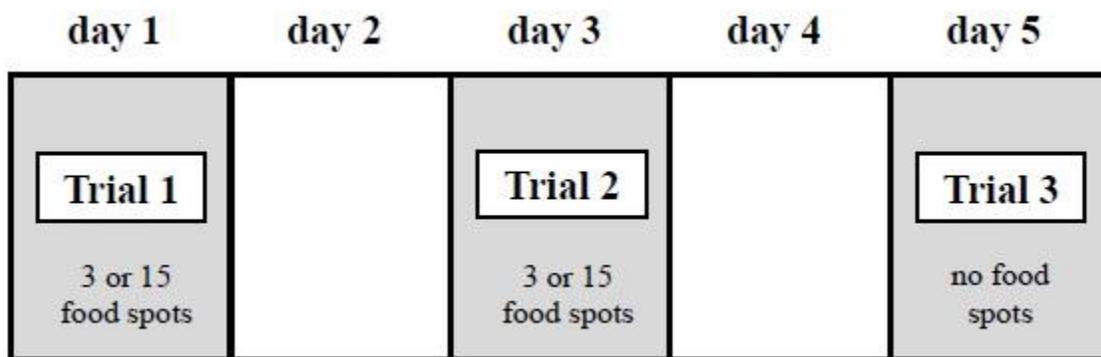
Figure 3.1 Schematic representation of the experimental design to assess exploratory behaviour in *A. leptorhynchus* and *A. albifrons*. Grey boxes represent days where experiments are performed. Three food spots represent *scarce* food conditions, while 15 food spots represent *abundant* food conditions.

initial data set with three behavioural parameters was reduced into one variable, the new principal component score. Linear regressions were then performed on the new scores against different independent variables (e.g. number of wall following events) to determine relationships between wall-following behaviours, exploratory behaviours, and predatory behaviours.

3.3 Results

All individuals from both *A. leptorhynchus* (n=18) and *A. albifrons* (n=18) exhibited wall-following behaviours when placed in a dark, novel environment. The proportion of time spent near the wall of the perimeter of the tank and refuge was greater than elsewhere in the tank. Results from *A. leptorhynchus* and *A. albifrons* are reported in terms of (1) movement patterns, i.e. spatial and temporal distributions within the test arena, (2) behavioural relevance of active wall-following, and (3) the range of variability in the movements between species across days.

3.3.1 Movement on day 1

All individuals from both species showed evidence of active wall-following around the perimeter and refuge during trial 1 (Figure 3.2). As a result, the mean wall-following preference in *A. leptorhynchus* was 0.76±0.05 (mean±SD) and 0.67±0.05 in *A. albifrons*. A fish with no preference for the wall or for any other area of the test area should exhibit a uniform distribution of wall distances when plotted. Distributions of radial distance from the center for both *A. leptorhynchus* and *A. albifrons* showed a higher frequency of near-wall distances than would be expected from a uniform distribution with no distance preference (Figure 3.3). *A. leptorhynchus* had a significantly higher displacement throughout the test area (Mann-Whitney, P<0.0001) (Figure 3.4a) and spent more time wall-following the outer and refuge walls than *A. albifrons*

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(Mann-Whitney, P<0.0001). *A. albifrons* however, exhibited a greater number of wall-following events than *A. leptorhynchus* (239.5±47.35 and 173.9±26.72 respectively) (Mann-Whitney, P<0.0001) (Figure 3.4b). The mean speed in which *A. leptorhynchus* travelled while wall-following was 1.40±0.38 BL/s, whereas the mean speed of *A. albifrons* was 0.97±0.3 BL/s (Mann-Whitney, P=0.0004) (Figure 3.4c). *A. leptorhynchus* travelled significantly faster when out in the open than *A. albifrons* (Mann-Whitney, P=0.0010) (Figure 3.4d).

Although *A. leptorhynchus* spent more time following the walls when moving throughout the test area*, A. albifrons* had significantly higher preference for being stationary near a wall (i.e. speed <0.05 BL/s) (Mann-Whitney, P<0.0001). These differences in behaviours could be related to different exploratory strategies. This means that *A. albifrons* had a higher number of times in which individuals were located close to the walls of the tank but not moving. *A. albifrons* also spent more time in the refuge during trial 1 than *A. leptorhynchus* although not significant with 97.85±71.21s and 90.57±54.94s, respectively (Mann-Whitney, P=0.9443).

3.3.2 Function of wall-following

Behavioural relevance of wall-following parameters was measured in both species combined for a total of 36 individuals. Three parameters typically used to quantify activity (latency to exit the shelter, speed in open, and total displacement) were combined using principal component analysis (PCA) (Sih et al. 2004) to provide a single score for each fish on a active-reactive continuum. Linear regressions were then performed on the scores against the data for preference for wall-following, which demonstrated that proportion of time spent wall-following significantly increased with activity (R^2=3626, P=0.0001) (Figure 3.5a).
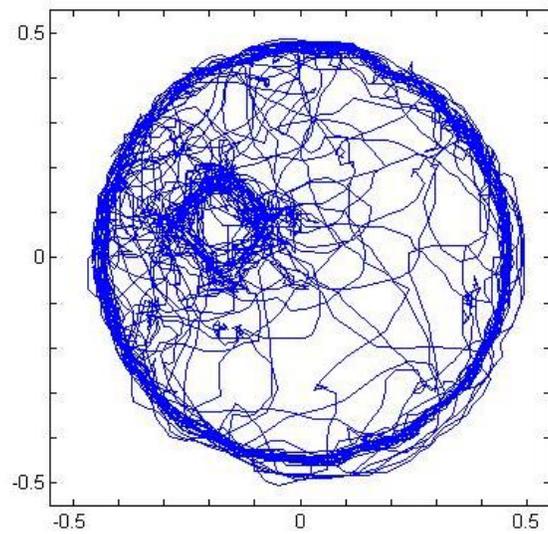
Figure 3.2 Actual swimming tracks of an individual *A. leptorhynchus* during a 20 min trial in a darkened novel environment.
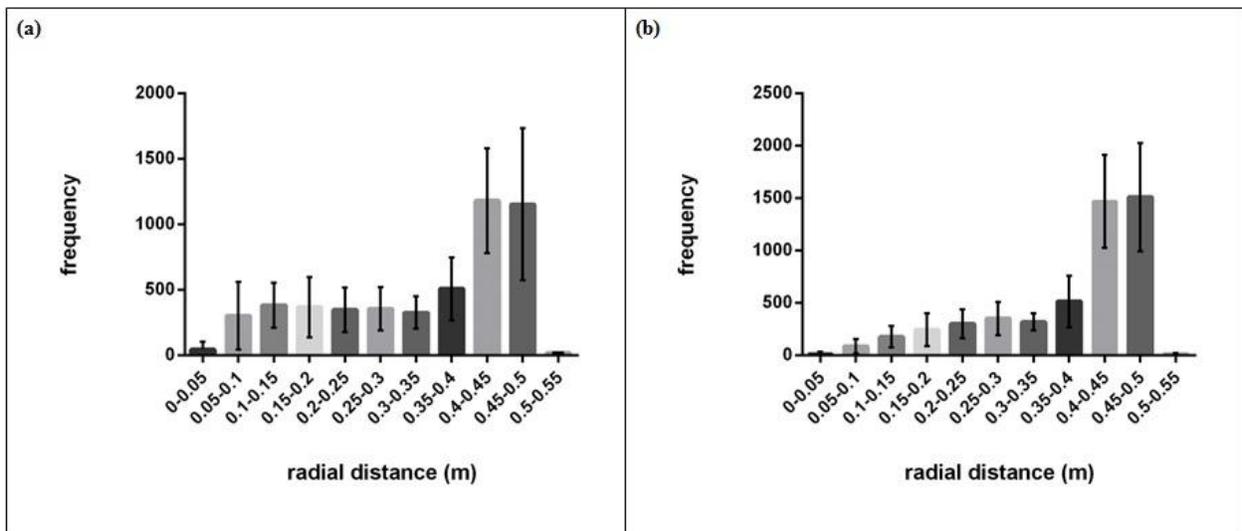
Figure 3.3 Frequency distributions of radial distances (m) from the center of the tank (0.5m radius) in (a) *A. albifrons* and (b) *A. leptorhynchus*. Means±SD are presented, n=18 for each species.
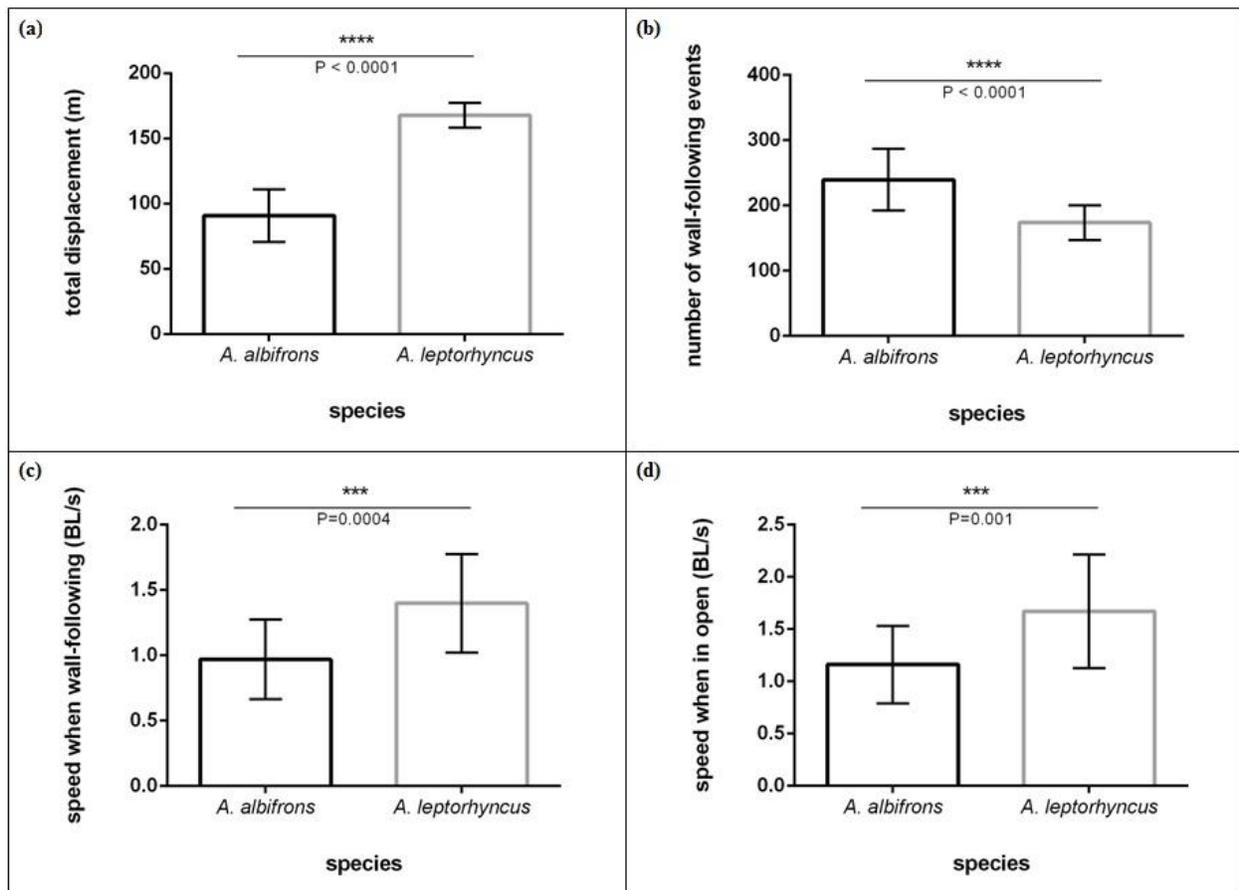
Figure 3.4 Bar graphs representing total displacement (a), number of wall-following events (b), speed when wall-following (c), and speed while in the open (d) in *A. albifrons* (black bars) and *A. leptorhynchus* (grey bars) during trial 1 (20 min). Mean±SD presented with P-values over bars, n=18 for each species.

The number of wall-following events parameter was also analyzed in a separate regression, with new scores provided by the PCA by substituting speed in open with time in open to avoid biasing the results. Looking at the relationship between the PCA score and the wall-following behaviours, the results demonstrate that the number of wall-following events increases with activity (R^2=0.1976, P=0.0066) (Figure 3.5b).

Distributions of the mean-power law exponents of the wall-following movements were then analyzed using movement data from when individuals were in the wall-following zone using the Maximum Likelihood Estimation method similar to that stated in Chapter 2. Using the same scores provided by PCA for the 'preference for wall-following', a linear regression was performed on the power-law exponents and active-reactive spectrum. The results demonstrate that the power-law exponents remain consistent as activity increased (R^2= 00.59, P=0.6550) (Figure 3.5c). Using the scores for the number of wall-following events, the power-law exponents again remain consistent as activity increased (R^2=0.0331, P=0.2880) (Figure 3.5d). Interestingly, the mean power-law exponents were within a similar range to those stated in Chapter 2, which were calculated using excursions into the open rather than movement around the periphery and refuge walls (1.41±0.068 in wall-following movements and 1.55±0.086 in excursions to open).

3.3.3 Movement across days

To determine if differences existed in the behaviours, both species were tested across three trials in both food conditions. *A. leptorhynchus* appear to exhibit a lot of variability in their exploratory behaviours across days, whereas the movements of *A. albifrons* across days suggest consistency
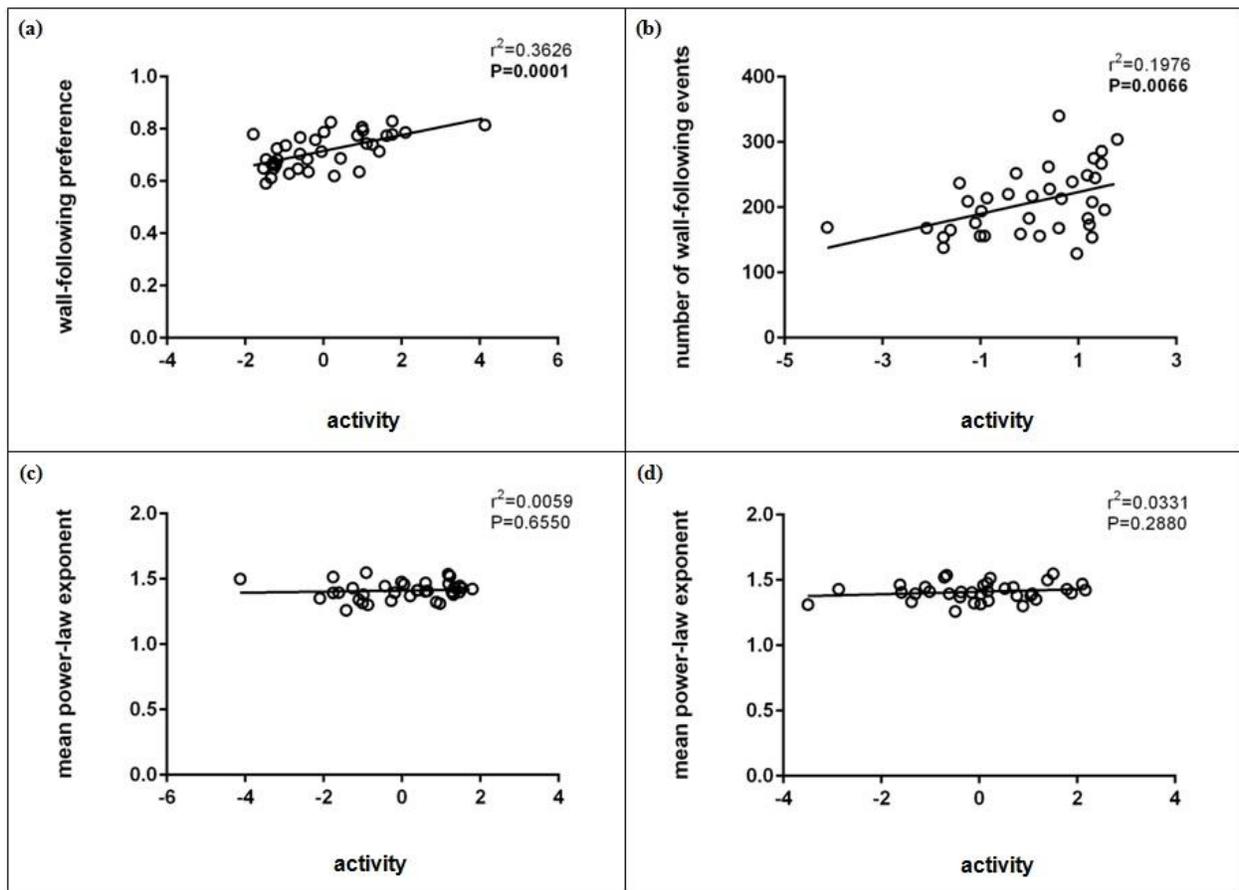
Figure 3.5 Linear regressions between PCA score of activity and (a) wall-following preference, (b) number of wall-following events, (c) mean power-law exponent for parameters used to calculate wall-following preference, and (d) mean power-law exponent for parameters used to calculate number of wall-following events. N=36

in their strategies. For example, there was no significant difference in total displacement across days for *A. albifrons* (Friedman test, P=0.6242) (Figure 3.6a).

In *A. leptorhynchus*, there was a significant decrease in total displacement between trial 1 and trial 2 (Dunn's multiple comparisons, P<0.01) and between trial 1 and trial 3 (Dunn's multiple comparisons, P<0.001), but no significant change in behaviour between trial 2 and trial 3 (Figure 3.6b). *A. leptorhynchus* also had significantly higher total displacements than *A. albifrons* in all of the trials. There were no significant differences in the mean speed of exploration in *A. albifrons* (Friedman test, P=0.2494). Mean speed in *A. leptorhynchus* significantly decreased between trial 1 and trial 2 (Dunn's multiple comparisons, P<0.01) and trial 1 and trial 3 (Dunn's multiple comparisons, P<0.001), but there was no significant change between trial 2 and trial 3. In *A. albifrons*, there was also no significant difference in the number of excursions made into the open area of the tank (Friedman test, P=0.7623) (Figure 3.6c), while *A. leptorhynchus* demonstrated a significant decrease in the behaviour between trials 1 and 2 (Dunn's multiple comparisons, P<0.05) and between trials 1 and 3 (Dunn's multiple comparisons, P<0.01) (Figure 3.6d). Similarities between species were demonstrated in the latency to exit the shelter at the beginning of the experiment, where no significant difference was present across any of the trials (Friedman test, P=0.0566 for *A. albifrons*, P=0.8283 for *A. leptorhynchus*). *A. albifrons* did demonstrate some variability in their exploratory movements in the mean amount of time spent out in the open (Figure 3.6e), where there was a significant decrease between trials 1 and 3 (Dunn's multiple comparisons, P<0.01) and between trials 2 and 3 (Dunn's multiple comparisons, P<0.05) (Figure 3.6f). Neither species had any significant differences in the

number of wall-following events across trials (Friedman test, P=0.7440 in *A. albifrons* and P=0.0698 in *A. leptorhynchus*) (Figure 3.6g, h).

In terms of specific wall-following behaviours, there was a significant difference in the preference for wall-following in *A. leptorhynchus* between trial 1 and trial 3 (Friedman test, P<0.01). The wall-following preference decreased from 0.76±0.05 during trial 1 to 0.63±0.13 during trial 3 (Figure 3.7b). In contrast, the wall-following preference in *A. albifrons* remained consistent across trials with an initial preference of 0.66±0.048 during trial 1 and 0.65±0.117 during trial 3 (Friedman test, P=0.4875) (Figure 3.7a). *A. leptorhynchus* spent significantly more time in the refuge during trial 3 than during trial 1 (Friedman test, P<0.0001), and the behaviour remained consistent in *A. albifrons* across all trials (Friedman test, P=0.0657). The preference for being stationary near the outer wall and refuge with a speed under 0.05 BL/s (i.e. not actively wall-following) was not significant across days for *A. albifrons* with an initial preference of 0.088±0.05 during trial 1 and 0.079±0.043 during trial 3 (Friedman test, P=0.6065) (Figure 3.7c). In contrast, a significant difference in the preference for being stationary was found in *A. leptorhynchus* between trial 1 and 3 with a preference of 0.022±0.013 during trial 1 which increased to 0.049±0.027 during trial 3 (Friedman test, P=0.003) (Figure 3.7d). Speed of movement while actively wall-following was consistent across days with no significant changes occurring in either species (Friedman test, P=0.2231 in *A. albifrons*, P=0.0657 in *A. leptorhynchus*) (Figure 3.7e, f).
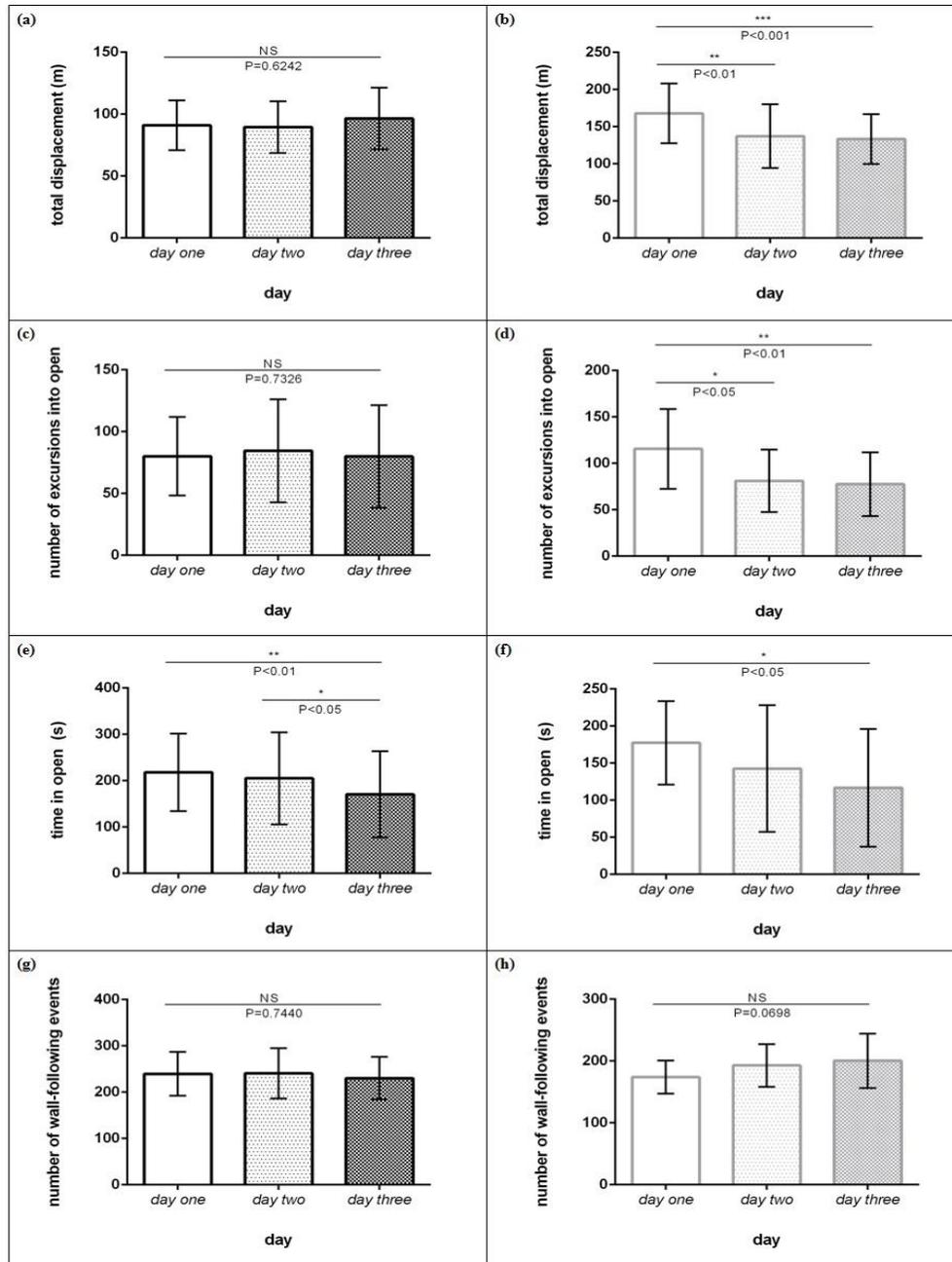
Figure 3.6 Bar graphs representing total displacement (a,b), number of excursions into open (c,d), time in open (e,f), and number of wall-following events (g,h) as a function of day in *A. albifrons* (left column) and *A. leptorhynchus* (right column). Mean±SD presented with P-values located above bars.
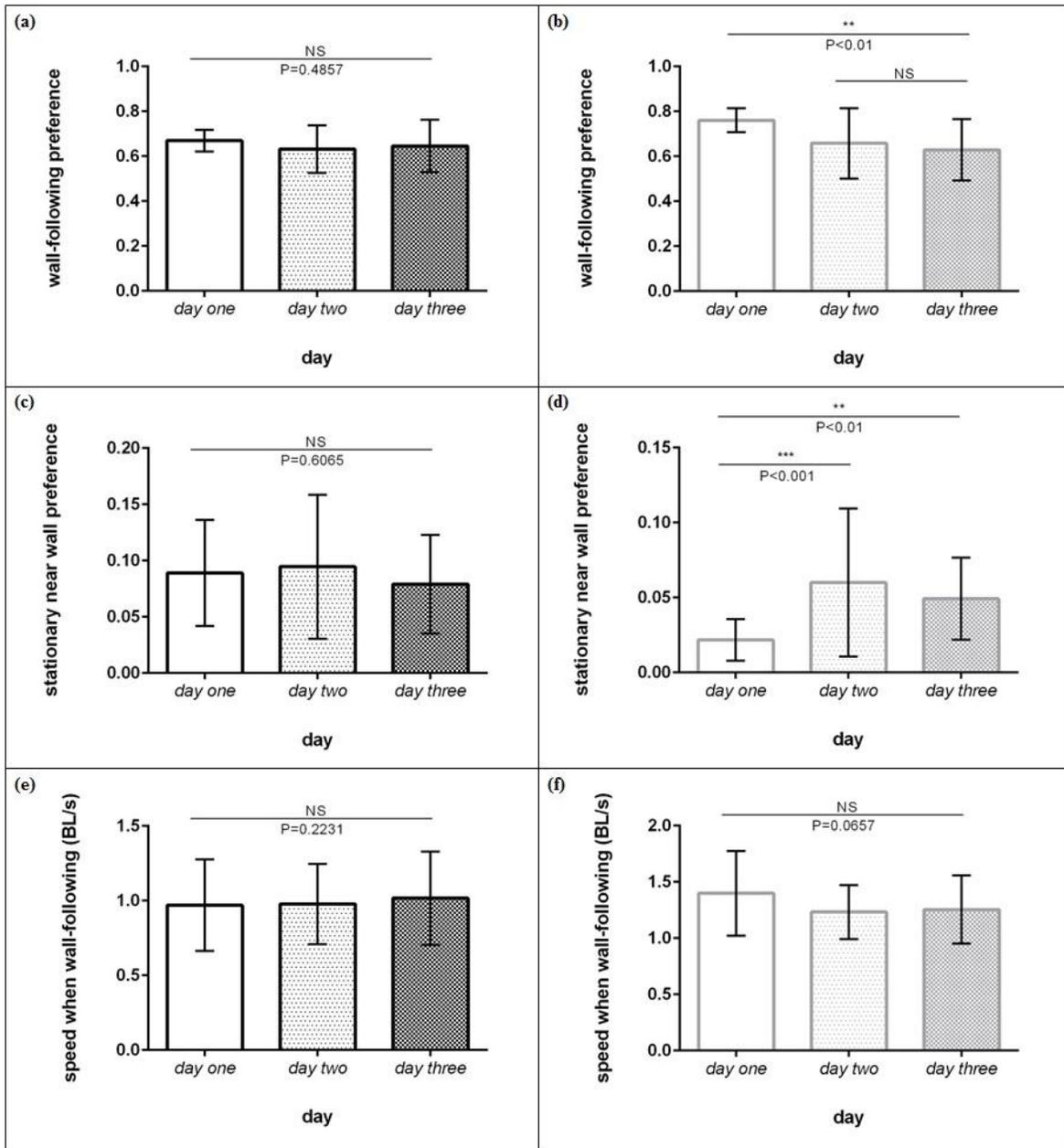
Figure 3.7 Bar graphs representing preference for wall-following (a,b), proportion of freezing (time spent stationary near wall) (c,d), time in open (e,f), and speed when wall-following (g,h) as a function of day in *A. albifrons* (black bars; a,c,e) and *A. leptorhynchus* (grey bars; b,d,f). Mean±SD presented with P-values located above bars, n=18 for each species.

3.4 Discussion

3.4.1 Patterns of wall-following behaviours

Both *A. leptorhynchus* and *A. albifrons* spent more than 60% of the total trial time actively following the peripheral and refuge walls. This is illustrated in figure 3.3 where there is a clear preference for being close to the outer portions of the tank. Although both species shared a preference for wall-following, *A. leptorhynchus* had a higher proportion of time spent wall-following than *A. albifrons*. This is expected since in Chapter 2, it was determined that *A. albifrons* had a slightly higher amount of time spent in the open zone, although not significant. Interestingly, *A. albifrons* had a higher number of wall-following events. A possible explanation for this is that *A. albifrons* also had more events of being stationary near the walls than *A. leptorhynchus*, which would terminate a wall-following 'event' due to the speed requirement (Chapter 2). It has been suggested (Sharma et al. 2009) that wall-proximity coupled with the absence of movement could be used as a protective function, especially in species whose predators are geared towards detecting visual motion. However, it is also possible that spending a longer time in close proximity to the wall with slow movement could serve as an exploratory function used as a thorough examination to build general spatial knowledge and find shelter or escape routes.

 A. leptorhynchus travelled at a higher speed when wall-following than *A. albifrons*, which is consistent with the previous results from Chapter 2. *A. leptorhynchus* also travelled at a higher speed when out in the open. A possible reason for why *A. leptorhynchus* would travel faster is that the perceived risk of predation is higher in the species.

3.4.2 Behavioural relevance of wall-following

The predator avoidance hypothesis proposes that wall-following behaviours serve as a protection function against predators. This explanation is plausible, since both species have a number of natural predators in their environments including electric catfish (Malapteruridae), electric eels (*Electrophorus electricus*) as well as other larger weakly electric fish (Moller 1995). *A. leptorhynchus* have been observed performing possible fright responses when introduced into the refuge at the beginning of the experiment. However, after 10 minutes of acclimation every individual made the decision to exit the refuge and move freely throughout the test arena instead of just remaining in the refuge, therefore it is unlikely that individuals were performing a fright response. Being in close proximity to a wall could decrease the number of directions from which a predator might attack. Both species also demonstrated many reorientation movements, in which they switched the direction of travel while following the walls. It is possible that switching the directions often could confuse a predator and reduce the amount of time the tail is left exposed. However, it is more likely that the reorientation movements were to better sample the environment using the electroreceptors and therefore this is inconsistent with the predator avoidance hypothesis (Lannoo & Lannoo 1993).

The exploratory hypothesis proposes that wall-following behaviours represent strategies for short-term localization of needed resources and long-term acquisition of the spatial configuration of the environment. In *A. leptorhynchus*, there is a significant decrease in the proportion of time spent wall-following during trial 3 from trial 1. This could be due to increased familiarity with the environment. *A. leptorhynchus* therefore would have used the first two trials to acquire long-term spatial information about the location of needed resources. More studies need to be done

since different results were obtained for *A. albifrons*. Perhaps *A. albifrons* are able to adapt faster and changes are harder to discriminate. The results also demonstrate an increase in the time spent in the refuge in *A. leptorhynchus* during trial 3. This suggests that after the localization of resources such as refuge for safety during the first two trials, *A. leptorhynchus* spent less time exploring the test arena during trial 3 and spent more time in the safety of a refuge. There was also a significant increase in the amount of time spent feeding in *A. leptorhynchus* during trial 2 from trial 1, suggesting decreased perception of risk.

To test the exploratory and predator avoidance hypotheses directly, both species were combined and using typical activity parameters, PCA scores were computed. Species were combined to obtain an overall active-reactive scale due to the large variability. Using the new scores, activity was then linearly regressed with the proportion of time spent wall-following and the number of wall-following events. The results demonstrated that individuals who were more active also had a higher preference for wall-following and had a higher number of wall-following events. It is expected that more active individuals would have higher rates of exploration and show greater levels of exploratory behaviours than reactive individuals. The results we showed were consistent with the exploratory hypothesis in the fact that active individuals demonstrated higher amounts of the wall-following behaviours. A future study could be to test the species separately to determine if species differences exist in the function of wall-following. Another interesting study to continue with the exploratory hypothesis would be to analyze the proposed benefits of wall-following. For example, to determine if wall-following behaviours do in fact lead to enhanced abilities to find goals.

An interesting point to note is that no relationship was found between activity level and mean power-law exponents when looking at the movements of individuals while wall-following. Similar to the results from Chapter 2, the power-law exponents suggest Lévy flight strategies when exploring and staying in close proximity to the walls. It is expected that different strategies would be used depending on whether an individual was searching in the open or wall-following, however, similar exponents are revealed.

Another explanation for wall-following behaviours is that they are an incidental consequence of how a fish's movements are redirected every time it comes into contact with walls of a concave environment. If this were the case, the distribution of the proportion of time spent wall-following would be distributed more Gaussian, following a normal distribution. In fact, wall-following distributions are characterized by a heavy-tail, suggestive of Lévy-flight. This potentially provides evidence against the incidental path of the fish's movements. A possible experiment to test this would be to add an object to the wall that provides a convex path. If fish follow the wall regardless of shape then it would suggest that fish are able to actively regulate their distance from the wall, however if individuals leave the path of the wall then it would suggest that they are unable to actively regulate the distance and that their wall-following behaviours are an artifact of tank shape. Without performing this experiment, there is still evidence in our results that both species are actively regulation the distance from the walls if you observe the movements around the refuge. The refuge is 10cm x 10cm square box and does not have any concave walls. The displacement plots demonstrate that individuals are actively moving around the refuge (Figure 3.2).

3.4.3 Variability of wall-following behaviours across days

Looking at the movement of both species across days, we found that *A. albifrons* showed a lot of consistency in the behaviours, whereas the behaviours in *A. leptorhynchus* were much more variable as was expected based on the predictions. In almost all of the behavioural parameters observed, *A. leptorhynchus* demonstrated significant changes in the behaviours between trial 1 and trial 3. The changes in behaviour observed in *A. leptorhynchus* are possibly due to the spatial learning aspect of exploration of novel environments. Exploration serves as a purpose of gaining information about the environment and the location of important resources. As novel environments become more familiar, it is expected that the amount of exploration would decrease. This is visible in the behaviours of *A. leptorhynchus* demonstrated a larger total feeding time during trial 2 than during trial 1. This observation was probably because individuals could learn the density of resources in their environment by having a large amount of exploratory behaviours during trial 1. In *A. albifrons*, the consistency in the behaviours could possibly be due to their behavioural type. Individuals who are more active are expected to act independent of the situation and consistently across time (Sih et al. 2004). Further testing will be necessary in order to make confident statements about possible suites of correlated behaviour in this species. Another possible explanation for the consistency of the behaviours in *A. albifrons* could be due to their capability of adapting well to different environments. Variable habitats have been shown to favour higher amounts of exploration (Simon et al. 1994). Living in a wide range of habitats, *A. albifrons* are exposed to a variety of different environmental conditions, including changes in conductivity and temperature of the water, changes in prey abundance and distribution, and

predator interactions (Moller 1995). It is possible that being able to adapt quickly can be advantageous in these situations.

Wall-following behaviours remained consistent in the movements of *A. albifrons*, whereas the behaviours of *A. leptorhynchus* appeared to be more variable. For example, the proportion of time spent wall-following in *A. albifrons* remained around 60% of the total trial time, however the proportion decreased in *A. leptorhynchus* significantly during trial 3. There was also an increase in the amount of time spent in the refuge during trial 3 in *A. leptorhynchus* suggesting a change in behaviour from actively exploring the environment on the first day to a more goal oriented behaviour of predator avoidance on the last day. Although there is a significant difference in the amount of time spent frozen near the wall in *A. leptorhynchus* across days, there is much variability in the behaviour across individuals.

3.4.4 Conclusions

In the present study we showed that there are behavioural differences in the wall-following parameters tested. It is possible that the evolution in wall-following in these two closely related species were different due to differences in habitat locations and levels of activity. *A. leptorhynchus* prefer to spend a larger proportion of time wall-following continuously rather than moving into the open zone of the test arena, whereas *A. albifrons* have a higher number of smaller wall-following events and spend more time exploring the open zone. In general, the behavioural responses of *A. leptorhynchus* appeared more variable than *A. albifrons* across days. This could be due to the differences in perceived behavioural traits in which *A. albifrons* who are potentially more active learn more quickly than the reactive *A. leptorhynchus*. This study

provides evidence in support that wall-following behaviours function as exploratory behaviours in weakly electric fish, whose senses are short-range. Individuals who were more active on the spectrum showed a higher proportion of time spent wall-following as well as a higher number of wall-following events.

It is unlikely that other senses were used throughout the experiment. The possibility of odor cues being used by the fish in this study was reduced by the addition of clean water at the start of each experiment with water of similar conditions to that of the home tanks. Any visual cues are unlikely due to the darkened environment that each individual was placed in, and the fact that experiments were performed during the dark portion of the photoperiod. It is unlikely that the lateral line was used because of the low conductivity levels in each experiment ($\sim$150 µS), which have the potential to decrease sensitivity of the mechanoreceptors (McIver et al. 2001).

Chapter 4. General Discussion

Until recently, studies on weakly electric fish have focused primarily on the neurobiological aspects of the unique sensory modality. The characteristics of the electric organ and the self-generating electric field have been well described, and there is continuing work being done in determining the links between neural processes, motor outputs, and behaviour. Despite this, there are a lack of studies describing these species from an animal behaviour and ecological perspective. One of the first studies to thoroughly characterize aggression and boldness in weakly electric fish was Shank (2013, thesis). This study provided evidence for a boldness behavioural syndrome in male *A. leptorhynchus*. I have focused on the characterization of exploratory behaviours in two closely related species of weakly electric fish, *A. leptorhynchus* and *A. albifrons*. More specifically, this study explored similarities and differences in the behaviours between the two species, as well as the function of wall-following in weakly electric fish, whose senses are short-ranged.

Personal observations in the laboratory suggest that *A. albifrons* are more active than *A. leptorhynchus*. In housing facilities, it is common to see *A. leptorhynchus* housed together in small groups of 3 to 4 individuals. On the other hand, it is expected that *A. albifrons* be housed in individual tanks due to the high levels of aggression observed between conspecifics. In the wild, these species also exhibit differences in aquatic habitats. Using wild-caught individuals, it was possible to make predictions based on habitats as well. We have shown differences are also found in the exploratory behaviours of these two closely related species of weakly electric fish.

A. albifrons spent more time out in the open area, had a higher number of feeding events, and a higher number of wall-following events. Future studies looking at the complexity of the habitat and how that affects the exploratory behaviours would be a great addition to this study. Also, a further characterization of the behavioural traits of *A. albifrons* would be interesting to compare with previous work done on *A. leptorhynchus*. Interestingly, the extrinsic motivation of abundant or scarce food did not have an effect on the characteristics of the exploratory strategies of either species. One suggestion as to why these behaviours are similar is due to the limited sensory range in weakly electric fish. In a novel dark environment, weakly electric fish rely primarily on the self-generated electric field to sense objects. The sensory range of the electric fish extends approximately half a body length in these two species (Snyder et al. 2007), and it is possible that all environments appear scarce in terms of the resources.

MacIver et al (2001) showed that detection performance improved (longer detection distances and lower miss rates) during prey-capture behaviour in *A. albifrons* at lower water conductivities, which results in an increase in the range of the receptive field. The mean detection distance almost doubled between high-conductivity (600 μS cm^{-1}) and low-conductivity (35 μS cm^{-1}), and the miss rate decreased from 11% to 2% in *A. albifrons* (MacIver et al. 2001). It has also been suggested that other electrolocation tasks are improved with lower water conductivity including the ability to discriminate capacitative targets (von der Emde et al. 1998) and the distance at which conspecifics are detected (Moller 1995). Several species of non-electric fish, including the Mexican blind cave fish (*Astyanax sp.*) use the mechanosensory lateral line for detecting the weak flow fields produced by their prey. In this system, fish generate a hydrodynamic flow field that becomes distorted by nearby objects (Sharma et al. 2009).

Mechanosensory sensitivity is expected to be affected only at very low conductivities, at which a low amount of Ca^{2+} in the water has been shown to reduce hair cell sensitivity (MacIver et al. 2001). If mechanosensory cues were dominant in prey detection in weakly electric fish, then detection performance in these species should be mostly insensitive to changes in water conductivity, or to decrease with lower water conductivity with the effects of low Ca^{2+} concentration. MacIver et al (2001) demonstrated that the prey detection performance in weakly electric fish actually increased as the conductivity decreased, suggesting that electrosensory cues dominate.

Wall-following behaviours have been observed in a variety of species across many taxa (Patton et al. 2010). Interestingly, a number of the species in which wall-following is found are those that have short-range senses (Sharma et al. 2009). These species, such as the Mexican blind cavefish, exhibit wall-following behaviours when introduced to a novel environment. Different sensory modalities were used by these species in order to detect and follow vertical surfaces. For example, the Mexican blind cavefish rely on the hydrodynamic flow of the lateral line to detect distance and location of objects (Sharma et al. 2009). Cockroaches use mechanosensory receptors located on the antennae to sense their surroundings. The function of wall-following in these species is not entirely clear. We have found that weakly electric fish, another species with a short-range sense, also exhibit wall-following behaviour. Both *A. leptorhynchus* and *A. albifrons* demonstrate a preference for active wall-following when placed in a darkened novel environment. Behavioural differences between the two species were observed. *A. leptorhynchus* had a higher preference for continuous wall-following, whereas *A. albifrons* had a higher number of smaller wall-following events that were broken up by movements out into the open area of the

tank. When travelling into the open and along the walls, *A. leptorhynchus* had a higher speed than *A. albifrons*. Similar to what is stated above, there was consistency in the movements of *A. albifrons* across days whereas *A. leptorhynchus* showed variability in behaviours.

Movements of these two species suggest that the behavioural function of wall-following in weakly electric fish is for exploration. It was demonstrated that individuals that had a higher proportion of time spent wall-following and a higher number of wall following events were also more active. Future research should focus on a more comprehensive test of the different hypotheses of wall-following behaviours in weakly electric fish. Performing tests in a goggle-shaped arena could be used to the test if weakly electric fish are able to regulate their distance from the wall or if the wall-following observed is an artifact of the concave tank shape (Patton et al. 2010).

This study is the first to extensively characterize the exploratory behaviours of two closely related species of weakly electric fish. It is also the first to characterize the function of wall-following in these species. By understanding how these species explore their environments, we can gain insight into the evolution of behaviours in species with unique sensory modalities.

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