Spatial cognition in three-dimensional environments

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Author contributions

The work within this is thesis is primarily my own. Five co-authors contributed to one or more of my chapters and their contributions are as follows:

Theresa Burt de Perera and Robert Holbrook contributed ideas and provided feedback for all of the chapters of this thesis.

Tim Guilford gave comments and feedback on chapter 3, and was part of discussions of the results in chapters 4 and 5.

Emiel Van Loon assisted with analyses and provided feedback on chapter 4.

Sarah Schumacher collected part of the data for chapter 3.

Abstract

Animals must navigate between different locations if they are to gather enough resources to survive. The natural world is three-dimensional; however, recent findings from studies of three-dimensional spatial cognition indicate that surface-bound animals remember information derived from the vertical dimension with lower accuracy than information from the horizontal dimensions. In contrast, non-surface-bound swimming and flying animals have been shown either to remember both with similar accuracy, or in some cases, remember vertical information more accurately than horizontal information. This has prompted questions about (i) the factors influencing spatial cognition in animals with different movement ecologies and (ii) what cues non-surface-bound animals use for navigation in three dimensions. In this thesis I explore these questions, principally through behavioural observations of freely-swimming fish in the laboratory. My findings reveal that factors influencing spatial cognition include (a) a difference in cues available in the vertical and horizontal dimensions, (b) asymmetry in the energetic costs of vertical versus horizontal travel and (c) animals' ecological niches. The influence of movement ecology on spatial cognition is complex: in spite of moving principally in the horizontal dimensions, benthic fish preferentially use vertical cues when information derived from the vertical and horizontal dimensions conflict. Hydrostatic pressure varies linearly with depth and represents a stable and reliable cue in an often-variable environment, thus fishes' sense of pressure could explain their vertical preference. Finally, in the absence of information about pressure and over small distances, fish may use horizontally- and vertically-arranged visual landmarks as approximate guides rather than indicators of the exact position of a location. Taken together my results provide new insight into the mechanisms underlying fishes' navigation in three dimensions and shed some light on how evolutionary history and day-to-day movement contributes to spatial cognition.

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Chapter 1

General Introduction

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Introduction

For the majority of mobile animals, food, mates and suitable nesting sites are scattered heterogeneously throughout the environment. Therefore, animals must move between different regions of their habitat if they are to acquire these resources to survive and reproduce. Regular travel exposes animals to increased risks of predation and conflict with other individuals, and depletes energy reserves. Accordingly, individuals that minimise the time it takes them to travel to locations, by memorising the distance and direction between familiar places, face lower fitness costs than if they were to search unsystematically (Benhamou 1994).

Animals have access to a diverse range of environmental cues that provide spatial (distance and directional) information for navigation. Some cues are global, in that they are located at a distance from the goal destination, while other cues are local and found close to the goal (Braithwaite and Burt de Perera 2006). How closely associated a cue is with a goal often influences the type of spatial information that can be derived from that cue. For example, global cues constitute distant panoramas like mountain ranges, or solar and magnetic compasses. These cues provide coarse information about approximate heading direction, such as whether animals are travelling North or South (Graham and Cheng 2009; Vlasak 2006). In contrast, local cues such as discrete landmarks that are close to or at an intermediate range from the goal can provide more precise information; either by directly signalling the location of the goal, as a beacon does (Collett 1987; Cook and Tauro 1999), or via indirect information, provided that the spatial relationship between the landmark(s) and the goal is known (Collett et al. 1986; Spetch et al. 1996, 1997).

The relative use of local and global cues depends on the type of navigational challenge being faced. For example, during long-distance migrations where animals return to natal breeding grounds (often to the same specific nest site) they can use the solar compass to establish the correct heading direction. As they begin to approach the region where they intend to nest they may switch to using local cues, such as visual landmarks, to pinpoint a specific nest site (Bingman and Cheng 2005; Lohmann et al. 2008) (figure 1); or, as is the case with pigeons, information from the solar compass may supplement information derived from local visual landmarks (Armstrong et al. 2013).

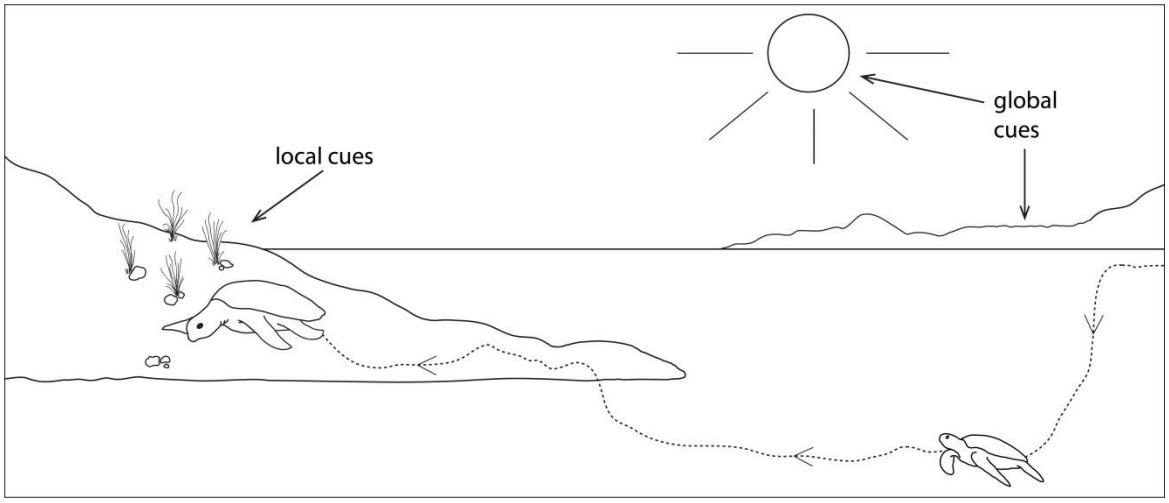


Figure 1. Global cues like the sun or distant panoramas give information about approximate heading direction. Local cues like plants and rocks give fine-scale spatial information about the immediate surroundings.

Animals can detect environmental cues via a variety of sensory systems. In some cases, cues may only be detectable by a single sensory modality; for example, invisible odour plumes can only be perceived via the olfactory system. In other cases, animals may use multiple sensory systems to detect a cue; for example, electric fish can sense obstacles in their environment using their electric, olfactory, lateral line and visual senses (Moller

2002; Schumacher et al. 2016). Using multiple senses simultaneously to perceive the environment provides animals numerous benefits: firstly, it can improve the accuracy of animals' mental representations of their environment. For example, bats (Danilovich et al. 2015) and humans (Sun et al. 2004) are better at estimating distances when they supplement visual information with biosonar and proprioceptive cues, respectively. Secondly, one system can take over for another as the principle source of information for navigation when the other becomes temporarily unavailable. For example, proprioceptive (Quirk et al. 1990), electrolocation (Moller 2002; Schumacher et al. 2016) and biosonar (Danilovich et al. 2015) cues can substitute for visual information in dark or turbid environments. In some circumstances these sensory substitutions can become permanent, as was the case with cave-dwelling blind Mexican cave fish (*Astyanax mexicanus*), whose lateral line system, over evolutionary time, developed a heightened sensitivity to perturbations in water flow in the absence of visual cues (Yoshizawa et al. 2014).

A third reason that using multiple sensory systems can be beneficial is that under some ecological conditions certain cues provide less reliable information than others. In these circumstances it is common for animals to weight sensory information according to how reliable they perceive it to be (for a review see, Cheng et al. 2007). For example, in environments with strong water currents, visual landmarks could be at risk of becoming displaced, meaning they will not provide consistently accurate spatial information. As a result, animals can benefit from resorting to other sensory systems that do not suffer from the same limitations (Girvan and Braithwaite 2000). This behavioural flexibility allows animals that must regularly return to their nest, a refuge, or self-replenishing food sources to continually recognise and remember the spatial position of these locations. This means that they can revisit these sites irrespective of ambient light levels, weather conditions and

shifting visual landmarks, all of which are features that could potentially change between visits in the natural environment (Schumacher et al. 2016).

1. Navigating in a three-dimensional world

In the past, much of the research into animal navigation has concentrated its efforts on investigating spatial learning in two-dimensional contexts, namely in animals travelling over horizontal planes (Brown et al. 2007; Collett 2009; Girvan and Braithwaite 2000; Gould, et al. 2010). Although this research has provided useful insights into some of the mechanisms underlying spatial cognition in animals, in reality, the world is three-dimensional and composed of both vertical and horizontal elements. As such, experiments that examine problem-solving in three-dimensional environments are more likely to reveal the strategies adopted by animals confronting real-world navigational challenges. My research focuses on deepening our understanding of the behavioural mechanisms that underpin navigational behaviour in three-dimensional environments.

Moving from a two-dimensional to a three-dimensional world view poses some challenges. The addition of the vertical dimension expands the size of the navigable space from a flat plane to a three-dimensional volume (Jeffery et al. 2013). With this expansion comes an increase in the overall amount of information available, and potentially a multiplicative increase in the complexity associated with navigation. For example, on a plane, a location can be represented by two Cartesian coordinates corresponding to its position in the X (forwards/backward) and the Y (left/right) dimensions, whereas in three-dimensional space a location is represented by three coordinates: the two in the X and Y dimensions as well as a third in the Z (up/down) dimension (Jeffery et al. 2015). Therefore, there is a high probability of an individual returning to their starting position after a series of random steps (where the direction of a step is not influenced by any

events preceding it) when moving in a one or two dimensions, but a much lower probability of returning to a starting position after random movements in three dimensions, due to the multiplicative increase in the directional combinations that could make up a sequence of random steps (Pólya 1921; Rayleigh 1919). Similarly, because of the increase in the rotational perspectives that can be adopted in three dimensions (yaw, pitch and roll), compared to just yaw in two dimensions, animals moving in three dimensions will approach regions of their environment from various different viewpoints that animals travelling in two dimensions will not experience (Vidal et al. 2003, 2004).

There is an element of vertical travel in most animals' natural movements; however the degree of vertical travel depends, in part, on the animal's movement ecology (Chapter 2; Chapter 3: Davis et al. 2014). Animals that are constantly in contact with a substrate, such as humans, rats and crabs, move with three degrees of freedom (figure 2a): two translational (forwards/backwards and left/right) and one rotational (yaw) (Holbrook and Burt de Perera 2013). This means that their vertical movements are dictated by the topography of the surface that they move over, be it undulating ground, the branches of a tree or the sea floor. In contrast, animals that are able to disengage from the substrate, such as those that can swim or fly, move with six degrees of freedom (figure 2b): three translational (forwards/backwards, left/right and up/down) and three rotational (yaw, pitch and roll) (Holbrook and Burt de Perera 2013). As such, in comparison with surface-bound animals, flying and swimming animals have more direct control over their degree of vertical travel, although the movements they can make are still limited by the increased energetic costs incurred whilst moving against gravity (Chapter 2; Grobéty and Schenk 1992; Jovalekic et al. 2011).

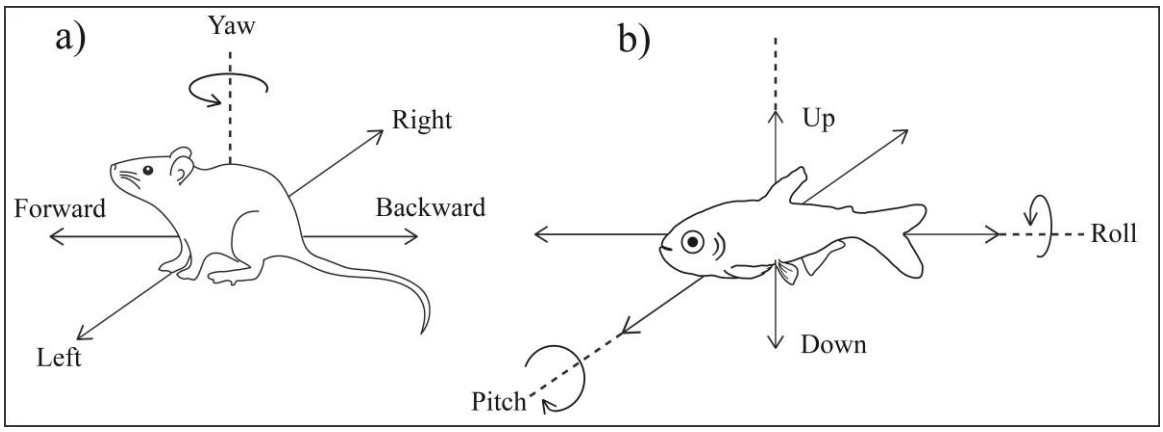


Figure 2. Taken from chapter 2: Schematic diagram of the translational (solid lines) and rotational (dotted lines and circular arrows) degrees of freedom in a) rats (surface-bound) and b) freely-swimming fish (non-surface-bound).

2. Fish as a study system

The majority of research into three-dimensional navigation has been undertaken in rodents (Grobéty and Schenk 1992; Hayman et al. 2012, 2015; Jovalekic et al. 2011; Wilson et al. 2015), and the findings have provided a fascinating window into the three-dimensional spatial cognition of an animal that moves with three degrees of freedom. Comparatively less is known about three-dimensional navigation in animals that move with six degrees of freedom as they have been less widely studied. However, the number of experiments investigating them is slowly rising, including studies of hummingbirds (Flores-Abreu et al. 2013, 2014), bats (Geva-Sagiv et al. 2015; Yartsev and Ulanovsky 2013), and fish (Holbrook and Burt de Perera 2009, 2013). My research on freely-swimming fish expands our knowledge on this relatively new field, as well as examining the unique conditions under which fish navigate.

2.1 Energetic costs during vertical and horizontal travel

Terrestrial surface-bound and non-surface-bound (flying) animals face an increased energetic cost when they move in the opposite direction to gravity. Evidence from studies in terrestrial animals indicates that the energetic costs associated with vertical movement may be responsible, at least in part, for the rate at which animals learn vertical information (Grobéty and Schenk 1992) and the accuracy with which they perceive it (Brandt et al. 2015; Jackson and Cormack 2008; Proffitt et al. 1995) compared to horizontal information (Chapter 2).

Fish are somewhat unusual among non-surface-bound animals in that many fish species do not face the increased energetic costs that other animals experience during vertical travel. The presence of the swim bladder, a gas-filled sac, allows fish to fine-tune their whole-body density relative to the surrounding fluid through passive increases or reductions in the volume of gas in their swim bladder as they move to different depths. As a result, fish that possess a swim bladder are able to counteract the effects of moving against gravity and thus move vertically and horizontally with similar energetic effort (Alexander 1972). In the presence of reduced energetic limitations in the vertical dimension, fish learn vertical and horizontal information at a similar rate (Holbrook and Burt de Perera 2009) and with similar accuracy (Holbrook and Burt de Perera 2013), and thus present an excellent model species to investigate true three-dimensional navigation under conditions where vertical and horizontal dimensions are energetically equivalent.

2.2 Movement ecology in the piscine clade

Fish inhabit a rich variety of habitats, from shallow rivers cluttered with rocks and plants to barren regions of open sea. Populating environments with widely-varying ecological

niches has meant that fish have developed adaptations in body shape, size and movement ecology (Smith and Skúlason 1996). Consequently, fish offer the opportunity to investigate the influence of movement ecology on spatial learning, as although they are all capable of moving with six degrees of freedom, not all fish exploit these. For example, benthic fish such as Plaice (*Hippoglossoides platessoides*) and Sole (*Solea solea*) spend much of their time in contact with the substrate, and thus in effect move with three degrees of freedom as in surface-bound animals, whereas pelagic fish move throughout the water column. Comparing my work on the performance of benthic fish in three-dimensional tasks (Chapter 2: Davis et al. 2014) with existing work on the performance of pelagic species under identical experimental conditions (Holbrook and Burt de Perera 2009) has provided the opportunity to begin to disentangle the relative influences of day-to-day movement and evolutionary history on spatial cognition in fish.

2.3 Species selected for experiments

Finally, from a species-specific point of view, both *Corydoras aeneus* (benthic species) (figure 3a and b) and *Astyanax mexicanus* (pelagic species) (figure 3c) were good logistical choices as candidates for my experiments. Both species are highly motivated by food, and the protocol of all of the experiments in this thesis depended on fish being able to form strong associations between particular locations and a food reward prior to data collection. Furthermore, both species were easy to keep and breed in captivity, and coped well with regular handling after an initial habituation phase which lasted between two and four weeks.

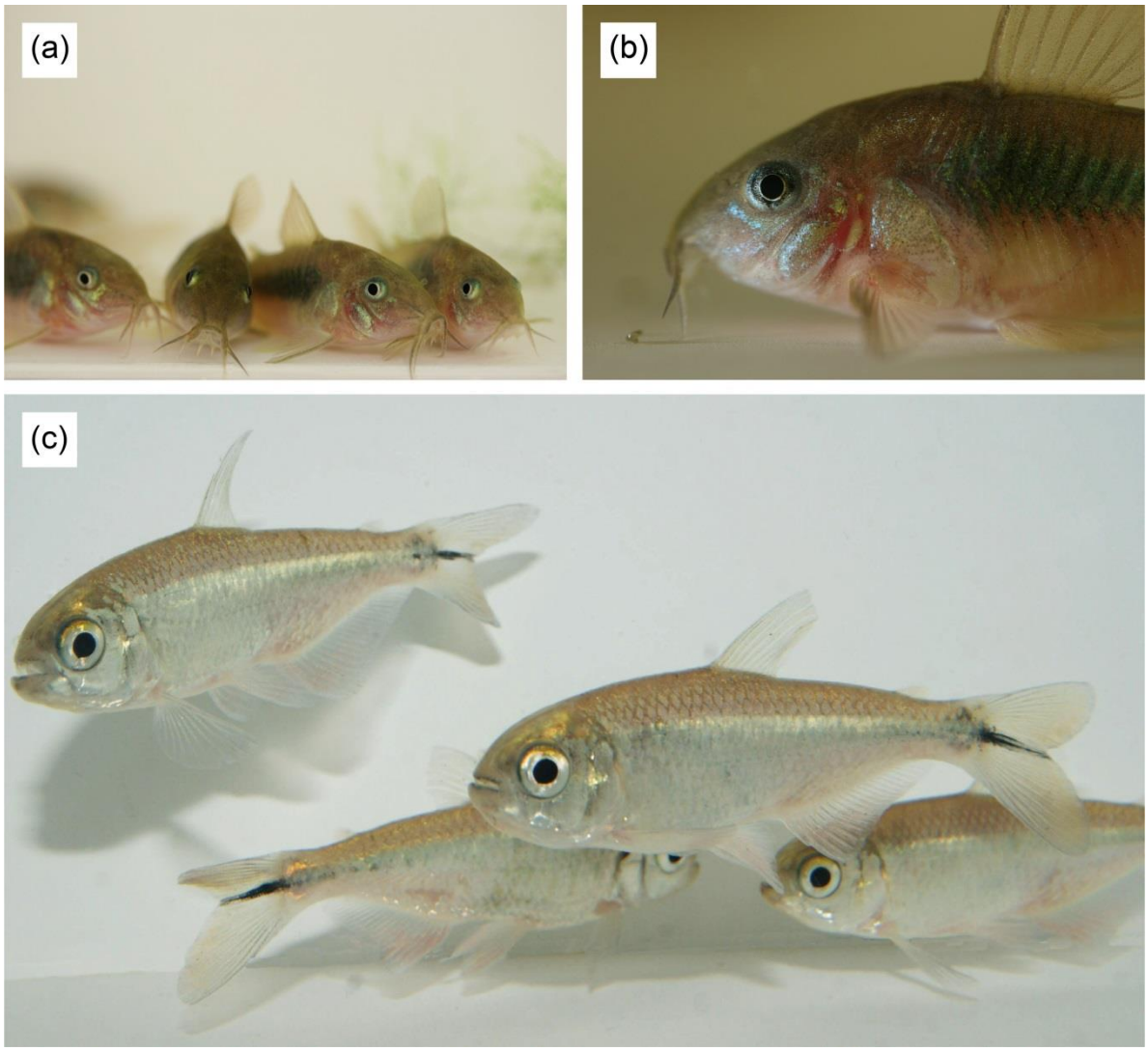


Figure 3. Subjects of the experiments in chapters 3-5: (a) and (b) Bronze corydoras (*Corydoras aeneus*); (c) Mexican tetra (*Astyanax mexicanus*).

3. Structure and aims of this thesis

Increasingly over the last two decades, researchers exploring spatial cognition and navigation in animals are considering both vertical and horizontal components of space in their investigations. Their findings have revealed differences in the way that surface-bound animals and flying and swimming animals encode the vertical component of three-dimensional space and perform tasks which include a vertical element. This has prompted

questions about what cues non-surface-bound animals are using for navigation and what features of their movement ecology and/or environment influence their spatial learning.

This thesis seeks to shed light on both issues by answering the following questions:

(1) How does an animal's movement ecology influence its spatial learning? (Chapters 2 and 3)

Chapter 2 reviews the findings of studies investigating three-dimensional spatial cognition in surface-bound and non-surface-bound animals. The aim of chapter 2 is first, to identify which factors are responsible for surface-bound animals learning and remembering vertical information less accurately than horizontal information, and second, to establish why these factors do not seem to influence the spatial learning of non-surface-bound animals in the same way. The factors I consider include: (i) the physical properties of the environment during development, (ii) the spatial cues available in different environments, (iii) the sensory systems that animals possess and the inherent biases of these systems, (iv) the difference in the energetic costs of travelling in different dimensions, (v) the ecological niche of an animal, and (vi) the inconsistencies between the different experiments to have investigated three-dimensional spatial cognition.

In Chapter 3 I probe this idea further with an empirical investigation of spatial learning in a benthic fish, the bronze corydoras (*Corydoras aeneus*), which can move with six degrees of freedom but spends most of its time travelling over the substrate in two dimensions. This chapter explores how *C. aeneus* encodes vertical and horizontal information, specifically whether its spatial learning is more similar to surface-bound animals or non-surface-bound animals. Answering this question could go some way to

establishing whether it is an animal's day-to-day movement or their evolutionary history that influences how they learn vertical and horizontal information.

(2) Which behavioural mechanism underlies vertical and horizontal landmark use in fish? (Chapter 4)

Behavioural studies have shown that fish can learn and remember the vertical and horizontal components of a three-dimensional route with similar accuracy (Holbrook and Burt de Perera, 2013). However, the question of how fish use visual landmarks to determine their vertical (and horizontal) position remains unanswered. In Chapter 4 I investigate the behavioural mechanism underlying the use of vertically- and horizontally-arranged pairs of landmarks for navigation by a pelagic fish (*Astyanax mexicanus*). Evidence suggests that many animals use one of two strategies to relate the position of a goal to nearby visual landmarks: either (i) by learning the absolute distance between the goal and one of the landmarks in an array (Collett et al. 1986) or (ii) by learning the relative position of the goal with respect to all of the landmarks in an array (Spetch et al. 1996, 1997). By using a landmark array transformation approach where the distance between the landmarks and the goal is doubled between training and testing, I investigated what strategy fish are using to represent the relationship between the landmarks and the goal, and whether these strategies differ if the landmarks are arranged vertically or horizontally.

(3) Can fish use pressure to determine their absolute depth? (Chapter 5)

It is well-established that the swim bladder can detect changes in the pressure of the surrounding water, responding to increases or decreases in ambient pressure by reducing or raising the volume of the gas inside the swim bladder respectively (Blaxter and Tytler

1972; McCutcheon 1966; Qutob 1962). However, in the past, researchers have predicted that fish could not use their swim bladder to ascertain their absolute depth via changes in pressure during vertical travel. Recent theoretical work has challenged this convention, providing a model for depth detection in fish using pressure cues alone (Holbrook and Burt de Perera 2011a; Taylor et al. 2010). In this chapter, I test empirically the hypothesis that fish can derive their absolute depth from hydrostatic pressure cues in the absence of all other spatial cues.

Finally, in Chapter 6, I summarise the key findings of this thesis by providing some answers to the questions (1-3) listed above. I then discuss the general implications of my findings and the relevance of these for the field of navigation, as well as highlighting further questions this research has precipitated and presenting ideas for the direction of work in the future.

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Chapter 2

The influence of movement ecology on animals' learning of vertical and horizontal spatial information

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Abstract

The degree of three-dimensional movement exhibited by animals depends, in part, on their movement ecology. For example, surface-bound animals such as humans are always in contact with the ground; consequently, their travel in the vertical dimension is largely dictated by the topography of the terrain. In contrast, non-surface-bound (flying and swimming) animals can move equally in all three dimensions. Research from the last 20 years has indicated that many animals learn and remember information about the vertical and horizontal dimensions with different degrees of accuracy, and that this may be influenced by their movement ecology. However, there has been no overview to determine whether these differences follow general patterns and there have been few attempts to explain the reasons behind them. Here, we review the literature on vertical and horizontal navigation, comparing the relative accuracy of these processes in surface-bound and non-surface-bound animals, and critically appraising the key contributing factors. We find that surface-bound animals learn vertical information less accurately than horizontal information, whereas non-surface-bound animals either learn vertical information more accurately than horizontal information, or learn both with similar accuracy. We propose that these patterns can be explained by a combination of factors that differ between the dimensions, including the energetic costs of travel and sensory system biases, as well as the extent to which an animal's ecological niche requires them to travel vertically. We suggest that the field would benefit from (1) studies where both behavioural and neurophysiological recordings are collected simultaneously, (2) studies of spatial cognition in closely-related species that occupy different ecological niches, and (3) more studies in a larger variety of species, to allow us to establish general principles to explain differences in spatial learning between species.

1. Introduction

Resources such as food, mates and nesting sites are distributed unevenly in the wild; therefore, animals must visit different parts of the environment if they are to exploit these resources to survive and reproduce. As they travel between regions, animals are vulnerable to predation, conflict with conspecifics and energy depletion. As a result, natural selection favours individuals that remember the spatial locations of frequently visited places, as this allows them to minimise the time that they spend travelling.

Spatial cognition is a multi-step process and a key component of successful navigation between familiar places. It involves using sensory systems to gather information about features of specific locations, such as the slope of the terrain or the positions of prominent landmarks. Once gathered, this information is then processed and stored until it is needed again, for example on a return journey (definition adapted from Healy and Rowe 2010).

The neural and behavioural mechanisms that underlie spatial navigation have been studied in a wide variety of vertebrates and invertebrates (Collett 2009; Healy 1998). In the past, the majority of this research has been limited to exploring navigation in the horizontal dimensions alone. However, the world is three-dimensional, comprised of both vertical and horizontal components, and consequently, for most mobile animals there is an element of both vertical and horizontal travel in their natural movement. Not all animals move in the vertical dimension to the same extent; their degree of vertical travel depends on their movement ecology, i.e. whether they are surface-bound or non-surface-bound (Chapter 3: Davis et al. 2014). Surface-bound animals are in continual contact with a substrate as they move, be it the ground, the seabed or the branches of a tree. Among surface-bound species are humans, lobsters and ants, and all such species move with three degrees of freedom (figure 1a): two translational (forwards/backwards and left/right) and

one rotational (yaw) (Holbrook and Burt de Perera 2013), meaning the extent to which they move vertically is dictated by the topography of the surface that they move over. In contrast, non-surface-bound animals such as those that can swim or fly are able to disengage from the substrate for extended periods (beyond leaps and jumps), and can thereby move with six degrees of freedom (figure 1b): three translational (forwards/backwards, left/right and up/down) and three rotational (yaw, pitch and roll) (Holbrook and Burt de Perera 2013). As a result, the vertical movement of non-surface-bound animals is not controlled by the topography of the land but by the animals themselves. In most cases the vertical movements of both surface-bound and non-surface-bound animals are constrained by the energetic costs associated with moving against gravity (Grobéty and Schenk 1992; Jovalekic et al. 2011).

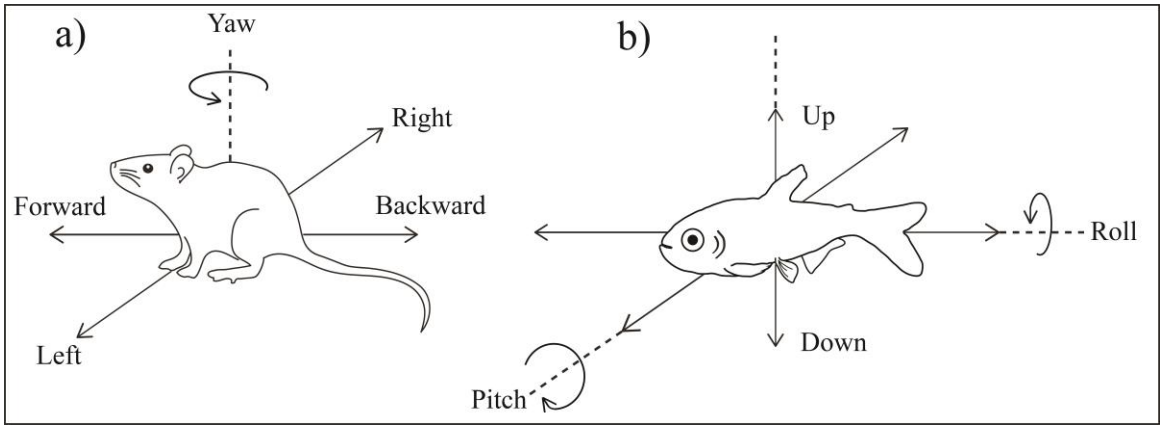


Figure 1. A schematic diagram of the hypothetical translational (solid lines) and rotational (dotted lines and circular arrows) degrees of freedom that a) rats (surface-bound) and b) freely-swimming fish (non-surface-bound) can move through. For many animals up/down and left/right movements cannot be performed independently of pitch and yaw rotations respectively.

Recently there has been a rapid increase in the number of studies investigating spatial cognition in three-dimensional environments. Many of these studies have suggested that animals may learn, encode, and remember vertical and horizontal information with different levels of accuracy, and that the degree to which they do this may depend on their movement ecology (Flores-Abreu et al. 2014; Jovalekic et al. 2011; Holbrook and Burt de Perera 2013; Yartsev and Ulanovsky 2013). However, because these comparisons have thus far been made on a case-by-case basis, it has remained unclear whether the accuracy with which animals learn vertical and horizontal information differs consistently between surface-bound or non-surface-bound animals. Moreover, the reasons that animals learn and remember vertical and horizontal information with different accuracies are largely unknown.

The aims of this paper are firstly, to review the literature on three-dimensional cognition, in order to identify where there are differences in animals' learning of vertical and horizontal information, and how these differences vary between surface-bound and non-surface-bound animals. Secondly, we attempt to identify the factors driving these differences, including the availability of cues, the sensitivity of sensory systems, and differences in species' ecological niches. We then discuss whether these factors ultimately shape three-dimensional spatial cognition via developmental or genetic routes. This review will help to elucidate the factors influencing animals' abilities to learn and remember vertical and horizontal information, and suggest new research avenues to uncover whether these abilities are primarily determined by genetic or developmental processes.

2. A review of vertical and horizontal spatial learning in animals

The studies that we review in this paper have taken two approaches to exploring animals' three-dimensional spatial cognition: 1) investigating how animals solve three-dimensional spatial tasks based on observations of their behaviour, either in the laboratory or in the wild; and 2) direct neurophysiological recordings of individual neurons involved in spatial cognition, either during passive exploration or as the animals actively solve navigational tasks based around three-dimensional apparatus in the laboratory. In much of the current research exploring three-dimensional navigation, rodents (mainly rats), humans and ants are models for surface-bound behaviour, whilst fish, bats, hummingbirds and bees are models for non-surface-bound behaviour.

2.1 Findings from behavioural experiments

2.1.1 Surface-bound animals

Experimental evidence suggests that both surface-bound and non-surface-bound vertebrates separate three-dimensional space into its constituent parts (Flores-Abreu et al. 2014; Grobéty and Schenk 1992; Hayman et al. 2011; Holbrook and Burt de Perera 2009). Holbrook and Burt de Perera have suggested that by partitioning spatial information into vertical and horizontal dimensions, animals may reduce the complexity of storing three-dimensional information (Holbrook and Burt de Perera 2009) however, there is currently no empirical evidence to support this theory. While it appears that both surface-bound and non-surface-bound vertebrates are encoding vertical and horizontal information separately there appear to be differences in the accuracy with which surface-bound and non-surface-bound animals learn and perceive vertical versus horizontal information.

Of the three behavioural studies investigating three-dimensional spatial cognition in rats, two found that rats remembered the horizontal component of a three-dimensional location more accurately than the vertical component (Flores-Abreu et al. 2014; Jovalekic et al. 2011). Notably, rats' lower accuracy in the vertical dimension could not be explained by them spending less time exploring vertically, as this same result was found irrespective of whether rats spent more time exploring horizontally (Jovalekic et al. 2011) or vertically (Flores-Abreu et al. 2014) over the experimental period.

The third study of three-dimensional spatial cognition in rats, by Grobéty and Schenk (1992), found that in the early stages of a learning task, rats were more accurate at remembering the vertical position of a food reward than its horizontal position. However, this effect disappeared in the later stages of the experiment as the rats became more experienced at the task (Grobéty and Schenk 1992). A possible explanation for this finding will be discussed later when we consider the energetic costs of vertical travel (section 3.3).

Experiments with humans and dogs have yielded similar results to those found in rats. In experiments based in multileveled buildings, both humans (Zwergal et al. 2015) and dogs (Brandt and Dieterich 2013) remembered the vertical component of a three-dimensional location less accurately than the corresponding horizontal component. Similarly, humans tended to overestimate vertical but not horizontal distances (Brandt et al. 2015; Jackson and Cormack 2007, 2008; Renier et al. 2006), a phenomenon known as the 'vertical-horizontal illusion' (Hicks and Rivers 1908, first described by Oppel 1855).

Desert ants (*Cataglyphis fortis*) use their number of steps to update an internal odometer used for distance estimation during path integration (Wittlinger et al. 2006). However, *C. fortis* does not appear able to path integrate in all three dimensions. Wohlgemuth (2001)

suggests that *C. fortis* disregards its movements in the vertical dimension entirely, simply remembering the relative horizontal ground distance it has travelled. Other researchers have corroborated these findings and suggested that although *C. fortis* may remember having to ascend or descend a slope on the way to food it does not incorporate this information into its path integrator. In other words, *C. fortis* remembers that there were vertical sections to its outbound journal but not how steep or how long these vertical sections were, or even whether the direction of the slope was upwards or downwards (Grah et al. 2005, 2007).

2.1.2 Non-surface-bound (swimming and flying) animals

There are fewer behavioural studies of three-dimensional spatial cognition in non-surface-bound animals, and the findings are somewhat mixed. *Astyanax mexicanus*, is a pelagic fish (species referred to as *A. fasciatus* in original work by Holbrook and Burt de Perera), which moves freely throughout the water column and learns both vertical and horizontal information at a similar rate (Holbrook and Burt de Perera 2009) and with similar accuracy (Holbrook and Burt de Perera 2013). However, experiments with freely-flying hummingbirds (*Selasphorus rufus*) showed that they remembered the vertical location of a food source more accurately than its horizontal location in both a three-dimensional maze (Flores-Abreu et al. 2014) and an open field navigation task (Hurly et al. 2010). In contrast, an earlier study found that hummingbirds appeared able to learn the horizontal but not the vertical position of a reward (Flores-Abreu et al. 2013), however, it is possible that this finding was a result of there being insufficient spatial cues available in the vertical dimension during the experiment (Burt de Perera et al. 2016).

In bees, attending to cues in the vertical dimension might be a species-specific trait. For example, honeybees (*Apis mellifera*) appear to ignore the vertical dimension. They

communicate the azimuthal direction and distance of a food source to other members of the hive with their waggle dance, but neglect to include any information about the height of the food source (Dacke and Srinivasan 2007). In contrast, the stingless bee (*Melipona bicolor*) communicates both the vertical and horizontal position of a food source to other members of the colony (Nieh et al. 2003).

Thus, overall, three-dimensional spatial cognition appears to be fairly similar within surface-bound animals, but varies among non-surface-bound animals; specifically, it seems that surface-bound animals remember horizontal information more accurately than vertical information, whereas non-surface-bound animals either remember all three dimensions with similar accuracy, as is the case with fish, or remember the vertical dimension more accurately, as is the case with hummingbirds.

2.2 Findings from neurophysiological experiments

2.2.1 Surface-bound animals

There are still very few studies investigating neurophysiological activation patterns in animals during three-dimensional navigation. As a result, all of the work that we discuss in this section about surface-bound animals has been undertaken in rats, and all of the studies on non-surface-bound animals have been conducted in bats. Hayman and colleagues found that both place cell and grid cell fields were vertically-elongated when rats explored a two-dimensional vertically-oriented pegboard and helix-shaped apparatus (both of which allowed rats to move forwards/backwards and up/down but restricted their left/right movement) (Hayman et al. 2012, 2015; Jeffery et al. 2013). Larger activation fields reflect a lower resolution of spatial information over a given area, in that place cell fields are smallest at the point when animals have access to the most sensory information, and become progressively more ‘diffuse’ as that sensory information becomes out-dated

(Geva-Sagiv et al. 2015; Ulanovsky and Moss 2011). Thus, Hayman and colleagues' findings that cell activation fields were vertically-elongated indicate that vertical information is represented at a lower resolution in the rat brain than horizontal information.

A more recent experiment by Hayman and colleagues investigating the arrangement of grid cell activation fields in rats found no difference in the pattern of grid cell fields between a flat plane and a sloped plane. They concluded from this that rather than grid cells' activation fields forming a repetitive hexagonal lattice throughout three-dimensional space (the way they do over a two-dimensional horizontal plane), the fields must be fixed to whatever surface the rat moves over irrespective of its incline (Hayman et al. 2015). Taken together these results suggest that rats encode vertical information at a lower resolution than horizontal information, and this may manifest in behavioural experiments as lower accuracy for remembering vertical versus horizontal information (Flores-Abreu et al. 2014; Jovalekic et al. 2011).

Unlike place and grid cells, head-direction cells are activated by turns of the head, as a result, the activity of these cells does not vary when a rat travels over a horizontal surface compared to when they climb vertically, as long as the head moves in the yaw plane (pointing nose left or right) (Stackman et al. 2000). There is also evidence that rats may possess cells that are tuned to turns in pitch (pointing nose up and down), however, head-direction cells in the rat that are sensitive to rotations in roll (nose tilting left and right) have yet to be found (Stackman and Taube 1998). Overall, This suggests that although rats' head-direction cells provide them with information about up/down and right/left head movements, rats still do not have information about torsional head movements and thus, they do not have a complete image of their head orientation in three-dimensional

space, but rather separate, cross-sectional image "slices" in the vertical and horizontal planes.

2.2.2 Non-surface-bound (swimming and flying) animals

In contrast to findings from rat place cell recordings, freely-flying bat place cell activation fields are not elongated vertically or horizontally, but are near-spherical in shape. This has led to the suggestion that bats encode spatial information at a similar resolution in all three dimensions (Yartsev and Ulanovsky 2013), although this has yet to be tested with behavioural experiments. Studies of head-direction cell activation in free-flying bats indicate that unlike rats, who possess cells that are activated at different angles of yaw and pitch, bats possess head-direction cells sensitive to turns in all three planes of rotation: yaw, pitch and roll (Finkelstein et al. 2014), providing them with three-dimensional information about up/down, left/right, and torsional head movements.

Overall, results from neurophysiological studies are similar to those of the behavioural studies that we have already discussed, in that neurophysiological experiments have revealed differences in the resolution of vertical versus horizontal information stored in the brains of surface-bound rats, but no differences in the resolution between vertical and horizontal information stored by non-surface-bound bats. As was the case with behavioural studies, the findings from neurophysiological experiments suggest inherent differences in the way that surface-bound and non-surface-bound animals encode information from the vertical and horizontal dimensions. However, the reasons for the observed asymmetry in encoding and memory between the different dimensions have, thus far, remained unclear.

In the following sections (3.1-3.4), we discuss the factors that may be contributing to the fundamental differences in the accuracy that surface-bound animals learn and remember vertical and horizontal information, and the reasons why these factors seem to influence non-surface-bound animals differently. We hope that by answering these questions we can begin to unravel how different sensory, ecological, and energetic constraints could shape animals' mental representations of the physical world.

3. Factors driving the learning of vertical and horizontal information

3.1 Cues available for three-dimensional navigation

Some cues are not accessible in all three dimensions. For example, the position of the sun can only provide information about an animal's location during horizontal travel; similarly, gravitational and hydrostatic pressure cues are only informative during travel in the vertical dimension (Jeffery et al. 2013). This leads us to ask whether the difference in surface-bound animals' abilities to learn and remember vertical and horizontal information is due to a difference in the spatial cues that are available for navigation in the vertical and horizontal dimensions.

If animals were to use one cue to navigate in the vertical dimension and another cue to navigate in the horizontal dimension, this could cause a discrepancy in their ability to navigate between these dimensions, particularly if the properties of the cues themselves affect how useful they are as a navigational cue. For example, it is often the case that the likelihood that a cue is perceived depends on the distance of the perceiver from the cue: olfactory and auditory cues become higher in intensity closer to their source, and visual landmarks change in relative size and apparent position at close or distant ranges. This means that although visual panoramas and very distant visual landmarks can be useful to indicate general compass directions (Graham and Cheng 2009) they are often not

sufficient to indicate precise locations, such as the nest site (Cook and Tauro 1999). In contrast, the hydrostatic pressure gradient present in aquatic environments is a global cue, in that its availability throughout the water is uniform (Chapter 4; Holbrook and Burt de Perera 2011a; Taylor et al. 2010). Thus, the likelihood of perceiving a change in pressure during vertical travel is completely independent of the location of the perceiver in the water. As a result, if animals have access to uniformly informative cues such as pressure in one dimension but not in the other, it is possible that this could cause a discrepancy in how accurately they are able to navigate in vertical versus horizontal dimensions.

Most studies did not identify whether there were differences in cue availability between the horizontal and vertical dimensions, such that the importance of this factor could not be tested. However, in the few studies where cue availability was documented, it appeared to be of little importance in determining the accuracy of vertical versus horizontal navigation. In experiments in which rats had access to the same visual landmark cues in both the vertical and horizontal dimensions (Flores-Abreu et al. 2014; Jovalekic et al. 2011), and all other cues, including odours, were removed, the rats remembered the vertical position of a reward with lower accuracy than its horizontal position. Furthermore, in the studies on fish, there were differences in cue availability, since hydrostatic pressure is only available as a cue in the vertical dimension (Chapter 5), and yet the fish learned vertical and horizontal information with similar accuracy (Holbrook and Burt de Perera 2013). One alternative explanation for this finding is that fish did not use pressure cues to locate the reward in this particular experiment, but instead relied on visual cues that provided both horizontal and vertical information; however, it is not possible to ascertain this from the study as it was not explicitly tested.

We have thus far discussed examples where cue availability differs; however, a second possibility is that a cue is equally available in all dimensions but is not equally *used* in all dimensions. In a study on humans where navigational strategy was observed and whole-brain activation was measured, the subjects relied principally on visual cues when they navigated in the horizontal dimensions, but switched to relying on other types of information, perhaps vestibular cues, when they navigated in the vertical dimension (possibly because the visual system is oriented in the plane of locomotion, which in the case of humans is the horizontal dimension: section 3.2; Zwergal et al. 2015). Thus, one possible explanation for humans' overestimations of vertical distances (Jackson and Cormack 2008) is that there is a difference in the quality of the information derived from visual versus vestibular cues. For example, visual cues can provide animals with long-range information, that is, at a considerable distance from their current position (subject to the animal's visual capabilities); however, vestibular cues provide information about movements as they happen and thus provide information over a very short-range. Whether there are differences in the quality of incoming vestibular and visual information, and whether this could be responsible for the lower accuracy with which many surface-bound animals perceive vertical information, remains to be confirmed with empirical research.

Overall, we suggest that a difference in cue *availability* seems unlikely to account for differences in the accuracy of vertical and horizontal navigation. However, these differences may potentially be explained by differences in the *quality* of the cues available in the vertical and horizontal dimensions; in particular whether the information the cues provide is uniformly available (irrespective of location) and is accessible over long distances.

3.2 Sensory system biases

The natural world is subjective, in that the way it is perceived is a product of both the cues available in the environment and the sensory systems that animals possess to detect them (Von Uexküll et al. 2010). Another explanation for differences in the accuracy of vertical and horizontal navigation may thus be that sensory systems have dimension-specific biases, such that they perceive vertical and horizontal distances with different accuracies.

In humans, the visual system is oriented in the plane of locomotion (i.e. the horizontal dimension) and therefore it gives a wider perspective than it does tall (Renier et al. 2006), such that it may measure horizontal information more accurately than vertical information. This could potentially explain the results of two independent experiments in which humans estimated vertical distances less accurately than horizontal distances (Brandt et al. 2015; Renier et al. 2006). However, a bias in the visual system may not be an adequate explanation, at least in isolation, for this asymmetry, because whilst humans appear to rely principally on their visual system for navigation in the horizontal dimensions, they resort to other sensory systems, most probably the vestibular system, for navigation in the vertical dimension (Zwergal et al. 2015).

The bias in the visual system could nonetheless play an important role in the accuracy with which humans learn horizontal and vertical information, if previous experience of visual cues leads to similar biases in other sensory systems. Renier and colleagues (2006) provided experimental evidence for this idea by investigating distance estimation in both blind and non-blind individuals. Both groups experienced the same cues: visual cues were obscured using blindfolds in the non-blind individuals, and both groups experienced a sensory substitution in which the vertical and horizontal distances of lines depicted on

screens were translated into different auditory tone frequencies. The authors reported that non-blind individuals routinely overestimated vertical distances relative to horizontal distances of the same length; however, congenitally blind individuals did not. They reason that although vertical representation biases may have originally resulted from implicit limitations of the human visual systems, these effects continue to persist because the biases of the visual system pollute other sensory systems, in this case the auditory system (Renier et al. 2006). If so, the vertical bias in the visual system could indeed explain humans' overestimation of vertical distances, even though vision is not the primary sensory system used in vertical navigation.

Behavioural and neurophysiological experiments indicate that non-surface-bound animals do not experience the same vertical perceptual errors as surface-bound animals; however the reasons for this remain unclear. Either non-surface-bound animals' sensory systems are not susceptible to the biases experienced by surface-bound animals or non-surface-bound animals have adapted to deal with these sensory biases so that they do not hinder vertical navigation. There is currently very little empirical data investigating the resolution of information coming in via different sensory systems. We suggest that investigating this could shed light on whether a difference in the quality of sensory information from different systems could be causing differences in vertical and horizontal learning in animals with different movement ecologies. One way to investigate this with behavioural experiments might be to control the sensory inputs that animals receive, reversibly blocking each sensory system in turn while animals learn a three-dimensional route. These animals can then be tested on their ability to remember the route, or estimate the distance travelled after learning this information under conditions where specific senses were unavailable. It may be possible to investigate this question in a similar way using neurophysiological recording experiments too. As before, researchers could limit

the sensory inputs that animals receive and then compare the shape and size of the activation fields of place and grid cells – characteristics thought to link directly to spatial scale and the resolution of incoming information – in order to determine how well information from different sensory systems is represented in the brains of different animals (Brun et al. 2008; Geva-Sagiv et al. 2015; Jung et al. 1994).

3.3 The costs of vertical travel

For many animals there is a fundamental difference in the cost of travelling vertically compared to horizontally, in that ascending sloping terrain requires them to expend greater amounts of energy than when travelling the same distance on flat terrain, simply because more energy is required to counteract the effects of gravity. One possibility is that the energetic costs of travel influences how animals learn about spatial information. This idea is confirmed by studies in humans by Proffitt and colleagues; they found that individuals carrying heavy backpacks perceived horizontal distances (Proffitt et al. 2003), and vertical slopes (Proffitt et al. 1995) to be greater than unburdened individuals. If energetic costs can influence distance perception in humans, perhaps the greater effort associated with moving vertically versus horizontally could provide an alternative and non-mutually exclusive explanation for the asymmetry in their encoding of vertical and horizontal information.

Grobéty and Schenk (1992) found that rats chose to remember the vertical component of a location before learning its horizontal component. However, this was only the case in the early stages of their experiment; as the rats became more familiar with the task their superior memory of the vertical component seemed to disappear. The authors propose that this result could be explained by the higher energetic costs associated with vertical travel. They suggested that because there is a greater risk associated with getting lost in

the vertical dimension, in that frequent upward travel depletes limited energy reserves more quickly than horizontal travel, it follows that rats should prioritise learning vertical information, if due to cognitive constraints, they are unable to learn both simultaneously (Grobéty and Schenk 1992; Jovalekic et al. 2011). Jovalekic and colleagues (2011) point out that such energetic costs could also explain their contradictory finding that rats prioritised learning *horizontal* information, since in their experiment, in contrast to that of Grobéty and Schenk, the rats were not forced to travel vertically to locate food, such that the energetic costs of travelling vertically were likely to be lower. Although such reasoning does not explain why a third study, by Flores-Abreu and colleagues (2014), found that rats were more *accurate* at learning horizontal than vertical information, these results are not directly contradictory, since *prioritising* the learning of information is not synonymous with 'better' learning of information (Rowe and Healy 2014).

Although a difference in energetic costs between vertical and horizontal travel may appear to be a plausible explanation for differences in animals' abilities to learn vertical and horizontal information, it may not be a solution that fits all cases. The relative difference in the energy expended during vertical compared to horizontal travel depends on the size of the animal in question. For example, running uphill compared to over flat ground requires a much smaller relative increase in energy expenditure in mice than in chimpanzees (Taylor et al. 1972). This suggests that, although energetic costs could potentially influence learning of vertical compared to horizontal information in larger animals like humans, the difference in energetic expenditure may not be sufficient to explain differences in vertical and horizontal spatial learning in some smaller animals. We have been unable to find any empirical studies that investigate energy expenditure during vertical and horizontal travel in the species we have discussed so far. As a result it

is difficult to come to any definitive conclusions about the effect of energetic costs on spatial learning in surface-bound and non-surface-bound terrestrial animals.

The relative energetic costs of vertical and horizontal travel are thought to be very similar for fish species that possess a swim bladder (Alexander 1972). This is because the presence of the gas-filled swim bladder allows fish to equilibrate their density with the density of the surrounding water through passive increases or reductions in the levels of gas inside the swim bladder at different depths (Blaxter and Tytler 1978). Once fish are the same density as the surrounding water they become neutrally buoyant, and as a result, do not incur the elevated cost often associated with moving vertically (Alexander 1972; Holbrook and Burt de Perera 2011). The similar energetic costs experienced during vertical and horizontal travel may explain why the Mexican tetra (*A. mexicanus*) learns vertical and horizontal information at a similar rate (Holbrook and Burt de Perera 2009) and with similar accuracy (Holbrook and Burt de Perera 2013).

Although it is possible that the energetic costs of travelling in different dimensions may contribute to some animals' differential learning of vertical and horizontal information, it is not possible to come to this conclusion definitively as there are so few studies measuring the difference in energy expenditure during vertical and horizontal travel, particularly in non-surface-bound animals like hummingbirds and bats. Studies of three-dimensional spatial navigation in hummingbirds suggest that they remember vertical information more accurately than horizontal information, as Grobéty and Schenk (1992) described in the early stages of their experiments with rats (Flores-Abreu et al. 2014; Hurly et al. 2010). However, unlike in experiments with rats (Grobéty and Schenk 1992), a hummingbird's superior memory of vertical information seemed to persist throughout the entire experimental period. This could be explained by following the same reasoning

as was used to explain the results in rats, i.e. that higher energetic costs in the vertical dimension cause the hummingbirds to prioritise learning vertical information. It is unclear whether terrestrial non-surface-bound (flying) animals are likely to experience similar energetic limitations to surface-bound animals when moving vertically. Investigations into the energetic demands of hummingbird flight suggest that the energy required for forward and hovering flight is similar (Suarez 1992), at least in *S. rufus*, however we have been unable to find any studies measuring energetic expenditure in these birds during purely vertical flight. One possibility is that hummingbirds are more similar to mice in that horizontal locomotion is so costly that the relative increase in energy expenditure during vertical travel is negligible (Taylor et al. 1972), if this is the case, energy expenditure may not be a sufficient explanation for hummingbird's superior learning of vertical information. Studies of spatial navigation in bats have yielded different results to findings in hummingbirds. Specifically, neurophysiological experiments on bats showed that they encode vertical and horizontal information with similar resolution and thus are likely to remember information from all three dimensions with similar accuracy, making them more similar to fish than to hummingbirds in this regard (Yartsev and Ulanovsky 2013). However, this result remains to be confirmed with behavioural experiments. As is the case with hummingbirds, we have been unable to find any studies comparing the energy required for vertical compared to horizontal flight in bats; however relative energy expenditure is likely to differ dramatically between different species based on body mass, wing span and flight style (Winter and Von Helversen 1998).

Aside from the questions that remain about energy expenditure in different animals it can be difficult to draw conclusions from studies of rats or humans and apply them to bats and hummingbirds. This is mainly because these animals differ vastly in their movement ecology, sensory systems, feeding habits and habitat preferences, as well as size, all of

which are likely to have their own influence on spatial learning. Finally, because three-dimensional spatial cognition has only been explored in a very small subset of species, it can be difficult to determine whether these findings represent general principles that can be applied in a wider context, or are specific to the species investigated so far.

Jackson and Cormack point out that ascending and descending vertically is associated with an increased risk of serious injury resulting from a fall, whereas this risk is much lower on horizontal terrain (Jackson and Cormack 2007, 2008). They propose that disproportionate risk rather than differential energetic costs in the different dimensions could contribute to the asymmetry observed in humans' estimations of vertical distances (Brandt et al. 2015; Jackson and Cormack 2007, 2008) and slopes (Kammann 1967), and perhaps manifest in their mental representation of vertical and horizontal dimensions in a similar way to that observed in rats (Hayman et al. 2011). Jackson and Cormack reason that natural selection should favour individuals that avoid taking routes associated with unnecessary risk. Individuals who perceive slopes to be steeper than they are in reality are more likely to avoid these routes, and by erring on the side of caution, might be more likely to survive and reproduce (Jackson and Cormack 2008). However, this reasoning implicitly assumes that given the correct information about vertical distances, humans would make sub-optimal decisions, either by underestimating the risk or overestimating the rewards of travelling on steep gradients. Further, the risk of serious injury is dependent on an animal's size. For example, smaller animals, such as rodents, have a much lower terminal velocity than humans, and are therefore unlikely to face the same risks of injury on steep gradients as humans. Consequently, we suggest that although the heightened risk of falling associated with vertical travel may have some influence over animals' perceptions of vertical distances; again, it is unlikely to be the only explanation

for the asymmetry in the accuracy with which surface-bound animals remember vertical and horizontal information.

3.4 Ecological niche

An animal's ecological niche is likely to impose important selective pressures that will contribute to the evolution of spatial cognition in three dimensions. For example, the extent to which an animal must travel in the vertical dimension depends, in part, on whether they are a ground-nesting and -foraging species whose food source varies primarily in the horizontal dimension, or a tree-nesting species whose food source varies in the vertical (and horizontal) dimension. Nieh and colleagues (2003) report that two species of Brazilian stingless bee differ in their ability to communicate the height of a familiar food reward to nest mates. *Melipona bicolor*, a canopy-foraging species, was able to recruit nest mates to the correct height of a food source, whereas *M. mandacaia*, a ground-foraging species, was not. This result could not be explained by a difference in their spatial learning ability *per se*, as both species remembered horizontal information equally well (Nieh et al. 2003). Instead, it seems likely that accurate encoding of vertical information (and thus, three-dimensional navigation) is selected for in species whose food source varies in all three dimensions, but not selected for in species where it does not. Analysis of the waggle dance of honeybees (*Apis mellifera*) found no evidence that they communicate information from the vertical dimension to nest mates, which is surprising given that they forage in the vertical dimension in a similar environment to *M. bicolor*. However, Dacke and Srinivasan point out that *A. mellifera* could be using acoustic signals rather than elements of their dance to convey vertical information, though this remains to be confirmed empirically (Dacke and Srinivasan 2007).

Based on the findings in the stingless bee we might expect that other animals that move with six degrees of freedom but spend most of their time foraging on the ground may be less competent at learning vertical information. However, this was not the case for the *Corydoras aeneus*, a benthic fish that can move with six degrees of freedom, but spends much of its time travelling over the riverbed and foraging for substrate-dwelling invertebrates. Contrary to expectations, *C. aeneus* encoded vertical and horizontal information similarly to a pelagic fish, *Astyanax mexicanus* (referred to as *A. fasciatus* in original work by Holbrook and Burt de Perera 2013). Both *C. aeneus* and *A. mexicanus* preferentially used vertical cues when vertical and horizontal cues gave conflicting information (chapter 3: Davis et al. 2014). This seems initially surprising in light of the findings in the stingless bee, and given that *C. aeneus* forages and travels mainly in the horizontal dimensions. However there may be selection on accurate vertical encoding in *C. aeneus* for reasons other than its foraging niche. It is known that *C. aeneus* sometimes inhabits hypoxic environments, and as a result has evolved a strategy where it swims to the surface and gulps air to oxygenate its blood (Kramer 1987). This means that individuals that recall vertical distances correctly are more likely to reach the surface before suffocation; therefore perhaps this is one of the factors driving the preferred use of vertical information over horizontal information in *C. aeneus*. Further investigation into spatial cognition in other benthic species that do not exhibit 'gas-gulping' might help to shed light on these findings.

There are still very few studies investigating three-dimensional spatial cognition in vertebrates that exhibit similar movement ecology but whose ecological niche means that they may travel vertically to different extents. However, there are many other examples of vertebrates, besides benthic fish, that move with six degrees of freedom but spend most of their time moving over the terrain that may help us elucidate the importance of

environmental niche-imposed selection on spatial cognition. For example, some wading birds like oystercatchers (*Haematopus ostralegus*) nest and forage on the ground; similarly, long-distance migrating birds spend a larger proportion of their migration travelling horizontally than vertically. By studying species that can move in three dimensions but spend most of their time travelling in two dimensions, it may be possible to establish the relative importance of day-to-day movement (due to their ecological niche) and movement ecology (due to their ancestral lineage). This, in turn, could help to separate the environmental and genetic factors contributing to spatial cognition.

Similarly, it would be useful to investigate three-dimensional spatial cognition in the reverse condition: in surface-bound animals that regularly travel in all three dimensions. Humans and non-human primates are potentially an extremely useful system for comparing the importance of ecological niche and movement ecology. Their brains are thought to be somewhat similar in their structure and function (Taube and Shinder 2013), and both have been found to possess place-responsive cells (humans: Ekstrom et al. 2003; squirrel monkeys: Ludvig et al. 2004) that function similarly to the place cells identified in rats (Hayman et al. 2011), bats (Yartsev and Ulanovsky 2013), and birds (Siegel et al. 2005). However, although humans and non-human primates are both surface-bound animals (according to our earlier definition), their movement ecologies differ considerably. Where humans spend most of their time travelling over horizontal surfaces, arboreal primates leap vertically and horizontally between the branches of trees (Howard and Fragaszy 2013). Therefore, we may expect arboreal primates to encode vertical information more accurately than humans, whose vertical distance estimation is less accurate than their horizontal distance estimation. Establishing whether there are differences in humans' and non-human primates' memories and representations of vertical information requires firstly, behavioural investigation into whether non-human

primates exhibit similar discrepancies between vertical and horizontal distance estimations as has been demonstrated in humans (Brandt et al. 2015; Hicks and Rivers 1908; Renier et al. 2006).

It is likely that experience will play an important role in animals' abilities to navigate and estimate distances in three dimensions, as a result, vertical distance estimation in humans may vary depending on an individual's daily activities. For example, those that regularly play sports that require them to judge the speed and distance of a ball moving through three dimensions are likely to be more accurate at judging vertical and horizontal distances than those that do not. Therefore it may be important when comparing arboreal primates and humans to compare individuals matched for their experience of judging vertical and horizontal distances on a regular basis.

There are currently no experiments investigating three-dimensional spatial cognition in species that exhibit different movement ecology depending on their life stage, but establishing how changes in day-to-day movement throughout different developmental stages influences spatial cognition, might further illuminate the importance of ecological niche and movement ecology. For example, frogs (tadpoles) and reef fish have a freely-moving juvenile life phase, where they will move vertically and horizontally to a similar degree and a somewhat surface-bound adult life phase where they are likely to spend more time moving horizontally than vertically. Undoubtedly, comparing the three-dimensional spatial abilities between the life stages of animals that undergo vast growth or complete metamorphosis will pose challenges. In particular, researchers will need to disentangle the possible effects of neural restructuring or extensive brain growth from experience-lead development. Nevertheless, if this can be done, the findings of studies in these animals may help to further resolve the relative importance of day-to-day movement

(imposed by ecological niche) and genetic pre-programming (imposed by phylogeny) on spatial cognition.

3.5 Summary of key factors

The evidence discussed throughout this section (3.1-3.4) has enabled us to come to some conclusions about which factors may be influencing the asymmetry in surface-bound animals' learning of vertical and horizontal information, and why these factors might influence three-dimensional spatial learning in non-surface-bound animals differently.

Firstly, a difference in the cues available in the vertical versus horizontal dimensions *per se* is not sufficient to explain the asymmetry in animals' memory for vertical and horizontal information. For example, fish have access to pressure information in only the vertical dimension, and yet learn and remember vertical and horizontal information with similar accuracy (Holbrook and Burt de Perera 2013). Instead, perhaps the quality of the information derived from the cues themselves is responsible for the discrepancy in animals' learning of vertical versus horizontal information. For example, vestibular cues (used predominantly during vertical travel) may yield lower quality information than visual cues (used predominantly during horizontal).

Alternatively, animals' own sensory systems could be responsible for their lower accuracy at remembering vertical information. For example, inherent biases in the visual system may lead to misrepresentation of vertical distances in the human memory (Renier et al. 2006), causing a mismatch in humans' accuracy when recalling vertical versus horizontal information (Brandt et al. 2015); whether this is also the case for other surface-bound animals remains to be tested. In some non-surface-bound animals, this problem may be reduced because they supplement their vision with other senses when navigating

in the vertical dimension, such as biosonar in bats (Danilovich et al. 2015) and hydrostatic pressure in fish (Chapter 5; Holbrook and Burt de Perera 2011; Taylor et al. 2010).

Another possible explanation for the misrepresentation of vertical distances is the discrepancy in the energetic cost between travel in the vertical compared to the horizontal dimensions (Proffitt et al. 2003); however, without empirical studies investigating the relative difference in animals' energetic expenditure during vertical and horizontal travel we cannot come to any definitive conclusions about this.

Ultimately, many of these factors are likely to be shaped by selection pressures which differ depending on an animal's ecological niche, in terms of the extent to which it must travel in the vertical dimension in order to survive. In animals that forage and nest in the vertical (and horizontal) dimension, such as bats and fish, evolution is likely to favour systems which allow these animals to learn and remember vertical and horizontal information with similar accuracy, whereas these selection pressures are likely to be much reduced in animals that spend most of their time travelling in the horizontal dimension.

4. Genetic and environmental influence on spatial cognition

So far we have discussed some of the environmental, physiological and ecological factors that may contribute to variation in the abilities of animals to learn and remember the vertical and horizontal components of three-dimensional space. Experimental evidence suggests that animals' sensory systems are among the factors that might be important in ultimately shaping animals' internal representation of vertical and horizontal space (Brandt et al. 2015; Renier et al. 2006). However, what remains unclear is whether the way that sensory perception influences vertical and horizontal navigation is ultimately

determined by evolutionary or developmental processes. For example, neural architecture and behavioural responses could be shaped primarily by an individual's evolutionary history (i.e. its genes), or by environmental cues experienced during sensory system development. In the former case, all individuals of the same species should encode three-dimensional space in the same way because their development is genetically pre-programmed and cannot be modified through experience; in the latter case, three-dimensional cognitive abilities should develop differently in individuals who have been exposed to environments where vertical and horizontal spatial stimuli are available to varying degrees. In this section, we discuss the evidence for the influence of environmental and genetic factors on vertical and horizontal navigation.

One way that researchers have been able to investigate the relative contributions of environmental and genetic factors is to observe whether adult surface-bound animals can form accurate internal representations in *truly* three-dimensional conditions, the way they can in two-dimensional conditions. By "truly three-dimensional", we refer to environments not simply composed of vertical and horizontal planes, but which instead give animals the opportunity to move in both vertical and horizontal dimensions freely and simultaneously. If adult surface-bound animals are able to form three-dimensional representation in these environments, it would indicate that despite having developed in an environment where vertical movement was restricted, these animals, given sufficient environmental experience, can adapt their mental representation to fit three-dimensional conditions.

There are logistical difficulties in observing surface-bound animals in truly three-dimensional conditions because their natural movement does not permit them to move unconstrained in three dimensions, the way flying and swimming animals can. In an

attempt to overcome this limitation, researchers have resorted to using virtual reality or placing animals in zero-gravity settings. These types of experiments help to separate whether the differences in surface-bound and non-surface-bound animals' abilities to learn and remember three-dimensional information are a result of (a) constraints in their movement or (b) constraints in their cognitive abilities (resulting from differences in brain structure and/or function). The key difference between these possibilities is that movement constraints influence the type of interoceptive (vestibular and proprioceptive) sensory information that the animals receive (environmental constraint), whereas cognitive constraints influence learning, memory storage and retrieval of information (genetic constraint).

Results from studies like these can indicate whether, over time, adults can begin to develop a three-dimensional representation of their environment; that is, the extent to which fully-developed surface-bound adults can apply their existing spatial cognitive architecture to three-dimensional contexts. Although few studies have directly set out with the objective of answering this question, there are studies we can draw on to get partial answers. These experiments have been undertaken in rats (Knierim et al. 2000) and humans (Vidal et al. 2003, 2004). The findings suggest that both of these species can eventually form a representation of their three-dimensional environment; however this representation takes longer to build and is more prone to error than representations in two-dimensional environments (i.e. a horizontal plane). Rats in zero-gravity conditions that were required to walk a path winding through rotations of yaw and pitch eventually developed stable place fields in three dimensions, but these took much longer to stabilise than it normally takes for fields in two-dimensional environments to stabilise (Knierim et al. 2000). Humans that experienced a virtual route taking them through rotations in yaw, pitch and roll could eventually recognise and identify the map of the route they had taken,

but only for simple routes. For example, routes with five turns instead of three vastly reduced humans' abilities to match their virtual experience with the correct physical map (Vidal et al. 2004).

One issue with the study by Vidal and colleagues is that when subjects are seated while they experience a virtual journey, as they were in this experiment, they do not receive the interoceptive (proprioceptive and vestibular) information normally available during a real journey, which could affect their ability to learn a route (Ludvig et al. 2004). For example, human horizontal distance estimation is improved when subjects have access to both visual and interoceptive cues (Sun et al. 2004). Similarly, animals' place cell firing patterns differ between conditions where they are with and without access to interoceptive information (Ludvig et al. 2004). Nevertheless, we do not feel this diminishes the importance of the findings in experiments by Vidal and colleagues, as subjects did not receive interoceptive cues in any of the conditions in the experiment (Vidal et al. 2004). Thus subjects' performances in the different treatment groups of this study are comparable with each other.

The findings that surface-bound animals' navigational performance in three dimensions never reaches that of their performance in two dimensions also extends to experiments where animals can move in all three dimensions but are in contact with a surface. For example, a study testing mice's memories of baited locations in a three-dimensional maze found that they could form short-term memories of locations where food had been depleted, but they could not commit this information to longer-term memory (Wilson et al. 2015).

Overall, the results of these studies suggest that three-dimensional spatial memory formation in adult surface-bound animals that experience truly three-dimensional

conditions is lacking in three areas: (1) the time that it takes to form the memories (Knierim et al. 2000), (2) the complexity of the information that can be remembered (Vidal et al. 2004) and (3) the length of time these memories can be stored and later recalled (Wilson et al. 2015). These results indicate that both genetic and environmental factors might be influencing spatial cognition. The fact that animals are able to build a three-dimensional representation of their environment after exposure to three-dimensional conditions suggests that environmental experience can eventually lead to animals adapting their spatial representation to their new environmental conditions. However, because animals appear never to learn and remember three-dimensional information as well as they learnt two-dimensional spatial information, this suggests some form of genetic constraint on their learning and cognition.

In support of behavioural experiments, neurophysiological work also suggests an important role for both the environment and genes in shaping three-dimensional spatial learning in animals. Research investigating the development and maturation of neural networks associated with spatial cognition in rats showed that the cells involved in encoding space are almost completely developed before the animals leave the nest (Langston et al. 2010; Wills et al. 2010). Head-direction cells emerge very early in development: after around 12 days of life. They are present prior to the opening of the eyes (Tan et al. 2015) and have matured into their adult form after 16 days of life (Langston et al. 2010; Wills et al. 2010). Place and grid cells emerge comparatively later in development, but both are capable of exhibiting adult activation patterns during rats' first explorations of their environment. However, the firing fields of these cells become steadily more stable with increasing experience gained through exploration of the environment (Langston et al. 2010; Rowland et al. 2011; Wills et al. 2010).

Taken together, these results suggest that both genetic and environmental factors are important in shaping neural aspects of spatial cognition, at least in rats. Firstly, the development of the neural architecture specialised for spatial cognition is largely genetically pre-programmed, with functioning head direction cells developing prior to experience of navigating in the external environment. Secondly, grid and place cell activation patterns become more stable and more similar to those of adults with direct environmental experience, suggesting that cues in the environment may help to refine spatial representations of specific environments. These findings corroborate findings in behavioural studies showing that environmental experience can modify spatial encoding of the environment but that the functioning of the cells themselves is fixed.

So far in this section, we have discussed experiments in which either (1) spatial memory was examined in surface-bound adults that were placed in truly three-dimensional conditions or (2) neural development was observed in surface-bound animals inhabiting their natural environment. Both methods help to illuminate the relative contributions of genetic and environmental factors on three-dimensional spatial cognition. However, another way to address this topic is to study the development of spatial cognition in individuals that experience different spatial conditions during their maturation. An important role for this type of experiment also is to shed light on the development of spatial cognition in wild individuals exposed to both two-dimensional and three-dimensional cues in early life, for example by comparing spatial cognition in rats inhabiting a horizontally-stretched underground burrow with that of rats inhabiting a vertically-stretched burrow inside a wall cavity. Studying spatial cognition in juvenile animals could thus be key to understanding the ultimate determinants of species' spatial cognitive abilities.

There are numerous studies investigating the relative contributions of genetic and environmental factors on two-dimensional spatial cognition in juvenile animals. Findings from these studies have revealed that varying (1) reliability of visual landmarks (Girvan and Braithwaite 2000), (2) the presence of geometric information (Brown et al. 2007) and (3) predator abundance (Brydges and Braithwaite 2009) in animals' rearing environments, can alter the speed of learning and the mechanisms underlying spatial learning. However, we have been unable to find any studies investigating three-dimensional spatial cognition in animals reared in environments where information from the vertical or horizontal dimensions has been selectively blocked during early development.

We propose that one way to investigate the effect of absent horizontal and vertical cues during development would be to rear animals in environments where their experience of vertical movement is limited in one condition and their horizontal movement is limited in another. Fish represent an ideal model species to investigate vertical and horizontal restriction as tank dimensions and water volume can be a simple way to constrain their three-dimensional movement (figure 3).

Vertically-restricted and horizontally-restricted groups of fish could be presented with spatial tasks which require them to solve a three-dimensional problem. If three-dimensional spatial cognition is exclusively a genetic trait, we would expect that both groups of fish would successfully solve the three-dimensional task, despite their experience of three-dimensional movement being limited during development. However, if normal three-dimensional spatial cognition is reliant on experience, we would expect these developmentally-restricted fish to be unable to solve the three-dimensional tasks they are presented with. These are examples of extreme outcomes.

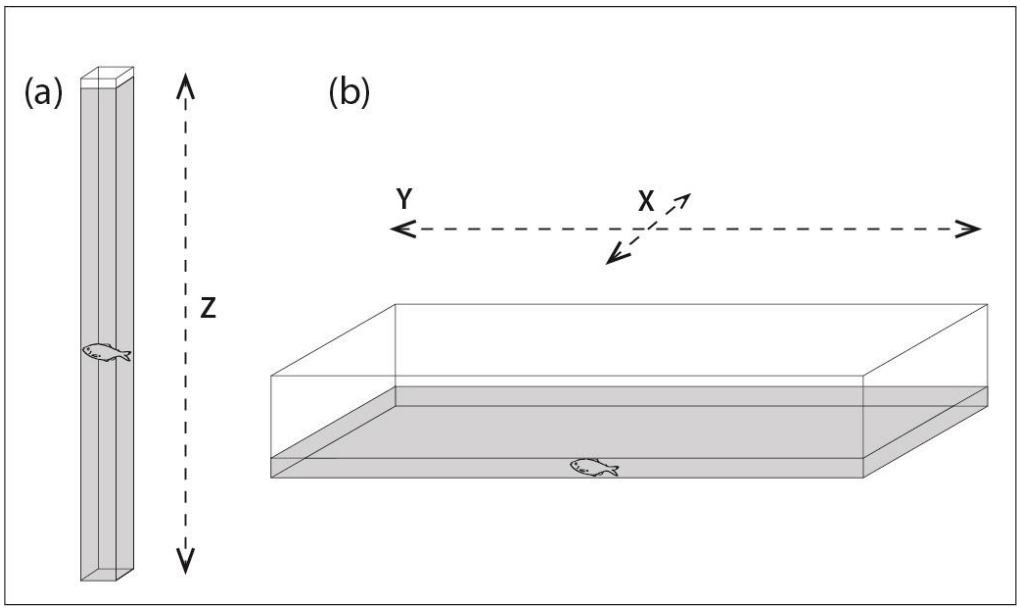


Figure 2. Horizontally- (a) and vertically-restricted (b) rearing environments for fish, enabling them to move in either the Z dimension, or the X and Y dimensions, respectively.

In reality, both genetic and environmental factors undoubtedly have a role in the resulting spatial cognitive abilities of animals, and the behavioural and neurophysiological studies discussed in this section corroborate this idea. However, experiments where vertical or horizontal information is selectively blocked could go some way to indicating the relative contributions of both factors on spatial learning.

5. Limitations of comparing studies

5.1 Developmental and testing environments

So far we have discussed (1) the differences in animals' abilities to learn and remember vertical versus horizontal information, (2) the factors that could be responsible for these differences and (3) the relative impacts of genetic and environmental factors on three-dimensional spatial cognition. However, the behavioural and neurophysiological studies

investigating three-dimensional spatial cognition are extremely diverse, and their idiosyncrasies can sometimes make it difficult to draw general conclusions from the work. In this section we discuss some of the limitations associated with drawing conclusions from the existing research, and suggest ways that these limitations could be addressed by future studies.

The animals involved in the experiments we have discussed so far are likely to have experienced vastly different conditions from each other during their early development. Some studies featured wild animals (Flores-Abreu et al. 2013; Hurly et al. 2010; Jacobs and Shiftlett 1999) while others used animals reared in captivity (Holbrook and Burt de Perera 2009, 2013; Jovalekic et al. 2011). There will, undoubtedly, be inconsistencies even between individuals reared in captivity at different institutions, as the conditions are likely to differ between laboratories according to facilities and accepted practices. There is extensive evidence that the environment that animals develop in can influence their resulting spatial cognition (section 4; Brown et al. 2007; Girvan and Braithwaite 2000; White and Brown 2014). For example, a paper by Brown and colleagues (2007) showed that although fish raised in circular tanks (instead of rectangular tanks) were no less able to use geometric cues for navigation, when geometric and featural cues conflicted they relied preferentially on featural cues. Therefore any inconsistencies in animals' rearing conditions between studies could be a potential factor contributing to the differences observed in spatial cognition in the adult animals, independent of factors connected with their movement ecology. Animals reared in environments with limited opportunity for vertical travel and exploration may either not develop the neural connections observed in their wild counterparts, which are necessary for encoding three-dimensional information (Tarr et al. 2009), or they may be less skilled physically at moving vertically, if they have not developed the motor skills and/or the muscles necessary to do so (Huck and Price

1976). It is therefore necessary to establish the contribution of environmental conditions during early development on three-dimensional spatial cognition by comparing spatial learning between captive and wild individuals of the same species. Only by doing this can we disentangle the effects of study-specific rearing environments from genuine species-specific differences in spatial cognition.

As with the rearing environment, the apparatus used to create the three-dimensional environment that animals are tested on varies considerably between studies, and can be categorised according to the freedom of movement afforded the test species. In some studies there is no explicit apparatus, and instead animals are observed in truly three-dimensional environments where they are able to move freely in all three dimensions (Hurly et al. 2010; Vidal et al. 2004; Yartsev and Ulanovsky 2013). In other studies, animals are tested in conditions where they can move in three dimensions but their behaviour is dictated by the apparatus present. This is either because they are naturally surface-bound and thus must be in contact with it as they travel (for example, the rats in the study by Flores-Abreu et al. 2014; Grobéty and Schenk 1992; and a study in mice by Wilson et al. 2015) or they are artificially constrained to be surface-bound because the food rewards are located on the apparatus (for example, the hummingbirds in the study by Flores-Abreu et al. 2013, 2014). Finally, in some studies movement is restricted to different degrees by the routes available in three-dimensional mazes (Hayman et al. 2012, 2015; Holbrook and Burt de Perera 2009, 2013; Jovalekic et al. 2011; Wilson et al. 2015) or buildings (Brandt and Dieterich 2013; Zwergal et al. 2015).

Experiments can be designed to investigate how animals navigate under environmental conditions with different degrees of freedom. However, problems can arise when trying to generalise findings between these studies. Firstly, there are currently no

neurophysiological studies of rats moving in truly three-dimensional environments in which their place cell and grid cell field dimensions were measured. These measurements have, thus far, only been made in environments where certain aspects of the rats' movement have been restricted, either vertically (sloping horizontal plane), horizontally (sheer vertical peg board) or both (helix), but evidence suggests that the shape of the enclosure or test arena can influence the activation patterns of grid cells (Krupic et al. 2015). Secondly, Taube and Shinder (2013) point out that in experiments where rats were observed moving over a vertical pegboard apparatus, their body orientation was horizontal (Hayman et al. 2011; Jovalekic et al. 2011) rather than vertical, which may have influenced the shape of their grid cell and place cell activation fields. Therefore, it can be difficult to draw definitive conclusions about whether the shapes of place cell fields are due to an animal's natural movement ecology or artefacts of (1) the apparatus the animal was observed on and (2) the way it travelled over it (Taube and Shinder 2013).

5.2 The objective of a spatial task

Many studies investigating three-dimensional spatial cognition address slightly different questions. For example, some investigate animals' abilities to memorise the three-dimensional location of a goal(s) (Flores-Abreu et al. 2014; Grobéty and Schenk 1992; Holbrook and Burt de Perera 2009, 2013), while others observe animals passively exploring their environment in search of food (Jovalekic et al. 2011; Wilson et al. 2015). Although the distinction between these studies may at first seem trivial, there is evidence from behavioural (Jovalekic et al. 2011) and neurophysiological (Marozzi et al. 1995) studies to suggest that the objective of a task may influence animals' spatial learning and neural activation patterns. For example, in a task where rats passively explored an environment looking for food, they learnt the horizontal part of the route first (Jovalekic

et al. 2011), whereas in a task where they were required to memorise the position of a food source, they learnt the vertical component of the journey first (Grobéty and Schenk 1992), perhaps to minimise the energetic costs that could result from performing random searches in the vertical dimension (Jovalekic et al. 2011). Similarly, in tasks where rats had to memorise a location, their place cell fields exhibited high directionality, in that the place cells would fire when a rat entered a region from one direction, but not when they entered it from another. This is thought to be a result of the sequential way that animals learn route information (Marozzi et al. 1995); for example, they must remember the order of a sequence of landmarks on the way to the food reward if they are to find it again. In contrast, the place cells of rats passively exploring their environment did not exhibit directionality, perhaps as a result of rats not needing to memorise the order of a sequence of landmarks (Marozzi et al. 1995).

5.3 Motivation

Behavioural studies have shown that hungry individuals value food more highly than satiated individuals (Pompilio et al. 2006) and as a result, hungry individuals are likely to be more motivated and better able to retain information about the location of food (Stewart et al. 1989). In experiments testing three-dimensional spatial cognition, specific locations are paired with a food reward, and subjects are restricted to a certain percentage of their body weight to facilitate learning and enhance memory; yet whilst a 90% body weight restriction is typically imposed (e.g. Flores-Abreu et al. 2014; Grobéty and Schenk 1992; Jovalekic et al. 2011; Wilson et al. 2015), not all studies follow this convention (e.g. Davis et al. 2014; Holbrook and Burt de Perera 2009, 2013). This poses potential problems when making comparisons between studies, as animals may be similarly capable of learning three-dimensional information but differ in the way they

perceive a food reward (Rowe and Healy 2014) which could manifest in behavioural experiments as a difference in their accuracy at returning to the location of a food reward.

5.4 A lack of overlap in neurophysiological and behavioural experiments

In most studies, researchers either observe behaviour or record neurophysiological activity, but few do both. This means that it can be difficult to correlate findings from behavioural and neurophysiological studies, particularly in those animals where we only have information on one of these aspects. For example, there are behavioural studies in fish (Davis et al. 2014; Holbrook and Burt de Perera 2009, 2013) and hummingbirds (Flores-Abreu et al. 2013, 2014; Hurly et al. 2010) but currently no neurophysiological studies in these species. This can create problems when drawing comparisons between animals for which we have neurophysiological data and animals we do not, such as fish and bats. Although we expect both fish and bats to learn three-dimensional information similarly because of their similar movement ecology, there is no way to corroborate similarities in their neural representations definitively without comparing neurophysiological activation in both species. Conversely, much of the research into three-dimensional spatial cognition in bats focusses on the neurophysiological aspects of their three-dimensional navigation (Geva-Sagiv et al. 2015; Yartsev and Ulanovsky 2013). Although there are numerous investigations into behaviour during navigation in bats, many of these experiments either do not explicitly investigate vertical and horizontal components of navigational behaviour (Jensen et al. 2005; Moss and Surlykke 2010), or focus on microbat species, which possess different sensory abilities (Lawrence and Simmons 1982; Mogdans 1988) to the megabats investigated in neurophysiological studies of navigation (Yartsev and Ulanovsky 2013).

Despite there being both neurophysiological and behavioural studies on rats, these two types of data are never recorded and collected simultaneously, and often take place on a different apparatus or in a different experimental environment (Grobéty and Schenk 1992; Hayman et al. 2012, 2015). One exception is that both rat behaviour (Jovalekic et al. 2011) and neural activation (Hayman et al. 2011) was recorded (over two separate experiments) as rats moved over a vertical pegboard. Behavioural observations indicated that rats spent most of their time moving horizontally where possible (Jovalekic et al. 2011) and neurophysiological recordings showed that grid and place cells were vertically-distorted during vertical travel (Hayman et al. 2011). Taken together, these results indicate that behavioural preferences map on to the neurophysiological findings well: the rats preferentially moved horizontally, and appeared to exhibit lower spatial resolution in their neural encoding of vertical space compared to horizontal space.

Investigating both neural and behavioural responses simultaneously during three-dimensional travel will reveal more than if both methods are conducted individually. Neurophysiological recordings can provide information about how these animals 'see' their environment, such as whether they encode vertical information at a lower resolution than horizontal information. This information can sometimes be difficult to obtain from behavioural studies alone, as numerous factors can influence ultimate behaviour independently of cognition, including energetic status and motivation (section 5.3; Ludvig et al. 2004). Similarly, neurophysiological recordings that are not accompanied by behavioural observations can be difficult to interpret, particularly as natural selection is acting on specific behaviours and not the resulting neural activation. Therefore collecting both neurophysiological and behavioural data will provide a clearer overall picture of how the information is coded and interpreted by the brain, and how this manifests in animals' navigational behaviours.

5.5 A lack of studies investigating neurophysiological activity in the wild

The dimensions of place cell and grid cell activation fields have been well-studied in a variety of species in the laboratory (Hayman et al. 2012, 2015; Siegel et al. 2005; Yartsev and Ulanovsky 2013). However, it might not always be appropriate to extrapolate information from tests in artificial environments to animals in natural settings. For example, although enclosure *size* does not appear to affect grid cell firing patterns (Geva-Sagiv et al. 2015) – apart from some minor transient disruptions thought to be explained by novelty (Barry et al. 2012) – grid activation patterns are affected by enclosure *shape*, with the symmetry and homogeneity of their activation patterns most affected in highly disproportioned enclosures such as those shaped like a trapezoid (Krupic et al. 2015). Because artificial test arenas often have very defined boundaries, they may influence grid cell activation differently from natural boundaries present in the wild. Recording grid cell firing patterns in natural environments will go some way to establishing whether the apparent shape and regularity of these cells' fields is an artefact of experimental arenas or an implicit quality of grid cell firing.

An empirical study of place cell firing in rats exploring different-sized enclosures has shown that place cells that exhibit single fields in small environments (68 cm) have multiple activation fields in larger environments (1.5 metres); indicating that these cells 'behave' differently in large versus small enclosures (Fenton et al. 2008). This finding illuminates the importance of studying animal navigation in the wild, or at least in enclosures that are large enough that results are comparable to studies in the wild. Using virtual reality simulations may present a good opportunity for investigating navigation in a large scale environment without the need to build huge enclosures or monitor animals in sometimes logistically challenging natural environments. However, Ludvig and

colleagues (2004) advise that this should be undertaken with caution as, in some cases, virtual reality may not be an adequate substitution for the real world (Ludvig et al. 2004). In designs where participants are seated or restrained, the resulting place and grid cell fields may not adequately represent normal neural activation patterns. This is because interoceptive signals like proprioceptive and vestibular cues, which are available during unrestrained self-motion, but absent when participants are restrained, appear to be important for regulating the regions where place cells become activated (Ludvig et al. 2004). Taken together, these findings suggest that neurophysiological monitoring in *very* large enclosures or in animals' natural habitats, is likely to best illuminate real-time brain activity and encoding of vertical and horizontal information during three-dimensional navigation in the wild.

6. Conclusion

We have reviewed the literature on three-dimensional spatial cognition in animals with different movement ecologies, i.e. surface-bound and non-surface-bound animals. Our overall finding is that surface-bound animals perform less well at remembering vertical information than horizontal information, whereas non-surface-bound animals either (1) remember vertical information more accurately than horizontal information or (2) remember information from all dimensions similarly. We have suggested factors that could be driving the differences in learning and memory of vertical information between animals with different movement ecologies. We propose that the most important amongst these factors are likely to include i) the cues that animals use for vertical versus horizontal navigation ii) the resolution of an animal's sensory systems, iii) the disproportionate energetic costs of travelling vertically versus horizontally, iv) animals' ecological niches, v) genetic constraints on neurophysiology, and vi) environmental experience.

Currently, the studies investigating three-dimensional spatial cognition are extremely diverse, with different species being investigated and the conditions under which they are tested varying among studies. Furthermore, some researchers collect data in the form of neurophysiological recordings, whilst others make behavioural observations. This diversity provides a wealth of information about spatial cognition in different animals and in different environmental contexts. However, what remains is to disentangle the relative contributions of the potential driving factors – such as animals' ecological niches, the energetic costs associated with vertical travel and the sensitivity of animals' sensory systems – and perhaps to identify other factors that have not been considered so far.

This is a relatively new and fast-growing field, and future work could be focused in the following areas: firstly, experiments in which behavioural and neurophysiological recordings are made simultaneously would help to increase the likelihood that causal, rather than correlative conclusions can be drawn from their findings. Secondly, more studies investigating navigation in wild animals and in natural habitats will clarify which findings are a result of experimental artefacts caused by experimental apparatus and enclosures, and also give us a clearer idea of how animals face real-world three-dimensional navigational challenges. Beyond this, more work in a larger variety of animals will help to increase the generality of findings, provided that experimental procedures between studies are appropriate for comparative analyses (see section 5) (Harvey and Pagel 1991). In particular, studies in closely-related species that occupy different ecological niches, or members of the same species occupying different types of habitats, would go some way to disentangling the relative contributions of environmental and genetic factors.

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Chapter 3

Three-dimensional spatial cognition in a benthic fish, *Corydoras aeneus*

Davis, V.A., Holbrook, R.I., Guilford, T. and Burt de Perera, T. 2014

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Abstract

The way animals move through space is likely to affect the way they learn and remember spatial information. For example, a pelagic fish, *Astyanax mexicanus*, moves freely in vertical and horizontal space and encodes information from both dimensions with similar accuracy. Benthic fish can also move with six degrees of freedom, but spend much of their time travelling over the substrate; hence they might be expected to prioritise the horizontal dimension. To understand how benthic fish encode and deploy three-dimensional spatial information we used a fully rotational Y-maze to test whether *Corydoras aeneus* (i) encodes space as an integrated three-dimensional unit or as separate elements, by testing whether it can decompose a three-dimensional trajectory into its vertical and horizontal components, and (ii) whether it prioritises vertical or horizontal information when the two conflict. Contrary to our expectation, our results indicate that *C. aeneus* is better at extracting vertical information than horizontal information from a three-dimensional trajectory, suggesting that the vertical dimension is learned and remembered robustly. Our results also showed that *C. aeneus* prioritises vertical information when it conflicts with horizontal information. From these results, we infer that benthic fish attend preferentially to a cue unique to the vertical dimension, and we suggest that this cue is hydrostatic pressure.

1. Introduction

The ability to navigate through familiar areas is central to a diverse range of animal behaviours, from foraging and predator avoidance to territoriality. One way that animals can navigate efficiently is to learn and remember cues from the environment, and encode these into a representation of space. Previous work has focussed on understanding the mechanisms behind spatial cognition in animals navigating predominantly in the horizontal dimension, but has largely overlooked animals navigating in the vertical dimension (Collett et al. 1986; Kamil and Jones 2000; Tommasi and Vallortigara 2000).

The experiments that have investigated three-dimensional spatial cognition in rats have produced conflicting results. Rats are surface-bound animals that move with three degrees of freedom: two translational (forward/backwards, left/right) and one rotational (yaw) (Holbrook and Burt de Perera 2011a). When presented with passive exploration tasks within a cubic lattice maze, rats navigated by the so-called "layer strategy" whereby all food rewards were obtained on each horizontal plane prior to moving vertically to explore the next (Jovalekic et al. 2011). In contrast, in similar experiments by Flores-Abreu and colleagues (2014) rats moved vertically more than they moved horizontally, and in spatial memory tasks, rats appeared to learn the vertical location of a reward before the horizontal location (Grobéty and Schenk 1992). Grobéty and Schenk (1992) suggest that vertical space may provide more salient information as it is paired with stronger kinaesthetic cues and greater relative energy expenditure. Therefore, when it comes to memorising the location of a reward, they suggest that although vertical movements may be learned more quickly, horizontal movements will be maximised where possible, to conserve energy (Jovalekic et al. 2011). According to both behavioural (Flores-Abreu et al. 2014; Jovalekic et al. 2011) and neurophysiological studies (Hayman et al. 2011;

Hayman et al. 2015), rats encode vertical information less accurately than horizontal information.

Unlike surface-bound rats, non-surface-bound pelagic fish move with six degrees of freedom: three translational (forward/backwards, left/right, up/down) and three rotational (pitch, roll and yaw) (Holbrook and Burt de Perera 2011a). In behavioural experiments where fish are deprived of informative visual cues, they have been shown to encode the horizontal and vertical dimensions with similar accuracy (Holbrook and Burt de Perera 2013). Fish prioritise the vertical dimension in tasks where horizontal and vertical cues conflict (Holbrook and Burt de Perera 2009). Unlike surface-bound animals, fish are unlikely to experience such a disparity in the energetic cost between vertical and horizontal movement, as changes in the molar amount of gas present in the swim bladder after vertical movement allows them to become neutrally buoyant irrespective of their depth (Alexander 1972; Holbrook and Burt de Perera 2011a; Schmidt-Nielsen 1971; Taylor et al. 2010).

Studies exploring three-dimensional spatial cognition in birds have used the rufous hummingbird (*Selasphorus rufus*) as a model. Hummingbirds feed from flowers that refill at different rates, and as such, rely on their spatial memory to best exploit resources and reduce the risk of revisiting flowers they have previously emptied (Healy and Hurly 1995). In a three-dimensional cubic lattice maze, *S. rufus* moved in all three dimensions equally, in contrast to rats, which spent more time moving vertically (Flores-Abreu et al. 2014). It is perhaps not surprising that there is a disparity in rats' and birds' use of space, as, like fish, birds move with six degrees of freedom, while rats being constrained to moving over a surface move with only three degrees of freedom. However, despite having a similar movement style, *S. rufus*' spatial encoding appears to differ from that of

fish, as, in experiments which put vertical and horizontal information into conflict, birds prioritised horizontal information (Flores-Abreu et al. 2013), but fish prioritised vertical information (Holbrook and Burt de Perera 2009, 2011b). Also, *S. rufus* encoded horizontal information less accurately than vertical (Flores-Abreu et al. 2014; Hurly et al. 2010), similar to rats (Hayman et al. 2011; Jovalekic et al. 2011), whereas fish encoded both dimensions with similar accuracy (Holbrook and Burt de Perera 2013). In a one-dimensional experimental paradigm *S. rufus* was able to find a rewarded flower within an array of unrewarded flowers when the array was in a horizontal linear arrangement but not when the array was in a vertical column. Experimental evidence suggests the reason for the disparity in vertical and horizontal array discrimination tasks is that birds inferred the location of a rewarded flower from its position relative to the heights of other flowers in a vertical array but learnt the absolute position of the reward in horizontal arrays (Henderson et al. 2006). Birds' consistency in finding the absolute position of the rewarded location in horizontal arrays decreased markedly when inter-flower distances were reduced from 30 cm to 5cm, suggesting that both the orientation *and* the scale of the array was important for bird's discrimination of the reward location (Flores-Abreu et al. 2013).

The studies that have considered three-dimensional spatial cognition do not yet provide a clear picture of the extent to which the vertical component of animals' representation of space is encoded. Here, we tested how a benthic fish, the bronze corydoras (*Corydoras aeneus*), learnt, stored and recalled spatial information in a restricted three-dimensional environment, in the absence of informative visual cues. In the wild, *C. aeneus* lives in shoals, inhabiting shallow pools and slow-flowing watercourses throughout South America and southern North America (Burgess 1992). They are benthic and feed mainly

on small invertebrates that occupy soft sediments (Mills and Vevers 1989). Whilst *C. aeneus* is able to move with six degrees of freedom, like many pelagic fish species, much of their time is spent associated with the substrate, moving with three degrees of freedom, more similar to surface-bound animals. Consequently, we surmise that many of the navigational challenges faced by *C. aeneus* will be associated with horizontal, two-dimensional movements and, as such, we expect that they may encode space differently to pelagic fish, which move freely in three dimensions. Using a fully rotational Y-maze, we tested how *C. aeneus* uses spatial information, and whether it prioritises vertical or horizontal information. We predicted that *C. aeneus* would prioritise horizontal information over vertical, unlike the pelagic *A. mexicanus*, (referred to as *A. fasciatus* in original work by Holbrook and Burt de Perera) which prioritises vertical information (Holbrook and Burt de Perera 2009).

2. Methods

2.1 Subjects

Subjects were 13 bronze corydoras (*C. aeneus*). All individuals were born in captivity and fed on sinking catfish pellets prior to experiments. We attempted to preserve natural feeding behaviour by feeding them food that sank to the bottom of the tank. We cannot guarantee that fish only fed at the benthic level, however, as fish may have sometimes swum up to reach the sinking food pellets before they had sunk to the tank floor. Fish were between 6-12 months of age at the beginning of training and were tested in two cohorts: the first (n=6) between November 2012 and January 2013, and the second (n=7) between May and November 2013. All fish from both cohorts were used in the second experiment; however the first cohort were not available for use at the time of the first

experiment and consequently the sample size decreased by half between the two experiments.

Individuals from both cohorts were divided between three holding tanks over the testing period and kept under a standard 12:12 light:dark cycle at a temperature of 24°C. Tanks housed groups of three or four individuals separated from each other by clear perforated screens enabling visual and olfactory, but not physical, interaction. Fish were not fed between training trials unless they did not feed over more than two consecutive trial sessions which amounted to approximately four days.

2.2 Apparatus

A Y-maze constructed from clear Perspex (figure 1) was placed inside a tank measuring 60 cm^3, with opaque white walls. The maze rotated 360° so that the arms could be positioned in any orientation, although the arms themselves were set at a fixed angle so could not be moved independently of each other. At the terminus of each arm of the maze was a detachable end cap. The rewarded arm of the maze was baited with a single red TetraMin® flake attached with petroleum jelly to the end cap. During early stages of training, subjects received two rewards simultaneously: the first being the food reward and the second being a view of conspecifics, the second reward gave the subjects the opportunity to shoal. The conspecifics were housed in a clear glass box placed at the end of the rewarded arm. The rewards were not visible to the fish from the start box, but only once it had made a correct decision and begun to swim through the appropriate arm. The maze was pivoted about the axis of the start box (figure 1) before each training session to remove any intra-maze cues, such as the start box door orientation. Throughout training

and testing the maze was constantly repositioned around the tank, rendering proximal and distal extra-maze cues unreliable.

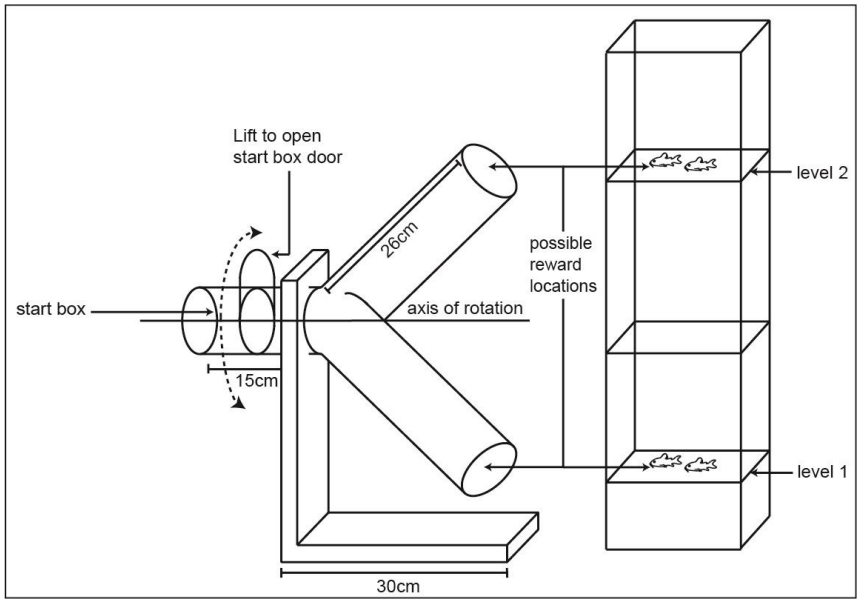


Figure 1. Experimental setup: the three-dimensional maze was fully rotational around the axis of rotation, indicated by the dashed arrow. The location of the reward was dependent on the direction in which the fish was being trained. Fish were trained in one of four positions: up and right, up and left, down and right and down and left. The conspecific fish were only ever in one position, at the bottom (level 1) if the subject was being trained down, or at the top (level 2) if being trained up. Similarly, flake was positioned on the end cap of the rewarded arm only.

2.3 Pre-training

The 13 individuals were split across four treatment groups; those trained up and right (n=3), down and right (n=4), up and left (n=3) and down and left (n=3). The subjects in each group were randomized between holding tanks and allowed 24-hours in the maze to acclimatise prior to training.

2.4 Training

Fish were placed in the start box and released into the maze after 60 s by removing the door. They had a choice of two directions; the correct rewarded direction, and the incorrect, unrewarded direction. A correct choice was recorded when the fish's tail crossed 10cm from the end of the rewarded arm. If the rewarded arm was chosen, the fish had 120 s to consume the food reward and interact with conspecifics. The end cap at the entrance of the maze was then removed and the fish was given 180 s to exit. If after such time the fish remained in the maze, it was rotated to encourage its exit. The training trial ended once the fish had chosen the rewarded arm or after 600 s had elapsed. Fish had a maximum of twelve training trials per day, and were trained on alternate days.

C. aeneus has a natural bias towards staying near the bottom of the tank, which was demonstrated by individuals taking more trials to reach criterion when their trajectory incorporated an upward component compared to a downward component (mean ± SE= 42.5± 25.4 and 11.2 ± 2.7 trials, respectively; Mann-Whitney U test: V=91, p=0.0016). To alleviate the potential for this bias, all individuals were trained in two phases. In the first phase fish were trained in one direction, and in the second phase they were trained in the opposite direction, as defined by their eventual experiment group (i.e. down and left, or up and right). Fish were deemed ready for the second phase of training and eventually testing once they had reached criterion. Following Holbrook and Burt de Perera (2009), fish had to select the rewarded arm nine out of the previous ten trials to reach criterion. Once an individual had reached criterion, it was presented with the maze in the absence of the reward. The unrewarded training trial ensured that the fish had learnt the direction to swim and were not just following olfactory cues emanating from the food reward. All test trials that the fish received were also unrewarded, however, training with a reward

continued between the unrewarded test trials to reinforce learning. After passing the initial criterion of nine out of the previous ten correct trials, for the remainder of the training period fish had to make the correct choice in four of the previous five trials, to ensure that all individuals remained at criterion immediately prior to testing.

2.5 Testing

In the first experiment we tested whether *C. aeneus* could identify the vertical and horizontal components of an integrated trajectory. We refer to the trajectories as 'integrated' here as they are composed of both vertical and horizontal components; however, they are not strictly 'three-dimensional' as fish do not have completely unrestricted three-dimensional movement during our experiments. Fish received three tests where the maze was oriented vertically, giving the choice of up or down and three tests where the maze was oriented horizontally giving the choice of left or right. If *C. aeneus* was able to separate the vertical and horizontal components from their previous training direction which incorporated both vertical and horizontal information, we expected they would choose the previously learnt vertical and horizontal directions when the maze was orientated in the vertical and horizontal configurations respectively. For example, fish trained up and right would be expected to select the right-hand arm when the maze was oriented horizontally and the upward-facing arm when it was orientated vertically. Just seven individuals (the second cohort) took part in experiment one as the first cohort were not available at the time of this experiment.

In experiment two we established whether *C. aeneus* prioritised either the horizontal or vertical direction when the two conflicted. During testing, the maze was rotated 180˚ from the training position so that a fish previously trained to swim up and right, during testing had the option to swim up and left or down and right. In other words, fish were

given a choice of either their previously learned horizontal direction or their previously learned vertical direction. All 13 individuals (both cohorts) took part in experiment two.

2.6 Statistical analysis

Each fish received three tests per experiment. To analyse data from the first experiment we calculated the proportion of trials in which the fish correctly identified the previously trained vertical or horizontal direction. We modelled the proportion of correct identifications using a mixed model with a binomial error structure, including dimension as a factor with two levels (vertical and horizontal), and individual fish as a random intercept. We tested (a) Whether fish chose the correct vertical direction more often than 50%, (b) whether fish chose the correct horizontal direction more often than 50% and (c) whether fish chose the correct vertical direction more often than the correct horizontal direction using Wald Z-tests (Bolker et al. 2009). To analyse data from the second experiment we calculated the proportion of trials in which fish preferentially chose to swim in their previously trained vertical direction or their previously trained horizontal direction. We modelled the proportion of trials that fish chose either vertical or horizontal using a mixed model with a binomial error structure. We tested whether fish chose to swim in their previously trained vertical direction more often than their previously trained horizontal direction using Wald Z-tests, as in experiment one.

3. Results

Results from experiment one indicated that *C. aeneus* was able to separate and identify the vertical component of the trained integrated trajectory, as fish chose to swim in the previously trained vertical direction more often than would have been expected by chance

(Binomial test: Z=2.89, p<0.003; figure 2a and 3a). In contrast, although some individuals did consistently correctly identify the previously learnt horizontal direction (figure 3b), overall *C. aeneus* did not choose the correct horizontal direction significantly more than would be expected by chance (Binomial test: Z=0.98, p= 0.327; figure 2b).

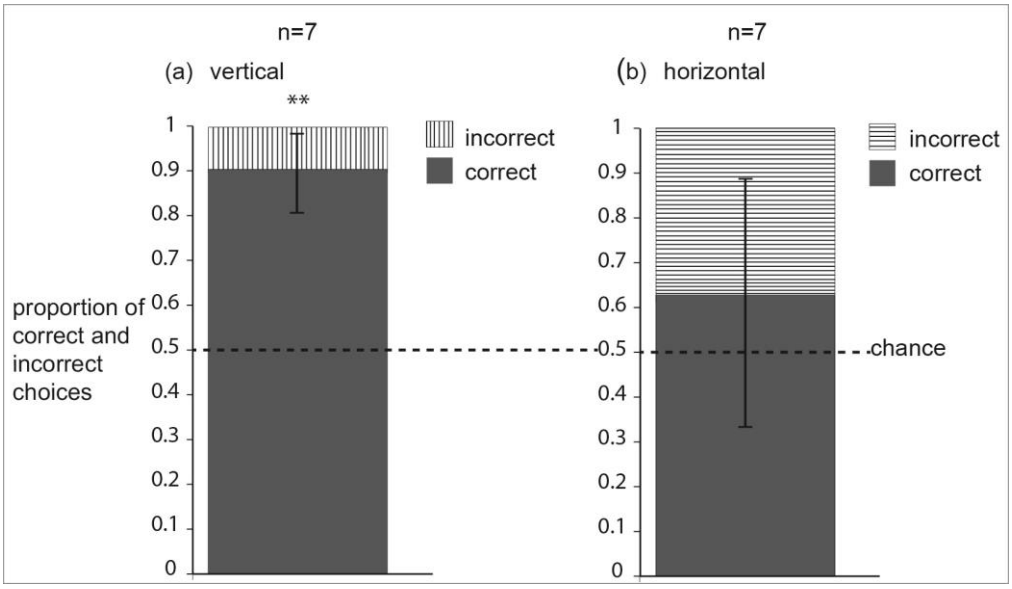


Figure 2. Experiment one (a) the proportion of trials in which *C. aeneus* chose the vertical direction to which they had been trained when the maze was positioned vertically, and (b) the proportion of trials in which *C. aeneus* chose the horizontal direction to which they had been trained when the maze was positioned horizontally. Error bars show the 95% confidence intervals.

The results of experiment two showed that *C. aeneus* prioritised vertical information over horizontal information, with all but two of the individuals (n=13) choosing the previously learnt vertical direction in all the test trials they received (Binomial test: Z=4.13 p<0.001; figure 4).

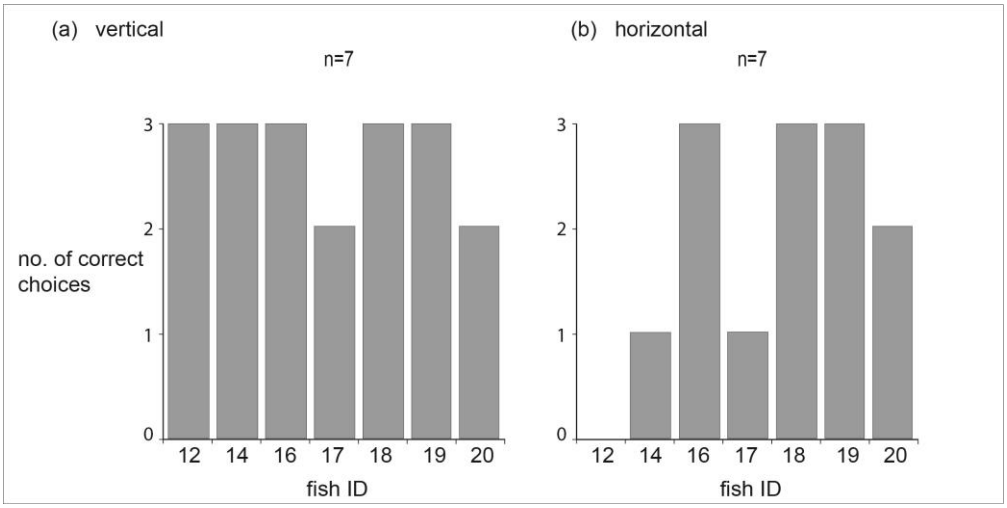


Fig 3. Experiment one: Frequency of successful identifications of the previously learnt (a) vertical and (b) horizontal direction from an integrated trajectory. Each fish received a total of six test trials: three with the maze oriented horizontally and three with the maze oriented vertically.

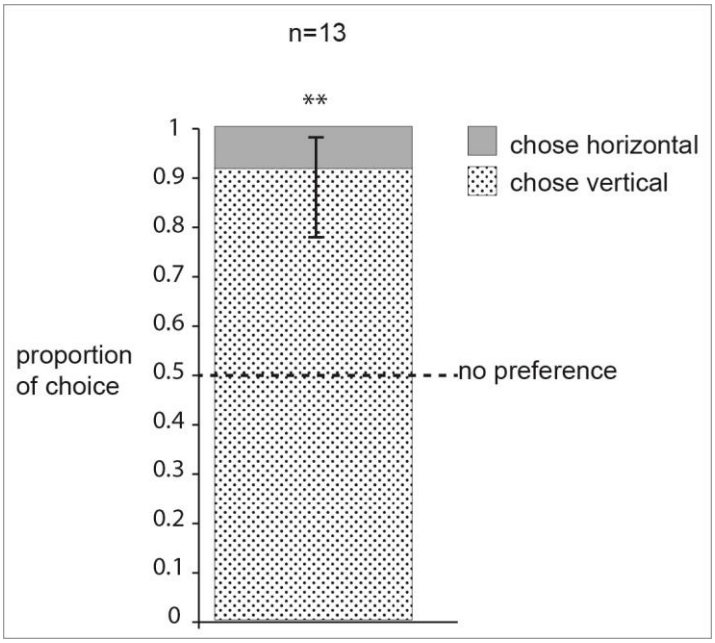


Fig 4. Experiment two: The proportion of trials in which *C. aeneus* chose the previously learnt horizontal direction compared to the previously learnt vertical direction. Each fish received three test trials. Error bar shows 95% confidence interval.

4. Discussion

The benthic-dwelling fish, *C. aeneus*, showed a disparity in its ability to separate and identify vertical and horizontal information from a learned trajectory that incorporated both. Here, we used the separation and identification of vertical and horizontal components of an integrated route as a proxy for the learning of this information. In our first experiment, we demonstrated that *C. aeneus* was able to identify the vertical component of the previously learned route in more than 90% of trials; in contrast, fish identified the previously learned horizontal direction in 61% of trials. Some fish successfully separated and identified the vertical component of a trajectory but not the horizontal component, suggesting that information from the different dimensions is stored separately. It is likely to be easier to store space as separate components rather than as an integrated three-dimensional unit as it reduces information-processing problems and cognitive burden (Holbrook and Burt de Perera 2009). Although our results suggest that overall *C. aeneu*s was less competent at learning horizontal information than vertical, when looking at our results on an individual basis, we see that nearly half of all individual fish within our sample successfully separated and identified the horizontal component of the previously learned route in all of the trials they were presented with (figure 3b). We cannot determine from this small sample whether this across-individual variation in the ability to learn horizontal information reflects population-wide differences, this would require further testing with a larger sample. We can say however, that the variation shows that not all fish learnt the horizontal information provided. Due to experimental constraints we were unable to increase this sample size (n=7), and so our result might reflect a type II error caused by low statistical power.

Our initial expectation was that, living a benthic lifestyle, *C. aeneus* might prioritise horizontal information over vertical information. However, data from our second experiment indicated that, instead, *C. aeneus* prioritised vertical information when vertical and horizontal information conflicted. This result can be interpreted in two different ways. First, as more than half of the fish in our sample did not appear to learn the horizontal component of the route, prioritisation of vertical information may have been due to the failure to learn horizontal information. The second possibility is that *C. aeneus* was demonstrating a genuine preference for prioritising vertical information over horizontal; we note that of the three fish that did successfully separate and correctly identify both the previously learned vertical and horizontal direction from an integrated route in all trials, all preferentially chose to swim in their previously learned vertical direction during conflict tests.

There are several factors that could explain why *C. aeneus* may have had a genuine preference for prioritising vertical information. First, hydrostatic pressure is a very salient and reliable cue unique to the vertical axis. Taylor and colleagues (2010) postulated that fish may be able to determine their depth using hydrostatic pressure information via neural signals transmitted from the stretch receptors of the swim bladder wall during ascent and descent. The rate of change in the volume of the swim bladder decreases as depth increases. Therefore, theoretically, fish may be able to determine their absolute depth from relative changes in swim bladder volume after descending at a given rate. Holbrook and Burt de Perera present a theoretical example of how this might work: a descent from one to two metres below the surface results in an increase in pressure on the swim bladder of 8.82%, whereas a descent from seven to eight metres below the surface causes an increase in pressure of 5.76 % (Holbrook and Burt de Perera 2011a). A

theoretical paper by Taylor and colleagues presents a compelling case for the possibility of fish detecting their absolute depth in this way, although it still remains to be tested empirically. The additional pressure cue might explain the preference for the vertical axis.

Alternatively, *C. aeneus*' prioritisation of vertical information could be explained by a phylogenetic constraint. *C. aeneus* (Siluriformes) and *A. mexicanus*' (Characiformes) are relatively closely related, being members of the same super order: Ostariophysi. The strategies employed to encode spatial information may have been phylogenetically conserved across taxa. For example, their preference for vertical information could have originated in a common ancestor and continued to persist in extant species.

In contrast to what we found here, Holbrook and Burt de Perera (2009) found that pelagic fish were able to separate horizontal but *not* vertical information from an integrated route. However, Holbrook and Burt de Perera ascribe this result to pelagic fishes' disinclination to swim upward rather than an inability to learn vertical information. In the wild, fish may be more exposed to additional predators nearer the surface, which could explain their reluctance to swim upwards (Holbrook and Burt de Perera 2009). Similar to Holbrook and Burt de Perera, we found that benthic fish were reluctant to swim upward, however this did not hamper their ability to separate vertical information.

Another possibility is that the degrees of freedom of movement an animal possesses may dictate how well they remember vertical information. For example, rats are surface-bound animals, moving with just three degrees of freedom. In contrast, both rufous hummingbirds (*Selasphorus rufus*) (Flores-Abreu et al. 2014) and the Mexican tetra, a pelagic fish (*Astyanax mexicanus*) (Holbrook and Burt de Perera 2013) move with six degrees of freedom and are better than rats at learning and remembering vertical information (Flores-Abreu et al. 2014; Grobéty and Schenk 1992; Jovalekic et al. 2011).

In the wild the vertical component of space is likely to be more relevant during hummingbird and fish movement than during rat's movement as the former move in an unconstrained way through three-dimensional space and so we expect selection on systems which allow these animals to navigate accurately in all three dimensions. For example, superior memory of vertical information cannot be explained solely by experience of moving vertically, as during experiments in a three-dimensional lattice environment rats spent more time moving vertically than horizontally (Grobéty and Schenk 1992) and spent comparatively longer moving vertically than hummingbirds in a similar experimental setup, and yet still remembered vertical information less accurately than hummingbirds (Flores-Abreu et al. 2014). Instead, rats' reduced accuracies in remembering vertical information could be attributed to a fundamental difference in the way spatial information is centrally processed. The hippocampus and entorhinal cortex of the mammalian brain are comprised of neurons that are thought likely to modulate navigational decisions. It is thought that these neurons encode information about current position in space (place cells), direction of gaze (head-direction cells) and distances between locations (grid cells: Hayman et al. 2011; Moser et al. 2008). Neurophysiological experiments indicate that rats receive lower resolution information about vertical space compared to horizontal. Grid cell functioning in particular is impaired or absent in the vertical axis, and grid cells are thought to be responsible for providing the distance information required for updating the internal odometer (Hayman et al. 2011). There are currently no empirical studies documenting the physiological properties of the brains of fish that move through three-dimensional environments, however we might expect that neurons in the parts of the brain that encode spatial information would encode vertical and horizontal information at a similar resolution (Burt de Perera et al. 2016), as has been

described in bats (Yartsev and Ulanovsky 2013)**,** because fish are able to move with six degrees of freedom.

In experiments where *S. rufus* had to find the absolute position of a rewarded location they were more accurate at pinpointing the vertical component of a location than the horizontal component (Flores-Abreu et al. 2014; Hurly et al. 2010). However, in experiments requiring birds to pinpoint the position of a rewarded flower within an array of other flowers, they could not identify the absolute position of the reward in three-dimensional space (Flores-Abreu et al. 2013). Instead *S. rufus* inferred the location of a reward from its position relative to the heights of the other flowers in a vertical array (Henderson et al. 2006). Holbrook and Burt de Perera demonstrated that *A. mexicanus* navigates with similar accuracy in both vertical and horizontal dimensions (Holbrook and Burt de Perera 2013). The difference in the three-dimensional spatial abilities of *S. rufus* and *A. mexicanus* suggests that aerial and aquatic environments might differ fundamentally in the availability of reliable cues in the vertical dimension, and in addition, that bird and fish sensory systems differ in that the latter may be able to quantify their absolute vertical position. We suggest that it is the presence of the hydrostatic pressure gradient in aquatic environments and the presence of the pressure-sensing swim bladder in particular, that provides the stable vertical cue necessary for the accurate three-dimensional spatial cognition demonstrated in fish (Holbrook and Burt de Perera 2011a; Taylor et al. 2010) We suggest that it is hydrostatic pressure that caused the superior learning and perhaps also the prioritisation of vertical information in the benthic fish, *C. aeneus*. In other words, we argue that it is not the type of animal movement *per se* that determines how three-dimensional space is encoded, but the type of information that is present in the vertical and horizontal axes of space, and the ability of the animals to exploit this information.

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6. Ethical declaration:

This experiment was approved by the Local Ethical Review Committee in the Department of Zoology, Oxford, and did not require a Home Office Licence.

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Chapter 4

Investigating how fish use vertically- and horizontally-arranged landmarks

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Abstract

Fish, like many other animals, use visual landmarks to navigate. However, unlike other animals, fish do not need to be in contact with a substrate to travel; instead, they move freely throughout their environment. Consequently, we expect that in the wild, fish will come across visual landmarks above and below them as well as to their right and left and ahead or behind them. We investigated how *Astyanax mexicanus* used vertically- and horizontally-arranged arrays of landmarks to determine the position of a food reward. We trained fish to find food positioned halfway between two landmarks, and observed where they searched after we doubled the distance between the landmarks. We expected fish to search according to either (1) the absolute distance of the food from one of the landmarks, or (2) the relative position of the food in relation to both landmarks. We were particularly interested in whether the strategy fish used to encode the relationship between the landmarks and the food differed in the vertically- and horizontally-arranged landmark conditions. We found that fish did not use an absolute or relative strategy in either the vertically- or horizontally-arranged landmark conditions, but rather searched unsystematically between the two landmarks for the food. Our results indicate that over small distances (comparable to those presented in this experiment) fish might perform generalised searches rather than encoding explicit relationships between a goal and nearby landmarks, perhaps because the river environment they inhabit means that food and visual landmarks are vulnerable to displacement in the current.

1. Introduction

Many animals are able to remember the location of significant places in their environment, such as their nest, rich foraging areas, and previously stored food caches. One way that they achieve this is to remember visual landmarks that occur in close proximity to their intended destination and store this information in a mental representation that they can refer back to at a later time (Healy 1998). Visual landmarks take different forms and provide different types of information depending on the scale of an animal's movement, its aspect during travel, and its perceptual abilities. Discrete entities at close range such as individual trees or flowers may act as beacons to signal the exact position of a goal (Cook and Tauro 1999), whereas distant panoramas such as forests or mountain ranges give less explicit information and instead signal the compass direction of a destination (Graham and Cheng 2009).

In situations where discrete landmarks are at close or medium range to a goal but are not 'beacons', in that they do not indicate its exact position, animals can assign quantitative parameters such as distance and direction to describe the relationship between a goal and the landmarks. Spatial relationships can be encoded in absolute or relative terms. In the former, animals encode exact distances and directions between landmarks and a goal (Collett et al. 1986; Spetch et al. 1996, 1997). In the latter, animals learn the position of a goal by a simple relational rule, such as that the goal is in the 'centre' of a set of landmarks (Cartwright and Collett 1982; Kamil and Jones 1997).

By manipulating landmarks that previously signalled the position of a goal, and observing the resulting shift in the location in which animals search for that goal, researchers have been able to infer the strategies that animals are using to encode spatial information (Collett et al. 1986; Spetch et al. 1996, 1997; Tommasi and Vallortigara 2000; Burt de

Perera 2004); this experimental approach is known as landmark array transformation. For example, animals trained to find a goal that lies halfway between two landmarks will either shift or maintain their search position when the distance between the landmarks and the goal is doubled, depending on whether the animal is using an absolute or relative search rule. Animals relying on absolute information have two potential shifted search positions corresponding to the absolute distance from the goal and each of the landmarks experienced during training (Collett et al. 1986). Animals using relative information will search in the same relative position, in this case halfway between the landmarks, irrespective of the absolute distance between them (Cartwright and Collett 1982; Spetch et al. 1997).

Both absolute and relative spatial encoding strategies exist in nature. Humans applied relative rules to remember the location of a goal in the context of a set of landmarks (Spetch et al. 1996, 1997), whereas gerbils encoded absolute vectors between the goal and each separate landmark in an array (Collett et al. 1986). Domestic chicks used a combination of absolute and relative strategies (Tommasi and Vallortigara 2000). There are mixed findings in pigeons: one study reported that similar to chicks, pigeons use a combination of absolute and relative strategies (Gray et al. 2004), whereas other studies suggest that pigeons encoded separate vectors between each landmark in an array−similar to gerbils−and then direct their search based on a new vector, which they calculate by averaging each of the separate vectors (Spetch et al. 1996; Spetch et al. 1997; Gray and Spetch 2006). In situations where only a few of the landmarks in an array were shifted and others remained in their original positions, birds searched at an intermediate position between where the goal should have been according to the unaltered landmarks, and where the goal should be according to the shifted landmarks (Cheng 1988).

Much of the research to date investigates how animals encode spatial information in horizontally-arranged arrays of landmarks (Collett et al. 1986; Kamil and Jones 1997; Tommasi and Vallortigara 2000), but have not considered the vertical dimension. As a result of their unconstrained movement, fish are likely to encounter landmarks in both vertical and horizontal contexts: above/below, left/right and behind/ahead. Here we use array transformations to investigate how the eyed Mexican tetra (*Astyanax mexicanus*) encodes the position of a food reward relative to an array of two landmarks arranged a) vertically (one above the other) and b) horizontally (side by side). We aim to test whether fish encode landmark information in a relative or an absolute way, and whether their strategy differs between vertical and horizontal arrangements of landmarks. We expect that landmarks play an important role during navigation for these fish in the wild, as they inhabit clear rivers that contain a variety of visual features. We expect landmark array expansions will reveal that fish use the same navigational strategy in both vertical and horizontal conditions, as they encode vertical and horizontal information with similar accuracy (Holbrook and Burt de Perera 2013).

2. Methods

2.1 Subjects

We trained 12 captive-bred adult Mexican tetras, *Astyanax mexicanus* (eyed morph) to find the position of a food reward based on its spatial relationship with two landmarks. We divided fish between three holding tanks over the testing period, ensuring that fish under different training regimes were evenly distributed across tanks. We kept fish under a standard 12:12 light:dark cycle at a temperature of 24^0C. We fed fish TetraMin® flake,

but did not give them food outside of testing and training unless they went for more than 8 training sessions without feeding; this amounted to approximately four days.

2.2 Apparatus

On three sides of the 60 cm^3 experimental tank we placed white Perspex sheets. We left the fourth side of the tank clear so that we were able to film the fish during tests. To reduce the fishes' view of the laboratory on the clear side of the tank we suspended a white cotton sheet behind the camera. We mounted a strip of red Perspex measuring 15 x 60cm onto one of the three opaque walls of the tank using clear fishing line, and this formed the base on which we attached an array of two landmarks and a red Tetramin® fish flake using petroleum jelly. We used two identical flat 1.5 cm^2 pieces of black plastic as the landmarks in the experiment. During training we placed a single flake in the centre of the hypothetical line joining the two landmarks at position X in figure 1. We always used a red flake for the reward, so that it was camouflaged once attached to the red Perspex, in order to reduce the likelihood of fish using it as a beacon and ignoring the landmark array.

Prior to all training trials and tests we removed fish from their home aquaria using a net and placed them in a clear plastic start box inside the experimental tank. We opened the start box by pulling the fishing line attached to the top which opened a sliding door; this way, we were able to release the fish at a distance from the tank so that we were out of view and caused minimal disturbance to the water in the tank.

2.3 Training

We divided the 12 fish into two groups: five of the fish were trained with the vertical array first and the seven remaining fish were trained with the horizontal array first (figure 2). In both vertical and horizontal conditions the arrays were presented to the fish perpendicular to the floor of the tank. During the training phase we positioned the landmarks 20 cm apart. We placed fish into the start box, approximately 40 cm away from the landmark array and the food reward (figure 1 shows only the vertically-orientated array). Between training and test trials we relocated the start box, positioning it at one of three vertical positions and three horizontal positions to scramble all laboratory and tank cues, so that only the landmarks of the array could inform fish of the position of the flake (figure 3).

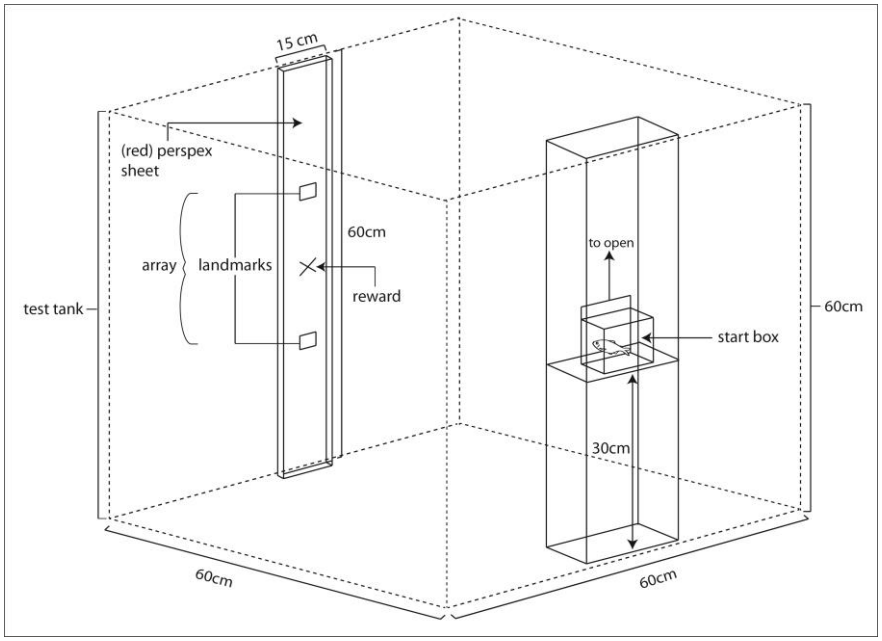


Figure 1. The red perspex sheet and landmarks *in situ*; the dotted line represents the walls of the experimental tank. The landmarks are shown in the training position (20 cm apart), the food lies exactly halfway between the landmarks (X). The perspex strip along with the

landmark array, reward and the start box were moved to a different wall of the tank between every training trial and test (except the wall we used for filming) to prevent fish from using landmarks in the laboratory to find the food. In the horizontal condition (not shown here) the perspex and landmarks were positioned horizontally instead of vertically.

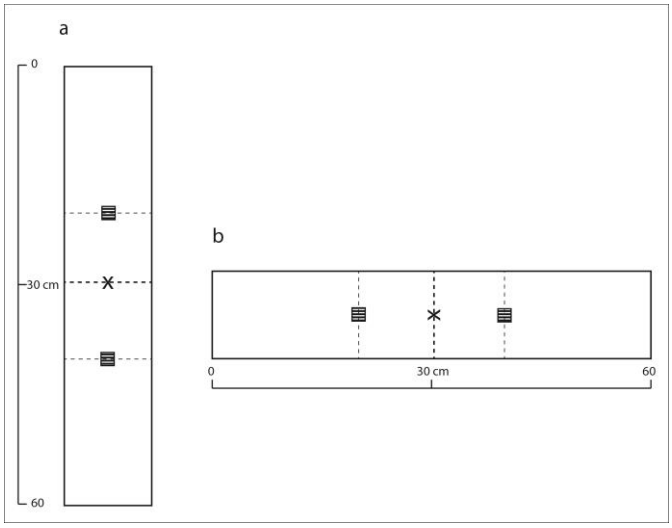


Figure 2. Perspex with landmarks attached in both vertical (a) and horizontal (b) orientations. The hatched squares represent the landmarks during the training phase of the experiment, and the X marks the position of the food reward.

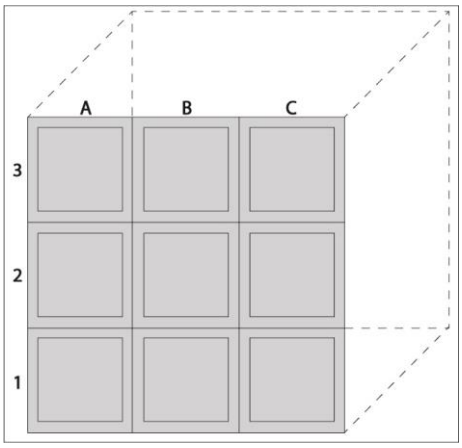


Figure 3. The squares in the grid represent the three horizontal (a-c) and three vertical (1-3) positions of the start box during training and testing on one wall of the tank. The release position of the fish was randomised on each training trial and test and could be

any combination of vertical and horizontal positions. The dotted line represents the other walls of the test tank.

We released the fish from the start box after 60 s by removing the door; we then gave it 600 s to find the food reward located between the two landmarks. Once the fish had found the food, we allowed it 120 s to consume it. We ended a training trial once a fish had found the food or 600 s had elapsed. Throughout training we moved the array (and the food) itself along the red Perspex through four different positions: 5, 10, 15, and 20 cm from the left wall or floor of the tank, depending on whether the Perspex was positioned horizontally or vertically, in order to reduce the chance that fish would use the dimensions of the tank or hydrostatic pressure as cues to find the food reward. In all training trials and tests, we positioned the landmarks centrally along the width of the Perspex, 7.5 cm from each long edge (figure 2). As well as shifting the array vertically or horizontally along the red Perspex sheet, we moved the entire Perspex (on which we had mounted the array and the food) around the tank to render external visual cues uninformative. We only tested fish once they had reached the criterion of having successfully retrieved the food on their first search attempt in nine out of the previous ten training trials.

2.4 Testing

During the test phase we presented fish with the array in the expanded condition; that is, we moved the landmarks so that they were 40 cm apart instead of 20 cm apart (figure 4). Each fish received twelve tests, six with the expanded array arranged vertically and six tests with it arranged horizontally. We did not displace the expanded test array vertically or horizontally between tests due to space constraints; instead, it remained fixed 10 cm

from the edge of the Perspex on both ends. We placed twenty food flakes 2 cm apart along the length of the hypothetical line between the two landmarks, and fish could access any flake that lay along this line. The continuous line of flakes was intended to give us an idea of the error that surrounded each fishes' search position, so that we could determine how precise fish were in their searching behaviour. If fish were using an absolute strategy, we would expect them to search at either the 10 cm or 30 cm positions on the array (with some associated error) depending on whether they were using the first (left or lower) or second (right or upper) landmark as a reference (figure 4). If fish were using a relative strategy we would expect them to search in the centre of the hypothetical line between the landmarks at distance of 20 cm from each (figure 4), again with some associated error. We refer to the locations at 10 cm, 20 cm and 30 cm as the 'predicted search locations' throughout the paper. We were interested in the distance of the fishes' observed search position from the nearest predicted search location.

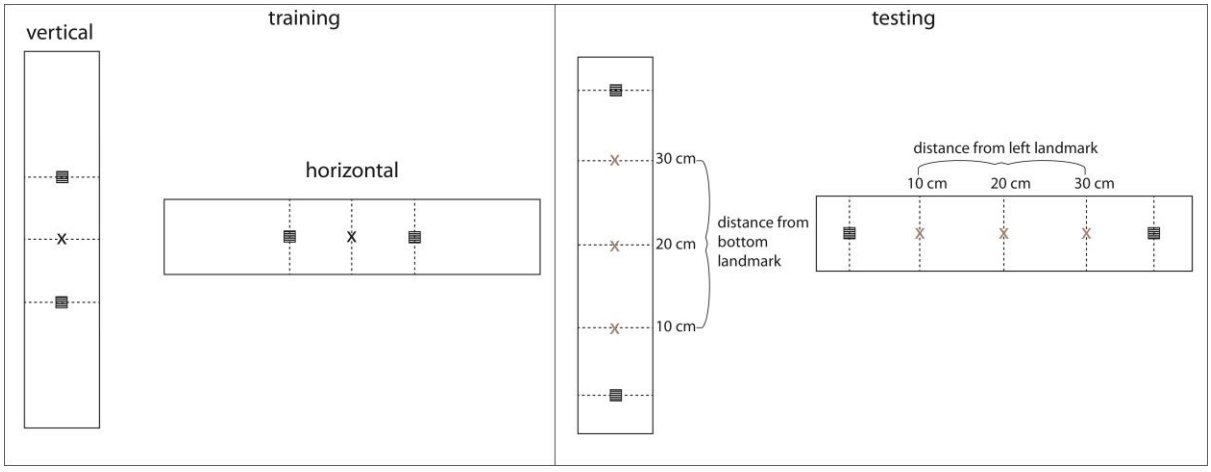


Figure 4. A schematic diagram of the landmark array during training (landmark 20 cm apart) and testing (landmarks 40 cm apart). The hatched squares represent the landmarks. The black X in the training condition represents the position of the food reward. The grey Xs in the testing condition represent the predicted search locations of fish orienting by the absolute distance from the first landmark (bottom or left landmark in vertical and

horizontal conditions respectively) (10 cm), the absolute distance from the second landmark (top or right landmark) (30 cm), or the relative position between the two landmarks (20 cm). The predicted search locations are measured according to their distance from the bottom landmark in the vertical condition and the left landmark in the horizontal condition.

We continued to train fish between tests to ensure that their search effectiveness remained at or above the pass criterion. Following the first test, each fish needed to pass further training trials in order to take part in subsequent tests: in each case, the fish's first contact with the perspex on their first search attempt had to be at the location of the food on four out of the previous five training trials.

2.5 Statistical analysis

We analysed the experiments with vertically- and horizontally-arranged arrays separately; however, the procedure we used to analyse the data was identical for both experiments. We used R 3.2.2 software for all our data analyses. Our analyses focusssed on two response variables: 1) the absolute position at which the fish searched (termed "search position"), to assess the *average* heights at which the fish searched, and 2) the distance between the fish's search location and the nearest predicted search location (10, 20 or 30 cm; we term this variable "dmin"), to assess whether the fish were using the expected search strategies. We describe these analyses in detail below.

We used a model selection approach to investigate whether the release location influenced fishes' search positions and/or dmin in the vertical and horizontal conditions. All of the models were linear mixed models and included individual fish as a random intercept to account for individual differences. We built models that reflected different

effects of the release position on (a) the search position and (b) dmin, and in each case selected the model with the lowest AICc value (Burnham and Anderson 2004). We formulated the following four models for testing in both the vertical and horizontal conditions:

1. release location has no effect
2. only horizontal release location has an effect
3. only vertical release location has an effect
4. horizontal and vertical release locations have additive effects

We investigated learning effects after multiple tests to check that the fishes' experience of the test conditions did not affect their search positions in later tests. To do this, we took the best-fitting search position model from the analyses described above, and used an ANOVA test to compare whether adding 'test number' as an explanatory variable significantly improved the fit of this model. To test for learning effects during training, we used a different dataset from the rest of the analyses: here, since fish did not have a choice of flakes during training, we assessed whether the number of previous training trials affected the time it took for the fish to find the flake (we term this "latency"). For this analysis we used an ANOVA test to compare a model containing 'training trial number' as an explanatory variable against a null model where training trial number had been removed; we included individual identity as a random intercept in both models.

Next, to test whether the fish searched according to one of the proposed search strategies (absolute or relative), we compared the distances to the nearest predicted search locations (the observed dmin values) to a simulated null distribution of dmin values assuming complete random searching according to a uniform distribution. For this comparison we used a Kolmogorov-Smirnoff test (a non-parameteric test), which quantified the

probability of obtaining the observed dmin values by chance if the fish had been searching randomly.

3. Results

We found that the distance fish searched from the nearest predicted search location (dmin) did not differ from what we would expect to see if fish were searching randomly in either the vertical (two-sample Kolmogorov-Smirnoff test, D=0.13, p=0.20) or horizontal conditions (two-sample Kolmogorov-Smirnoff test, D=0.12, p=0.29). Therefore our results suggest that fish did not search systematically for the food at the 10, 20 or 30 cm predicted search locations. Also, we did not see any significant individual effects on dmin in either the vertical (ANOVA, Chi^2=1.22, p=0.27) or horizontal conditions (ANOVA, Chi^2=0.59, p=0.44), suggesting that fish were not consistent in how closely they searched to the predicted search locations.

In the vertical condition, fishes' search positions were influenced by their vertical release location (model 3, p=0.015, marginal-R^2=0.07 and conditional R^2=0.45) and in the horizontal condition, fishes' search positions were influenced by their horizontal release location (model 2, p=0.011, marginal-R^2=0.12 and conditional R^2=0.15). For example, in horizontal tests, fish released on the left-, middle, or right-hand side of the tank had a slight left, middle, or right bias in their search position respectively (figure 5). Although we found a similar effect of release position in the vertical condition, where fish released at the bottom of the tank searched at lower depths than when they were released in the middle or at the top of the tank, the effect was much smaller than in the horizontal condition. We also saw an overall downward bias in fish searches outside of the effect of release position in vertical tests (figure 5).

Despite release location influencing search position, we did not find that the distance fish searched from the nearest predicted search location (dmin) differed from what we would expect if fish were searching randomly. Release location did not explain the variation in dmin in the horizontal tests; for example, a fish released at the bottom of the tank may have searched around 6 cm in one test and then around 12 cm in another (conditional R^2=0.16). However, individual choices (i.e. the individual-level random effect) did explain a lot of the overall variation in dmin during vertical tests (conditional R^2=0.45), suggesting that individual fishes' search positions were more consistent in vertical tests than in horizontal tests.

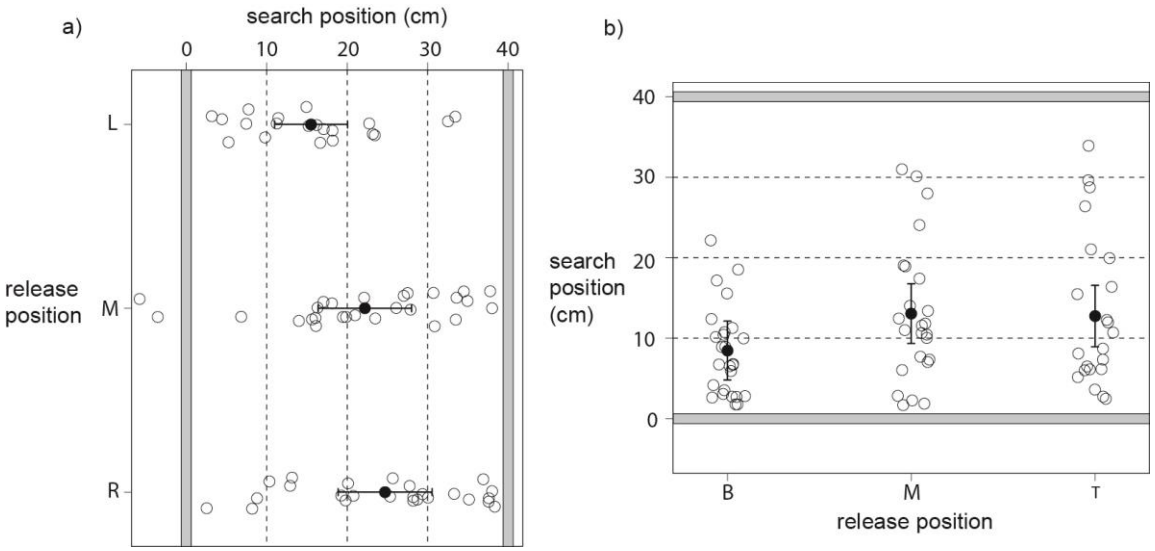


Figure 5. The distribution of fish search positions for each release location in a) horizontal: L=left, M=middle, R=right, and b) vertical: B=bottom, M=middle, T=top for all fish and all tests. Empty data points show the raw data and filled data points show model-predicted means and 95 percent confidence intervals. The horizontal dotted lines represent the predicted search locations if fish were using either an absolute (10 cm and 30 cm) or relative strategy (20 cm) to find the food reward. Landmarks were positioned at 0 cm and 40 cm in both vertical and horizontal conditions (shown by the grey bars). A jitter has been applied so that data points with identical values do not obscure each other.

Fish became faster at finding the food over consecutive training sessions (ANOVA, Chi^2=62.1, p<0.0001) suggesting that fish learnt the position of the food over time. However we did not see any effect of test number on fish search behaviour in the vertical (ANOVA: Chi^2=0.24, p=0.623) or in the horizontal conditions (Chi^2=0.037, p=0.858), suggesting that experience of the test conditions did not influence choices in later tests.

4. Discussion

We investigated how the Mexican tetra (*Astyanax mexicanus*) used two visual landmarks (arranged vertically or horizontally) to determine the location of a food reward, by observing their search position after we increased the distance between the landmarks. We predicted that fish would be using one of two strategies, either: a) learning the absolute distance from one of the landmarks to the location of the food reward or b) learning the relative distance between the two landmarks and the location of the food. Our results indicated that fish did not use either absolute or relative strategies when the landmarks were arranged vertically or horizontally; instead, they searched unsystematically between the two landmarks in both conditions.

We found that the vertical position at which we released the fish influenced where they searched when the landmarks were arranged vertically, and similarly, that the horizontal release position influenced where fish searched when landmarks were horizontally-arranged. While release position influenced the average height at which the fish searched, it had no effect on the distance between the search location and the closest predicted search location. In the horizontal tests, for example, fish searched more towards the left of the tank on average when released from the left (L) position instead of the right (R) position, but their distance from the left-most expected search location in the first case

(10cm) was similar to their distance from the right-most expected search location in the second case (30cm; left panel, Figure 5). Overall, this suggests that the release position affected which flake was chosen, but not whether the fish used absolute and/or relative search rules.

Experiments with flying and ground-based terrestrial animals have shown the use of both absolute and relative strategies (Collett et al. 1986; Spetch et al. 1996, 1997; Gray and Spetch 2006). The unsystematic search behaviour that we observed in *A. mexicanus* could be explained by fundamental differences in the physical characteristics of aquatic and terrestrial environments. *A. mexicanus* occupies streams and small rivers in southern North America, and although the rivers they inhabit are generally slow-flowing, landmarks (and food) could be vulnerable to displacement by the current. Evidence suggests that fish from river environments rely less on landmarks to inform them of their location compared to fish that inhabit ponds or other aquatic habitats devoid of flow (Girvan and Braithwaite 1998; Burt De Perera and Macías Garcia 2003), probably because landmarks are less stable in river environments and could potentially give misleading information. It could be argued that our experimental design - in which we manipulated the absolute distances between the landmarks and the food reward between training and testing - could have futher violated how reliable the fish percieved the landmarks to be. However, we think our experimental approach is unlikely to have had such an effect on our results, as we did not see a significant influence of experience over consecutive test trials on searching strategy.

Another possible explanation for the unsystematic variation we saw in searching behaviour lies in the way in which fish code information about the spatial relationships between elements in an array. Evidence suggests that some animals encode separate

vectors between the goal and each landmark in an array, weighting them according to some criteria that indicates the quality or reliability of the information they provide (Cheng et al. 1987). When faced with an expanded (or contracted) array, pigeons and Clark's nutcrackers calculate a new vector based on some intermediate of the weighted vectors that they encoded during training, and then direct their search behavour based on this (Spetch et al. 1997; Kamil and Jones 2000). Spetch and colleagues suggest that this method of encoding space could explain the apparently random search positions along the distance between landmarks (Spetch et al. 1997). Their experimental design was different to ours in that the landmarks and the goal formed an isosceles triangle, with the two landmarks forming the base and the goal positioned perpendicularly to form the third vertex of the triangle. After the expansion, pigeons continued to search at the correct perpendicular distance from the two landmarks (maintaining the height of the isosceles triangle), but as in our experiments, their search was spread randomly over the imaginary line between the landmarks.

Mexican blind cave fish (*A. mexicanus*) are the eyeless morph of the species that we used in our experiments. Burt de Perera found that cave fish treated arrays of four landmarks as a single unit of information: as soon as one or more landmarks in the array were displaced, the fish behaved as if they were in a novel environment (Burt de Perera 2004). We think it is unlikely that the eyed morph of *A. mexicanus* are responding to the manipulated array in the same way as their blind counterparts, because their sense of vision is functional over much larger distances than the lateral line system relied upon by blind fish. Consequently, irrespective of the distance between the landmarks in our experiment, eyed fish could percieve them simultaneously and gain information about the goal in the context of one or both landmarks. In contrast, landmarks would not have to

have been moved very far apart before blind fish could only perceive one at a time rendering them incapable of relating the position of one landmark to the goal or any other landmarks.

We camouflaged the food reward as well as possible by mounting it on to perspex of a similar colour and embedding it in petroleum jelly to reduce olfactory cues. We cannot rule out the possibility that the apparent unsystematic nature of fishes' search locations were a result of fish directly percieving food from their position in the start box during training and testing. This is because although it was difficult for human eyes to distinguish the food from the Perspex we cannot say definitively that it was just as difficult for *A. mexicanus* to see the food from that distance, as we have been unable to find studies investigating visual capabilities in *A. mexicanus*. However, we observed a decrease in the time it took fish to find the food over consecutive training sessions; if the fish were able to percieve the food visually or olfactorily from the point at which we released them, we would expect to see no change in the latency to find the food over successive training sessions, suggesting that at least in early parts of our experiement fish were unable to percieve the food from their position in the start box. It is likely that at close range the fish were able to percieve the food; however, because we arranged the food uniformly over the full 40 cm between the landmarks and ensured that all flakes were of an identical shape and size, we would not expect the appearance of any particular flake to bias the area in which the fish chose to search.

Cook and Tauro point out that animals treat landmarks that they percieve to be close to the location of a goal as beacons (Cook and Tauro 1999). If this was the case in our experiment, fish would have approached the landmarks directly and then performed a random search around the general vicinity, rather than encoding information about the

spatial relationship between one or both of the landmarks and the goal. Although we cannot rule out the possibility that fish treated our landmarks as beacons, we think this is unlikely, as the fishes' swimming trajectories did not suggest that they were heading directly to either of the landmarks during testing or training, and a similar experiment at comparable spatial scales to ours showed that gerbils can accurately pinpoint a goal using the spatial relationships between itself and nearby landmarks (Collett et al. 1986).

There is evidence to suggest that the environment that animals' experience during development can influence their spatial learning (Girvan and Braithwaite 2000). The fish used in our experiments were reared in captivity; however they had access to visual landmarks in the vertical and horizontal dimensions throughout their development so we do not expect their rearing environment to have negatively influenced their use of landmarks in our experiment.

Although we found that fish searched unsystematically irrespective of whether landmarks were arranged vertically or horizontally, we did see a difference in the spread of search positions between the vertical and the horizontal conditions. In the horizontal treatment, fish searches appeared to be clustered around the central region between the landmarks; however, in the vertical treatment, there was a downward bias in fishes' search positions (figure 5). Fishes' downward search bias in vertical tests is likely to be due to their disinclination to swim high up in the tank rather than a difference in the way fish encode vertically- and horizontally-arranged landmarks. Restricting their search to lower depths is likely to be an anti-predator response, as in the wild, swimming closer to the water surface may increase the risk of encountering predators (Holbrook and Burt de Perera 2009).

Our results show that fishes' search behaviour is similar in both vertically- and horizontally-arranged arrays of landmarks, as we expected; however, contrary to our predictions, fish searched unsystematically between the two landmarks. Our observations could indicate that over small distances in unpredictable environments, it may be more valuable for fish to broaden their search for a goal, either by performing random searches within a known area, or by averaging information from different sources to generate an intermediate search location. In either case, these results would suggest that fish may not confine their search to one place but instead perform generalised searches over a predetermined area. Another possibility is that fish were able to percieve the food reward from their position in the start box after repeated training and testing. We suggest that because it is difficult to rule out this second possibility it is difficult to draw any definitive conclusions from our study and that further work where food is not available during the testing phase may help to shed light on the answer to this question.

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Chapter 5

Fish use hydrostatic pressure to sense their absolute depth

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Abstract

Fish move freely through the water and must navigate in three dimensions. A sense of depth would offer a substantial adaptive benefit to fish during three-dimensional navigation. Hydrostatic pressure provides a global cue that varies linearly with depth, so it should be possible, in principle, for a fish to quantify its depth from a measurement of absolute pressure. However, it has been assumed that fish cannot sense absolute depth using pressure, the main dispute being that the volume of gas inside the swim bladder (the likely site of hydrostatic pressure detection) is varied to regulate neutral buoyancy, so it cannot simultaneously act as a long-term steady reference. Recent theoretical work proposes that absolute depth might be derived during vertical movement by combining a measurement of speed with a measurement of the fractional rate of change in swim bladder volume. Here we provide the first empirical evidence that the Mexican tetra, *Astyanax mexicanus* can determine its absolute depth, relying exclusively on its sense of pressure. We found that *A. mexicanus* accurately learnt the depth of a food reward using absolute pressure alone. Furthermore, when we experimentally manipulated the surrounding pressure, these fish shifted their search accordingly. Our results highlight new sensory information that these fish are using during navigation.

1. Introduction

The basis of navigation in all animals hinges on knowing the spatial relationship between an individual's current location and an intended destination. Although all animals inhabit a three-dimensional world, many, including humans, are constrained to travelling over surfaces with three degrees of freedom: two translational (forwards/backwards and left/right) and one rotational (yaw) (Holbrook and Burt de Perera 2013; Taylor and Krapp 2007), meaning that navigation is principally in two dimensions. However, non-surface-bound (flying and swimming) animals that travel freely through all the dimensions of space can move with six degrees of freedom: three translational (forwards/backwards, left/right and up/down) and three rotational (yaw, pitch and roll) (Holbrook and Burt de Perera 2013; Taylor and Krapp 2007), and therefore must navigate in three dimensions. The addition of the vertical dimension enlarges the size of the navigable space from a two-dimensional plane to a three-dimensional volume (Jeffery et al. 2013), potentially leading to a multiplicative increase in the complexity of navigation (Holbrook and Burt de Perera 2009, 2011b, 2013).

If animals possessed reliable information about their vertical position, it would be a huge advantage for three-dimensional navigation. Non-surface-bound terrestrial animals use a variety of different cues to determine their vertical position, depending on the conditions of the environments they inhabit and the sensory systems that they possess. These cues include: visual motion (Dacke and Srinivasan 2007), visual landmarks (Healy and Hurly 1998; Hurly and Healy 1996; Hurly et al. 2010; Schnitzler et al. 2003) and biosonar (Geva-Sagiv et al. 2015). In contrast, fish inhabit aquatic environments and thus have access to hydrostatic pressure cues. Pressure varies linearly with vertical distance in the water column (Holbrook and Burt de Perera 2011a; Taylor et al. 2010), so the pressure at

a given location indicates its depth. The pressure cue is unique in that its availability throughout the water is homogeneous, irrespective of the location of the perceiver (Chapter 2; Holbrook and Burt de Perera 2011a; Taylor et al. 2010). The global availability of pressure makes it an extremely reliable and informative cue for fish navigating in the vertical dimension; however, whether fish actually use pressure to quantify their absolute depth has remained unknown.

Fish are able to sense minute changes in pressure via their swim bladder (Blaxter and Tytler 1972; McCutcheon 1966; Qutob 1962), but it is thought that they cannot use pressure to quantify their absolute depth, since the molar amount of gas in the swim bladder varies to maintain neutral buoyancy, making it unsuitable as a reliable reference in the long term. However, Taylor and colleagues present a theoretical model explaining how fish might use their swim bladder to determine absolute pressure and therefore quantify their depth despite the fluctuating volume of gas inside it (Taylor et al. 2010). By using the fractional rate of change of swim bladder volume combined with vertical speed, fish could locate their precise vertical position. This is because the percentage change in swim bladder volume for a given vertical movement decreases with increasing depth, so a particular change in swim bladder volume at a known speed will be unique to a particular depth. This physical model is independent of the molar amount of gas inside the swim bladder (Taylor et al. 2010), and thus can represent a reliable strategy for depth perception.

Here, we investigate empirically whether fish can quantify their absolute depth using only hydrostatic pressure. We test firstly, whether fish can learn the vertical position of a food reward located at a given depth. Secondly, we test whether fish move their search location upwards when the pressure is increased, and downwards when the pressure is decreased.

The results of these experiments reveal whether fish can use hydrostatic pressure for navigation in the vertical dimension.

2. Methods

2.1 Subjects

We divided 27 captive-bred adult Mexican tetras, *Astyanax mexicanus* (eyed morph) between seven holding tanks, ensuring that fish under different training regimes were evenly distributed across tanks. We separated individuals from each other by clear perforated screens, allowing them visual and olfactory, but not physical, contact. We kept fish under a standard 12:12 light:dark cycle at a temperature of 24ºC. To ensure adequate motivation for the training and test tasks (see below), we did not provide fish with food (TetraMin® flake) outside of training unless they went for more than 16 training trials without feeding, which amounted to approximately four days.

2.2 Apparatus

We filmed the training trials and tests through a transparent wall of the test tank (figure 1); the other walls were opaque. We suspended a white sheet over the transparent wall of the tank, behind the video camera, to block fishes' view of the laboratory. Fish began all training trials and tests in a 14 cm^3 start box made from clear plastic, consisting of five fixed faces and a sixth face that formed a sliding door. We released fish from the start box by pulling the fishing line attached to the top of the sliding door (figure 1); this firstly allowed us to open the box out of view of the fish, and secondly, ensured that we caused minimal disturbance to the water in the tank. We suspended the start box from the top of the tank with clear fishing line, varying its height so that it was positioned either 20, 30 or 40 cm from the bottom of the tank during testing and training. This prevented fish from

using the start box position as a cue or following a memorised trajectory to reach the food. On the wall opposite the start box we placed a long piece of white Perspex (120 cm tall and 5 cm wide) upon which we attached red TetraMin® food flakes with petroleum jelly. This formed the array of food flakes that the fish would have to choose from, with each flake located at a different depth. We covered all but one of the flakes with microscope cover slips so that they were visible but inaccessible (i.e. could not be eaten). To ensure that the absence of a cover slip was not an indicator of the position of the accessible flake, we placed a cover slip directly onto the Perspex in the position of the accessible flake, and then positioned the flake over the top of it. We trained fish to remember the position of the accessible flake, which remained at a constant depth between training trials.

We obscured the fishes' view of the bottom of the tank by installing a fake floor made of opaque white Perspex that we suspended at two depths, either 17 cm or 5 cm from the bottom of the tank. By fitting the fake floor, we prevented fish from being able to use the bottom of the tank as an indicator of the position of the accessible flake. We used the small rectangular hole (2×4 cm) cut into the back edge of the fake floor to pass the Perspex, on which the arrays of flakes were mounted, through so that it could sit on the bottom of the tank beneath the fake floor. We also suspended a Perspex ceiling from the top of the tank of similar design to the fake floor, except that there was an additional larger square hole (15 cm^2) cut into its front edge. This extra hole allowed us to lower the start box containing the fish through into the tank without having to remove the whole ceiling (figure 1). We then placed a lid on top of this square hole so that the fish could not see the laboratory above them from their position in the start box. As with the fake floor, we designed the ceiling so that it could be suspended from two different positions: either 63 cm or 54 cm from the bottom of the tank. Between all training trials and tests, we

changed the height of both the ceiling and the floor so that fish could not use the distance from either to find the food reward.

The total height of the tank was 90 cm. As a result, we were limited by how much we could vary the height of the ceiling, fake floor and start box while still maintaining an adequate selection of flakes for fish to choose from that were sufficiently far enough apart that they could detect a change in pressure between them (Taylor et al. 2010). The constantly shifting ceiling and floor meant that the total number of flakes that fish could choose from on any given training trial or test varied from between three and six (figure 2).

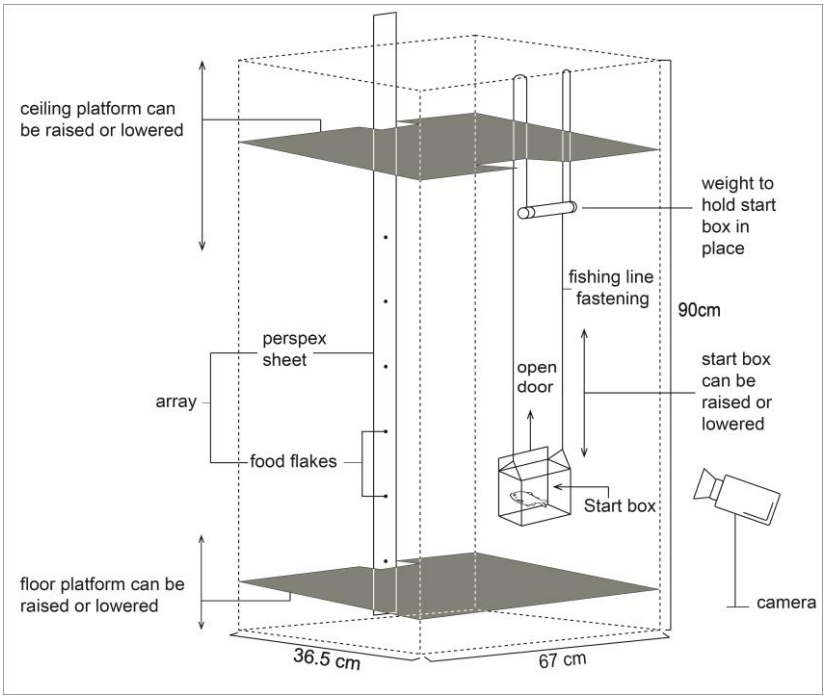


Figure 1. A schematic diagram showing the experimental setup. The dotted outline represents the test tank dimensions.

2.3 Training

We divided fish into two treatment groups; each group received different training regimes. The 'low group' (n=15) were rewarded with accessible food 45 cm from the bottom of the tank and the 'high group' (n=12) were rewarded with accessible food 30 cm from the bottom of the tank. Group numbers were uneven as some members of the original sample of 30 had to be removed from the experiment after they became ill. The reason for the groups' different training regimes was due to the vertical space limitations of the tank: we needed to ensure that in the test phase of the experiment there was enough room in the tank, even with the moving floor and ceiling, to have as large a potential search area for the fish as possible.

We allowed fish 60 s to settle in the start box; after this time we lifted the door and gave them 600 s to select a flake. We ended the training trial either once the fish had chosen a flake or after 600 s had elapsed. We considered the first flake that the fish's mouth came into contact with to be the fish's 'choice'. If the fish chose the accessible flake, we allowed it 120 s to consume the flake and considered it to have passed that training trial. If the fish chose one of the inaccessible flakes; we considered it to have failed that training trial and allowed it to continue its search for the remaining time. Fish received their first test once they had passed the training criterion – namely, once they had passed nine of the previous ten training trials. Between tests we continued fishes' training, setting the pass criterion at four out of the previous five trials, to ensure that they continued to remember the location of the training flake during the subsequent tests. We removed any fish from the experiment that failed to reach the criterion after 100 trials and placed them into separate aquaria.

We constructed three arrays that differed only in the distance between the flakes. We placed flakes 10 cm apart in array 'A', 15 cm apart in array 'B' and 11.6 cm apart in array 'C'; this was to prevent fish from simply 'counting' the flakes or learning the position of the accessible flake relative to the other (inaccessible) flakes. We only presented two of the three possible flake arrays to the fish: individuals in the 'high group' received arrays 'A' and 'B' while those in the 'low group' received arrays 'B' and 'C' (figure 2). Presenting identical arrays to all fish was not possible due to the limited dimensions of the tank. We trained the 'high group' with the water at a depth of 66 cm and the 'low group' at a depth of 81 cm.

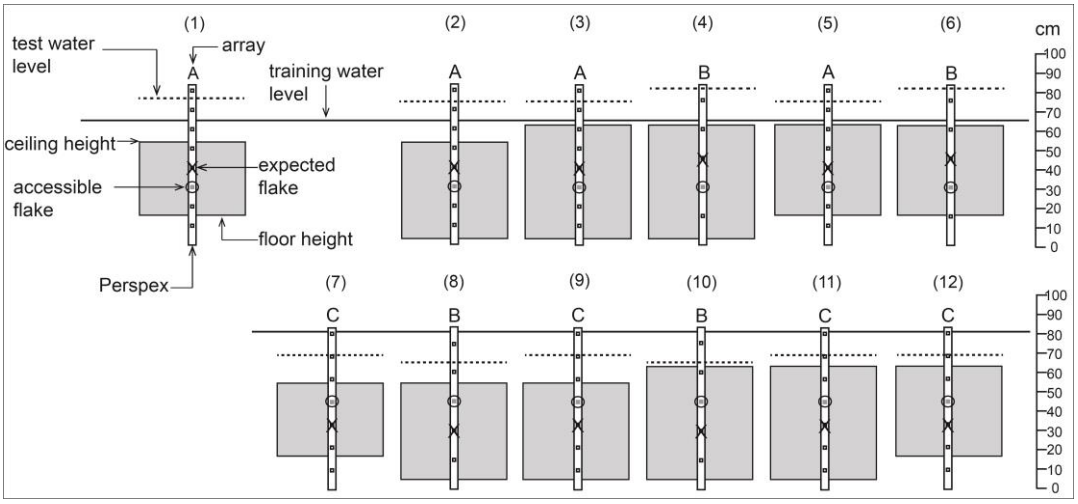


Figure 2. Scale diagram of all the different combinations of ceiling depths, floor depths and flake arrays. There are 12 different combinations in total; we presented fish in the 'high group' with combinations on the top row (1-6) and the 'low group' with the combinations on the bottom row (7-12). The top and bottom of the large grey boxes mark the depths of the floor and ceiling (fish could search anywhere between these, inside the bounds of the grey box). The letters refer to the three flake arrays (A, B or C). The small empty squares represent the flakes covered with cover slips, the circled filled square represents the flake accessible during training, and the black X represents the flake we

expected the fish to select during the test if they responded according to the pressure change that we imposed. The long solid line along the top of all the combinations represents the level of the water during training, and the dotted lines represent the level of the water during testing.

During the training phase, we had hoped to block all spatial cues, except for pressure. However, this was not possible, for two reasons. Firstly, to ensure that fish learnt to associate a specific depth with food, we had to provide them with uncovered, accessible food at that location. This made it possible that the fish could just follow olfactory cues emanating from the accessible flake to reach the correct location. Secondly, fish may have had limited access to visual cues through the clear wall of the tank that we used for filming tests and training trials. Because of these limitations, it was conceivable that fish could pass the training criterion by using visual or olfactory cues to locate the food reward rather than using pressure. In an attempt to eliminate this possibility, we included a test phase in the experiment.

2.4 Testing

During the tests we (1) covered all the flakes to remove any differences in olfactory cues and (2) added ('high group') or removed water ('low group'), leading to an increase or decrease in pressure, respectively. If fish had learnt the vertical location of the reward using pressure alone, they should shift their search for the flake in the direction that the pressure cues dictated. However, if fish searched in the same location as they had searched during training, this would have indicated that fish were using the distant external visual landmarks, as these remained in the same vertical position for both training and testing. Finally, if the fish had used differences in olfactory cues during training, we would expect them to search randomly during the tests, since all flakes were

covered during the tests and thus no differences in olfactory cues would have been detectable.

Ten of the original 27 fish passed the training criterion and moved on to the test phase, where we presented them with three filmed tests each (the remaining 17 fish were excluded from further experiments). We added water to the tank for fish in the 'high group' (n=4), causing them to experience an increase in relative pressure in tests compared to training, and removed water from the tank for fish in the 'low group' (n=6), causing them to experience a decrease in pressure. If fish used pressure to determine the position of the food reward, we expected to see a deviation in their search location during the test phase of the experiment, with the search location of fish in the 'high group' being displaced upwards (figure 3a) and the search location of fish in the 'low group' being displaced downwards (figure 3b).

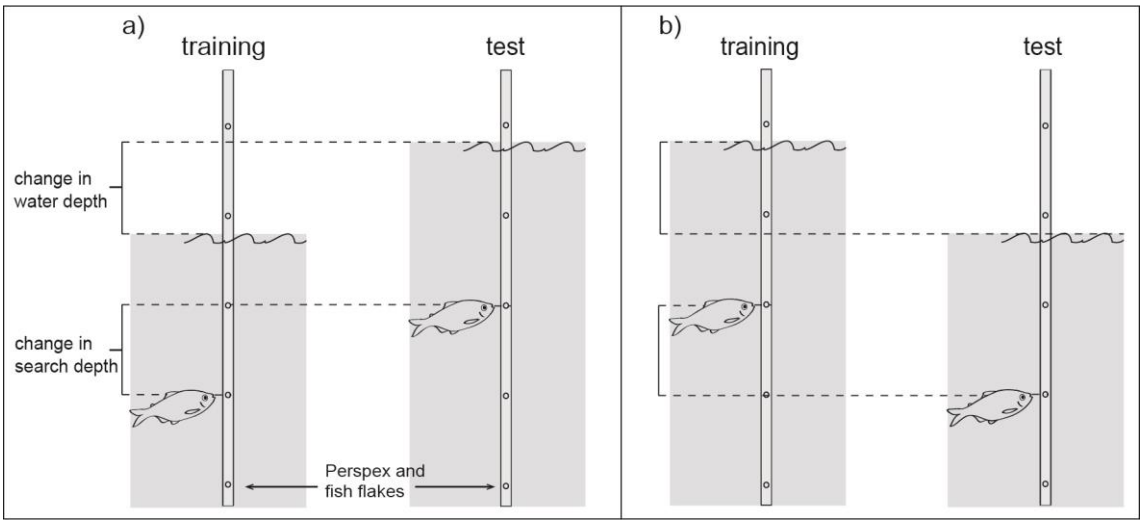


Figure 3. Predicted shifts in search location between training trials and tests after water has been added (a) or removed (b).

The amount of water we added ('high group') or removed ('low group') depended on the flake array we presented to the fish on each test, as it corresponded with the between-

flake distances of the different flake arrays. For example, on tests where we gave fish array 'A' ('low group') we removed 10 cm from the tank water level, and on tests where we gave fish array 'C' ('high group') we added 11.6 cm of water. We presented fish from both 'low' and 'high' groups with array 'B', either removing ('low group') or adding ('high group') 15 cm of water to the tank in the test condition (figure 2). Because we gave the fish three tests but only two flake arrays, we had to present the fish with one array once and the other array twice. We balanced this across fish, where possible, so that approximately one third received array 'A' twice, a third received 'B' twice and a third received 'C' twice. We refer to the flake located where we would expect fish to search if they were using pressure as the 'expected flake' (the position of the Xs in figure 2)

2.5 Statistical analysis

Only the ten individuals that passed the training criterion were included in the test analyses. To test whether the total number of tests (three tests per each of the ten fish) where the expected flake (according to pressure) was chosen more frequently than we would have expected by chance, we tested two null hypotheses. The first was the 'random' hypothesis, where we tested the probability of finding our result if fish were choosing flakes randomly. The randomly-behaving fish would choose any of the flakes with equal probability (1/f, where f = number of flakes available during a training trial). Therefore, the probability of choosing the accessible flake depended on the number of flakes that the 'random' fish had to choose from on a given training trial.

The second null hypothesis was the 'training' hypothesis, where we tested the probability of finding our result if the fish were behaving in the tests as they had during training trials. Unlike the 'random' null choices, the 'training' null choices also accounted for differences in search behaviour between individuals. To generate the 'training' null

expectation, we matched each of the 30 tests (three tests per each of the ten fish) with training trials whose conditions were identical to those of each test, based on three criteria: floor depth, ceiling depth and flake array. Matching the tests with the training trials using these criteria ensured that each subset of training trials was exactly equivalent to the test trial, except that there was no change in pressure. Matching the training trials and tests in this way also meant that for each test, we had comparable training trials where fish had an identical number of flake options to choose from in both the test and the training. Once we had matched each test with a subset of the training trials, we randomly drew a flake chosen by each fish during one of the matched training trials, and treated that as the flake chosen in the test. We did this for all 30 of the tests and calculated the number of these tests that were passed, i.e. in which the 'training' fish chose the expected flake (according to pressure).

Using each of the 'random' and 'training' null models for flake choices, we simulated the flake choices for all 30 tests 1000 times, which gave 1000 pass counts (ranging between 0 and 30 in each case). We then calculated the p-values as the proportions of the 1000 simulations in which the total number of passed tests (i.e. in which the expected flake was chosen) was greater than or equal to the observed number of passed tests in our real data (=17 out of the 30 tests).

3. Results:

Ten of the 27 fish in our experiment passed the initial nine out of ten training criterion. There was large variation in the speed of learning between different individuals, with fish taking a mean of 88 ± 34 (s.d.) training trials to pass the training criterion. These ten fish were then subjected to tests where the water volume was experimentally manipulated. Of the 30 tests (three for each of the ten fish), we found that in 17 of these tests, fish selected

the flake at the expected depth (i.e. according to the pressure change) (figure 4 and 5). This proportion was higher than would be expected if fish were (a) searching randomly ($p<0.001$) or (b) searching according to how they had searched during training ($p<0.001$). This indicates that fish shifted their search position according to changes in hydrostatic pressure.

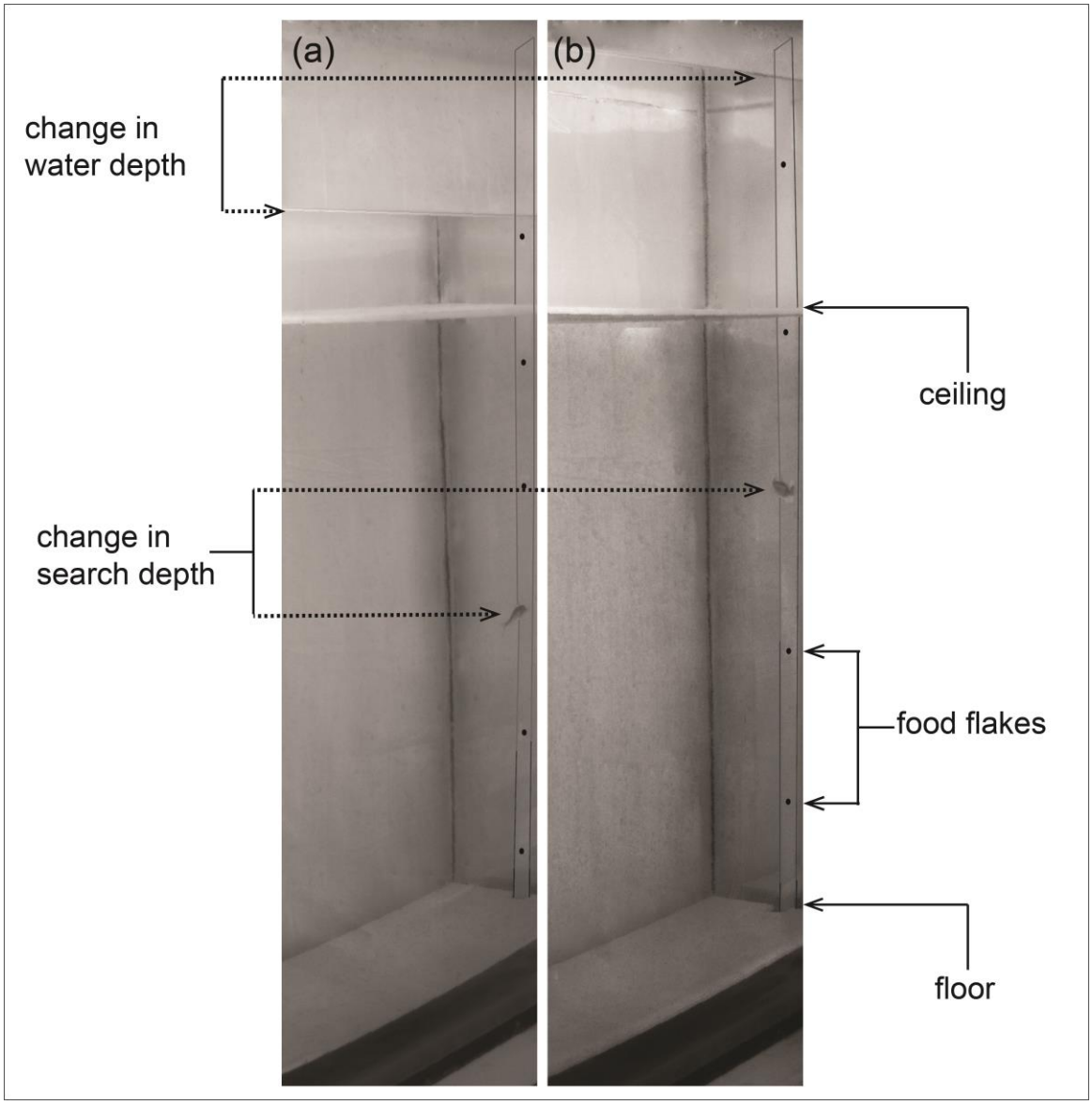


Figure 4. Stills captured from video recording of an individual from the 'high group' showing a training trial (a) and a test (b) where ceiling and floor depths matched.

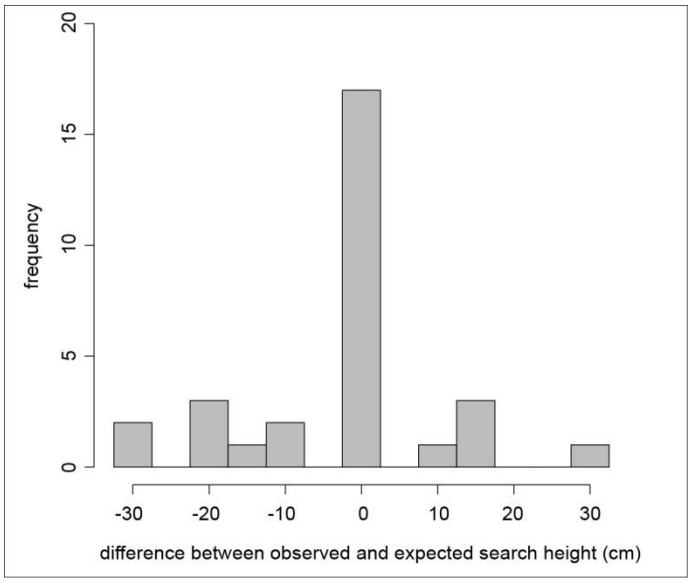


Figure 5. The difference in the observed and expected search locations of all fishes' tests (n=30). Here, 0 represents tests where there was no difference in fishes' observed and expected search locations.

4. Discussion

Our results demonstrate that fish can use hydrostatic pressure to establish their absolute depth and use this information to pinpoint their location in the vertical dimension. These findings provide an insight into how fish navigate in three dimensions. Contrary to speculations that fish may learn and remember vertical information contextually, that is, without details about distance and direction (Jeffery et al. 2013), our results show that this is unlikely to be the case. Instead, through their detection of hydrostatic pressure, fish – at least *A. mexicanus* – have access to metric information about their position in vertical space (Burt de Perera et al. 2013), which may explain their ability to remember and retrieve spatial information in the vertical dimension with high accuracy (Holbrook and Burt de Perera 2013).

Behavioural experiments have shown that fish store vertical and horizontal information separately, perhaps to reduce the complexity of learning, processing and storing three-dimensional information (Holbrook and Burt de Perera 2009). Fishes' abilities to use pressure as a navigational cue provides an explanation for the separation of vertical and horizontal dimensions while navigating through volumes, as pressure is only available in the vertical dimension and thus can provide information during vertical but not horizontal travel (Holbrook and Burt de Perera 2009; Holbrook and Burt de Perera 2011b). Because pressure represents a global cue that is uniformly available throughout aquatic environments, it provides a stable and reliable source of spatial information in otherwise variable environments (Holbrook and Burt de Perera 2011a). As a result, a sense of pressure is likely to account for fishes' preference for relying on vertical information in situations where vertical and horizontal spatial cues conflict (Holbrook and Burt de Perera 2009), a preference that persists even when fish are provided with extra (visual) cues in the horizontal dimension (Holbrook and Burt de Perera 2011b).

The physiological mechanism that fish are using to determine their depth via pressure is still unclear. Taylor and colleagues (2010) have suggested that fish may be able to integrate information about their vertical speed and the fractional rate of change of their swim bladder volume to determine absolute depth; however this remains to be tested empirically. Evidence suggests that at the surface of the water fish can detect pressure changes over vertical distances as small as 0.05 m (Blaxter and Tytler 1972; Qutob 1962). As Taylor and colleagues point out, to experience the same fractional rate of change at deeper depths fish would need to make vertical movements over larger distances: over a distance 0.1 m at a depth of 10 m and 0.5 m at a depth of 100 m (Taylor et al. 2010). Our results should encourage further scrutiny of the swim bladder, to determine if this is the site of depth detection in fish, and if this is found to be the case it may help to answer

important evolutionary questions about the function and its adaptive importance for animals that possess this organ (this comprises around half the Actinopterygii fish species; Nelson 2006). Repeating this experiment under conditions where the swim bladder is temporarily rendered unable to send neural signals to inform the animal of pressure changes may help to shed light on whether it is the swim bladder that plays the central role in depth detection, as some suspect (Holbrook and Burt de Perera 2011a; Taylor et al 2010). Finally, our results generate questions about where information derived from pressure cues is stored and how these cues are learned and combined centrally with cues received via other sensory modalities.

Our results reveal how fish that navigate through complex three-dimensional environments are able to simplify this task by sensing and utilising hydrostatic pressure, facilitating efficient and accurate navigation in the vertical dimension.

5. Acknowledgments

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Chapter 6

General Discussion

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1. Summary

In recent years there has been increasing interest in how animals navigate in three dimensions. Studies have revealed differences in three-dimensional spatial cognition in surface-bound animals, such as humans and rodents, compared to non-surface bound animals, such as those that swim and fly, like fish, birds and bats. These findings have led to questions about (i) what elements of an animal's movement ecology and their environment influences their spatial learning and (ii) what navigational cues are available to non-surface-bound and surface-bound animals.

The aim of this thesis was to explore these questions, principally in vertebrates, using fish as a model system. I investigated the environmental spatial cues available to fish as they navigate, and explored how fish with different movement ecologies – namely, pelagic versus benthic lifestyles – learn spatial information. In the opening chapter of this thesis I posed three questions that I attempted to answer with my research and experimental work: (1) "how does an animal's movement ecology influence its spatial learning?" (2) "which behavioural mechanism underlies vertical and horizontal landmark use in fish?", and (3) "can fish use pressure to determine their absolute depth?".

In this chapter I discuss my results in a wider context, providing a brief summary of my findings which complements the more extensive discussions within each separate study. After outlining my results, I go on to discuss further questions that my research has generated and suggest some potential areas for further investigation in the future.

2. Key findings and future directions

(1) How does an animal's movement ecology influence its spatial learning?

In chapter 2 I reviewed the literature on how surface-bound and non-surface-bound animals learn and remember information on the vertical versus horizontal dimensions. Overall, findings from both behavioural and neurophysiological studies suggested that surface-bound animals learn and remember information derived from the vertical dimension less accurately than information from the horizontal dimension. In contrast, non-surface-bound animals like hummingbirds are better at remembering vertical information than horizontal information, (Flores-Abreu et al. 2014) and studies of fish (Holbrook and Burt de Perera 2013) and bats (Yartsev and Ulanovsky 2013) revealed that they represent vertical and horizontal information with similar accuracy. I proposed that spatial learning in animals with different movement ecologies is likely to be under the influence of a combination of non-mutually exclusive factors, including (i) the resolution of the sensory systems that detect spatial cues and their inherent biases (Brandt et al. 2015; Zwergal et al. 2015), (ii) the asymmetric costs of travelling vertically versus horizontally (Proffitt et al. 1995; Proffitt et al. 2003; Grobéty and Schenk 1992), and (iii) animals' ecological niches. For example, whether they need to travel in both vertical and horizontal dimensions as they forage (Nieh et al. 2003) or whether they travel principally in two dimensions, because their food source is located on a substrate (Chapter 3: Davis et al. 2014).

My attempts to uncover the reasons for differences in how animals with different movement ecologies learn and remember vertical information have exposed some of the gaps in our knowledge. For example, some authors have suggested that a difference in the increased energetic costs associated with travelling vertically compared to horizontally

may influence the way that animals learn and remember spatial information from the different dimensions (Flores-Abreu et al. 2014; Grobéty and Schenk 1992; Jovalekic et al. 2011). While it seems plausible that energetic costs could influence spatial learning there are currently very few studies investigating the difference in energy expenditure during vertical and horizontal locomotion in different animals. The finding by Taylor and colleagues (1972) that mice experience a relatively smaller difference in energy expenditure during travel between the vertical and horizontal dimension compared to chimpanzees suggests that the importance of energetic costs may differ with an animals' size and locomotory style. As a result, it is important to perform direct tests of energy consumption during uphill and level-ground walking (or swimming and flying) in different animals before considering energetic constraints an important factor in spatial learning. To test this directly, one might measure gas and water output as individuals travel identical distances over areas of terrain of varying steepness. Levels of labelled hydrogen and oxygen in excreted carbon dioxide and water can then be monitored as an indication of the energy expended during these activities (Lifson and McClintock 1966).

A more general problem with current research into three-dimensional spatial cognition is that there are currently no studies that investigate activity in the brain whilst simultaneously reporting on behaviour. The separation of neurophysiological and behavioural studies makes it difficult to directly link underlying differences in the shapes and sizes of place and grid cell activation fields to the accuracy demonstrated in behavioural assays of three-dimensional navigation. If both behavioural and neurophysiological activity were to be recorded whilst animals solve a navigational challenge, it might be possible to match up brain firing patterns with specific behavioural events which could shed light on how the real world is represented in the brain. Studying both neural and behavioural aspects of freely-moving animals poses various technical

challenges, particularly if researchers want to observe the whole brain rather than obtain single cell recordings, as techniques to do this are still in their infancy (Nguyen et al. 2016). However, until we can gather behavioural and neurophysiological information simultaneously it will be difficult to form definitive conclusions about how navigation in three-dimensional environments maps on to the underlying neurophysiological activity, and in turn, what firing patterns tell us about animals' representations of three-dimensional space.

Very little is known about the relative contributions of genetic and environmental factors on three-dimensional spatial cognition in animals. For example, it is unknown whether rats born in a vertical burrow (such as inside a wall cavity) develop more accurate representations of the vertical dimension than their horizontal burrow-dwelling counterparts. Investigations of hippocampal development in juvenile rats suggests that many of the place, grid and head-direction cells are fully developed prior to rats leaving their nest (Langston et al. 2010; Wills et al. 2010). However, their firing fields become more stable and highly resolved with environmental experience. As a result, we might perhaps expect rats with differently-shaped burrows to develop similar cell functioning but differently-shaped activation fields. The field of three-dimensional navigation would benefit from further exploration of how much spatial cognition depends on day-to-day movement (i.e. environmental experience) and how much is pre-determined by genetic factors. I suggest that experiments investigating spatial cognition in animals of the same species that inhabit different environments or slightly different ecological niches would illustrate how these factors interact and influence spatial learning in adult animals.

Answering questions about the importance of experience and learning for navigation is also important for drawing conclusions from captive and wild animal spatial cognition studies. For example, the environmental conditions animals are exposed to prior to

experimental testing are likely to have an important influence on their performance during spatial problem-solving tasks that involve distance estimation, remembering the location of a goal or calculating a diversion in the event that the original route becomes impassable. Past experience and natural biases may be particularly difficult to determine in wild individuals where data of their movement has not been recorded throughout their lifetime.

As there is currently very little work on three-dimensional spatial cognition in surface-bound animals and even less on non-surface-bound animals, it is difficult to disentangle species-specific findings from general principles of animals' movement ecologies. As more studies emerge in different species inhabiting different environments, it will become possible to undertake comparative analyses investigating general patterns in three-dimensional spatial cognition in surface-bound and non-surface-bound species.

My findings from chapter 3 indicated that *Cordoras aeneus* learns and remembers three-dimensional spatial information in a way that is more similar to pelagic fish than to surface-bound animals. Specifically, *C. aeneus* preferentially attended to cues derived from the vertical dimension when information from the vertical and horizontal dimensions conflicted (chapter 3: Davis et al. 2014), as was the case in an identical experiment with pelagic fish (Holbrook and Burt de Perera 2009). These results could be explained by *C. aeneus'* ecological niche, for example it often inhabits hypoxic environments in which it must gulp air from the surface if it is to stay alive (Kramer 1987). Therefore, selection is likely to favour those individuals that can accurately judge vertical distances, to allow them ample time to reach the surface before the risk of suffocation. It is still difficult to disentangle the relative contributions of ancestry and the adaptive importance of accurate memory of vertical distances. Investigating spatial

cognition in other species of benthic fish that spend very little time moving vertically, such as *Pelvicachromis pulcher,* might help to shed light on the relative influence of these factors.

Questions remain about fishes' foraging style in three-dimensional environments. For example, rats allowed to explore a three-dimensional cubic lattice maze investigated each horizontal layer of the maze prior to moving vertically to the next layer (Jovalekic et al. 2011). In contrast, hummingbirds exposed to a similar maze spent a comparable proportion of their time foraging both vertically and horizontally. So far, there have been no similar studies of foraging style in fish. This could be investigated very simply by placing a cubic lattice maze rewarded with fish flake at each juncture between vertical and horizontal rods and observing the strategy fish use to search for the food, i.e. if they prioritise vertical or horizontal movements during their searches. The benefit of the cubic maze is the fish are able to move completely freely in the tank and are not required to have learnt a route before testing. This type of experiment could illuminate whether fish prioritise one dimension over the other in a passive spatial task, the way they do in a goal-focused spatial task (Davis et al. 2014; Holbrook et al. 2013) and whether this differs with their movement ecology i.e. in benthic and pelagic fish.

(2) What behavioural mechanism underlies vertical and horizontal landmark use?

In chapter 4 I investigated how a pelagic fish, *Astyanax mexicanus*, learnt the vertical and horizontal location of a food reward relative to a pair of landmarks. I predicted that fish would either learn the absolute distance from one of the two landmarks to the goal (Collett et al. 1986; Spetch et al. 1996, 1997), or learn the relative distance between the goal and both of the landmarks (Cartwright and Collett 1982; Kamil and Jones 1997).

Contrary to my hypotheses I found that fish did not use these strategies, instead favouring a randomised search over the area between the two landmarks (Chapter 4).

Studies of three-spined sticklebacks (*Gasterosteus aculeatus*) suggest that individuals that inhabit rivers−where visual landmarks may be at risk of displacement or obscured from view by sediment deposits−do not rely on visual landmarks to navigate (Girvan and Braithwaite 1998; Girvan and Braithwaite 2000). In the wild, *A. mexicanus* inhabits rivers with slow to moderate flow; thus one possible explanation for my finding that they search randomly could be that visual landmarks are not perceived to be reliable enough to indicate the position of the goal accurately. Another possibility is that over such small distances it may not be considered by fish to be risky to search randomly. Future work may help to elucidate the reasons behind these findings by investigating landmark use in fish over a larger spatial scale, as well as with species from lakes or ponds where there is no risk of currents displacing or obscuring landmarks.

One of the challenges I faced in the design of this experiment was how to camouflage the food, and as there is currently little information available on the visual capabilities of *A. mexicanus* it was difficult to determine whether I was successful in my attempts. Although I found that experience of the test condition did not have an effect on fishes' search locations I cannot completely rule out the possibility that *A. mexicanus* may have learnt to perceive the food from its start position overtime and experience of the experimental apparatus. One way to eliminate this possibility in the future would be to repeat the experiment without the presence of the food reward during testing phase. This could be done by training fish to touch the correct point between two landmarks to receive a reward in a different part of the tank. Once fish had learnt the correct location

the distance between the landmarks could be doubled and their new search position could be observed in the absence of a food reward.

(3) Can fish use pressure to determine their absolute depth?

In Chapter 5 I demonstrated that fish can determine their absolute depth using pressure cues alone. Fish were not only able to learn the depth of a food reward, but after experimental manipulation of the water pressure in the tank, they also responded by adjusting their search position, searching higher when the pressure was increased and lower when the pressure was decreased. These findings reveal a new means by which fish can obtain reliable and uniformly-available information about their exact vertical position as they navigate.

The exact mechanism fish use to determine pressure remains unknown; however, one proposed mechanism is that fish use their swim bladder. Fish are able to sense pressure changes via their swim bladder (Blaxter and Tytler 1972; McCutcheon 1966; Qutob 1962), a gas-filled sac that allows them to equilibrate their density with the density of the surrounding water, resulting in a reduction in the energetic costs associated with upward travel (Alexander 1972). It has been suggested that a second function of the swim bladder might be to determine absolute depth by integrating information about their vertical speed with the fractional rate of change in the volume of their swim bladder during vertical travel (Holbrook and Burt de Perera 2011a; Taylor et al. 2010). I did not test the importance of the swim bladder in determining depth with this experiment; future studies could investigate whether the swim bladder plays a role in depth detection by repeating my experiment under two different conditions, a control condition where the swim bladder is functional and another where the activity of the swim bladder is temporarily

suppressed. If the swim bladder is the site of depth detection, the second group of fish would be unable to determine their depth the way the fish in my experiment were able to.

3. Concluding remarks

There are currently very few studies of three-dimensional spatial cognition in surface-bound animals and non-surface-bound animals; however, interest in how animals navigate in three-dimensional environments has been growing over the last two decades. My work has (1) shown that fish can determine their absolute depth using a sense of hydrostatic pressure, and (2) begun to explore the factors that influence three-dimensional spatial cognition in animals with different movement ecologies (i.e. surface-bound and non-surface-bound animals). As the field grows and neurophysiological recording techniques improve, it will become possible to translate behaviour directly from neuronal activity, allowing us to form general conclusions about three-dimensional spatial cognition in animals with different movement ecologies as well as different cognitive abilities. First, it is important to carry out experiments to answer some of the basic questions that have so far remained unanswered: whether energetic expenditure is a plausible explanation for differences in vertical and horizontal spatial learning, if there is a difference in the quality of the information received from different sensory systems, whether the swim bladder is the site of depth detection in fish, how fish without a swim bladder determine their depth and the influence of experience and learning on animals' performances in spatial tasks.

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