Doctoral Degree in Cognitive and Brain Sciences

34th Cycle

ENVIRONMENTAL GEOMETRY IN FISHES AND TORTOISES: EFFECT OF LANDMARKS, BEHAVIOURAL METHODOLOGIES, AND SENSORY CHANNELS ON SPATIAL REORIENTATION

Supervisor:

Prof. Valeria Anna Sovrano

Ph.D. Student:

Greta Baratti, 200735

Academic Year: 2021-2022

Table of Contents

Chapter 1: Introduction

1.1 Spatial cognition

Animals inhabit life-spaces where daily activities such as getting food, mating, and hiding from predators are accomplished. Therefore, the capacity to move through space is essential for adaptation and required to handle several sources of spatial information. Position (where the animal is), direction (where the animal wants to go), and distance (how far the animal has to go) are used to move from-to places: orientation consists in aligning with a compass or particular position, to get around efficiently.

Orientation mechanisms such as *dead reckoning* or those based on the use of celestial and magnetic *compasses*, *landmarks*, and *cognitive maps* involve the capacity to interact with the environment while moving, to understand the surrounding area (e.g., how different locations are connected) (Collett & Graham, 1986; 2004; Wiener, 2011; Mouritsen, 2018; Wang & Spelke, 2003). Locations can be represented as egocentric (self-based) or allocentric (world-based), depending on the reference frame (Burgess, 2008).

Dead reckoning has been initially studied in ants (*Cataglyphis fortis*) by Müller and Wehner (1988) and consists in tracking relative distances and angle of motion in relation to the Sun, then calculating and memorising the absolute distance between two locations (e.g., the nest and a foraging site). Ants kept continuously track of their position along the path towards the foraging site, updating and integrating relative distances (e.g., turning movements) to come back to the nest travelling the shortest way. Dead reckoning has been observed also in mammals (hamsters: Etienne, 1992; Séguinot et al., 1993; humans: Loomis et al., 1993).

Celestial and magnetic *compasses* are used by “long-distance navigators” that usually cover extremely long (in time and space) distances. Homing and migratory phenomena in bird species are mainly steer by the Sun, stars, and geomagnetic field. For instance, night-migratory songbirds adapt their circadian rhythms to the movements of stars, after interpreting their centre of rotation as North (Mouritsen, 2018). Bird navigation had further aid in exploring how environmental geographies and species-specific spatial behaviours are connected (Wiltschko & Wiltschko, 2003).

The use of environmental objects as *landmarks* has been addressed in navigation studies for long (Chan et al., 2012). Tinbergen (1932; 1951) started observing that wasps (*Ammophila pubescens*) used a circular array of pine cones to find the nest in its centre. Different types of landmarks can guide navigation in specific ways, depending on their functional role. *Beacons* are objects that indicate a single nearby/exact location, providing accurate positional information: beacon-based navigation mainly consists in self-monitoring within a space and according to a specific cue. While associative landmarks are usually related to a context, behaviour, or action targeted to a goal (e.g., the red-marked arm of a maze provides food), orientation landmarks give information about heading directions in world-based coordinates.

Also, moving from-to locations with a purpose (e.g., reach a foraging site) can rely on unified environmental representations, better known as *cognitive maps* (Epstein et al., 2017). The view that the brain builds a cohesive image from a layout of spatial information (objects and/or relationships among them) has been advanced by Tolman (Tolman, 1948; Tolman & Honzik, 1930*a,b*; Tolman et al., 1946). Following this hypothesis, the use of cognitive maps during active explorations of space would allow animals to retain in memory a location, instead of a behavioural stimulus-response association.

The reference frame (egocentric or allocentric coordinates) and its relative representation in the brain (parietal or medial temporal), has led O'Keefe and Nadel (1978) to distinguish two systems for spatial navigation: the taxon system (striatum, parahippocampal gyrus) and the local system (hippocampus, retrosplenial cortex, anterodorsal thalamic nucleus, pre- and post-subiculum). Bicansky and Burgess (2018) found both egocentric and allocentric activity in the retrosplenial cortex, as a region mediating between viewpoint-dependent and independent representations. But generally, the parietal taxon system and the hippocampal locale system were argued to be separated, starting from functional dissociations: selective damages impaired one single domain, to result in place-cue learning dissociations (rats: Morris, 1981; Morris et al., 1982; Schenk & Morris, 1985; birds: Bingman et al., 1998; Vargas et al., 2004; reptiles: López et al., 2000*b*; 2001; 2003; fish: López et al., 2000*a*; 1999; Rodríguez et al., 1994).

However, another way for orienting has been a focal issue in spatial cognition studies over the past thirty years: the use of *environmental geometry* as a reference schema to localise one's current position (reorientation behaviour) or find goal-locations (searching for a hidden/salient object).

1.2 Geometric spatial reorientation

1.2.1 Basic concepts

Geometric spatial reorientation is a special phenomenon in the research field of animal cognition and consists in using large-scale environmental cues, usually, to find a goal-position after disorientation. The interest in studying the use of environmental geometry resides in animal ecologies: in natural contexts, animals would use geometric cues to orient and detect advantageous resources, since they could be referred to salient surfaces, which therefore acquire ecological meanings (Gallistel, 1990).

First observed in rats by Cheng (Cheng, 1986; Cheng & Gallistel, 1984), the spatial problem can be described as follows. Within a rectangular white room, disoriented animals can find the position of a target corner by using metric attributes, such as length, distance, or angular magnitude, in relation to left-right sense. In Figure 1, the target corner has a short wall on the right and a long wall on the left. Metric and sense provide geometric information that allow to reorient properly, but create a situation of spatial symmetry, since the diagonal (or rotational) corner at 180° has the same attributes than the target corner. If the room is perfectly rectangular, these two corners have the 50% probability to be chosen. In other words, if animals use spatial geometry to reorient, they will prefer the two geometrically correct corners rather than the incorrect ones, which have opposite geometric attributes (i.e., a short wall on the left and a long wall on the right.

The capacity to reorient with environmental geometry is widespread among vertebrates (Baratti et al., 2022; Cheng, 2008; Cheng et al., 2013; Cheng & Newcombe, 2005; Lee et al., 2017; Tommasi et al., 2012) and invertebrates (Sovrano et al., 2013; 2012; Wystrach & Beugnon, 2009; Wystrach et al., 2011*a,b*).

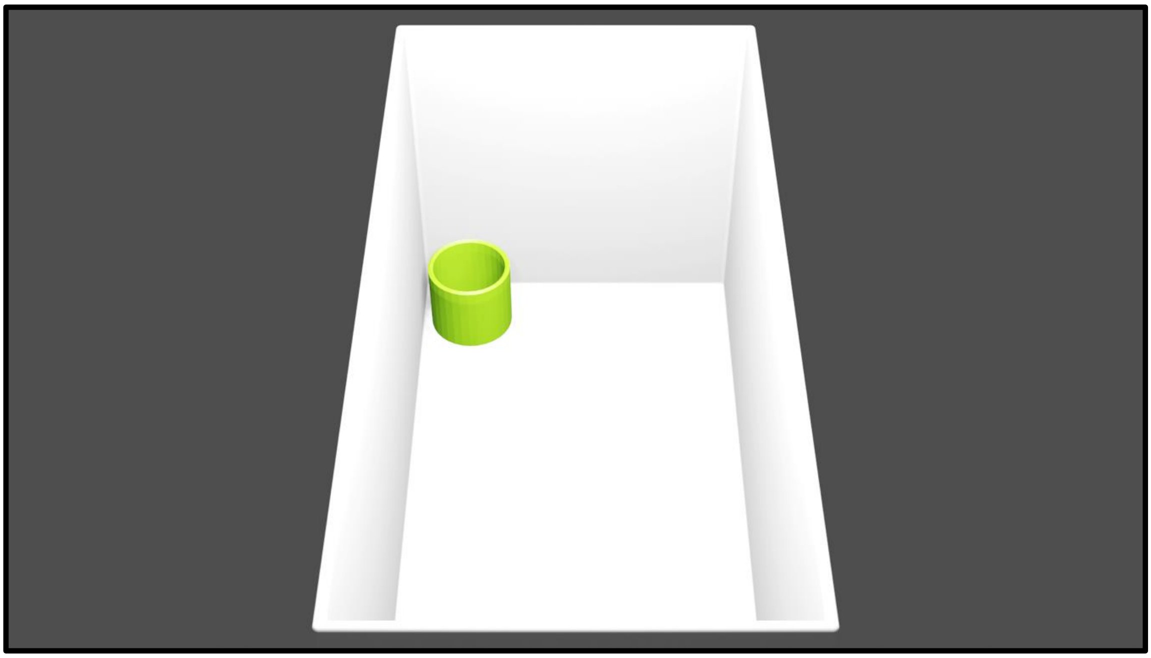


Figure 1. Rectangular white room. The target corner, i.e., where one salient object is placed, cannot be distinguished from the rotational corner, i.e., symmetric at 180°, if only geometric parameters are present (i.e., short wall right, long wall left).

In rectangular environments, the spatial symmetry can be brought through the aid of landmarks. Figure 2 shows two experimental scenarios where a conspicuous (A) or local (B) cues differently mark the space. In the former case, the target corner has a short blue wall to its right, while the short wall to the right of the rotational corner is white: animals will distinguish the two geometrically correct corner if they combine both the sources of information (geometry and landmark). In the latter case, the target corner is marked by one unique colour-pattern: animals would not need to use geometry to resolve the task.

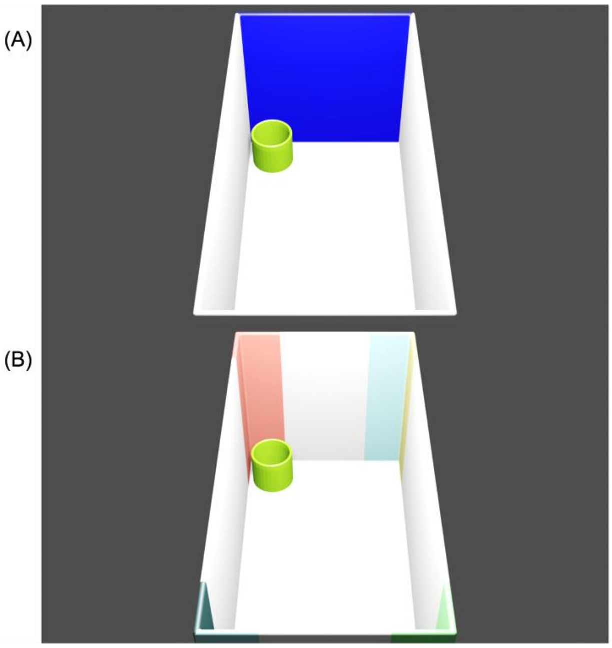


Figure 2. Rectangular white room with landmarks. (A) Conspicuous landmark: a blue wall. (B) Local landmarks: corner panels, characterised by unique patterns. The target corner can be distinguished in both cases, due to its special characteristics.

Cheng (1986) observed that rats got rotational errors while reorienting within a rectangular arena to find a location that provided food, irrespective of the behavioural task (spontaneous vs. experienced). In the presence of landmarks, their integration with geometry occurred over training only, and the performance of rats in two generalisation tests ("diagonal transposition" of the panels on the geometrically correct diagonal; "affine transformation", i.e., 90° displacement of each panel) supported reorientation behaviour as governed by independent cognitive systems. Following the modular view by Fodor (1983), the "geometric module" hypothesis was advanced: the coding of spatial geometry would be a product of such a central (encapsulated) system, while the coding of environmental objects would be a product of "featural subsystems". This orthodox definition, however, has been queried by evidence that other species than rats used both geometry and landmarks to reorient properly. Thirty years of research has led to a puzzling amount of data from several neuroscience fields, each of them providing a partial view about the point of contention: how animals code space geometries, also in relation to landmark-use?

1.2.1 Theoretical views and open issues

Geometric spatial reorientation is a controversial topic of spatial cognition research in living beings. After Cheng, a considerable amount of behavioural data was collected, and several theoretical views to interpret them were advanced (Cheng, 2008; Cheng et al., 2013; Cheng & Newcombe, 2005; Tommasi et al., 2012).

One of the first interpretive view, the "modularity-plus-language", was based on reorientation studies in humans. Hermer and Spelke (1994; 1996) reported the use of environmental geometry alone both in children and adults, while the capacity to merge geometry with a conspicuous landmark in adults only. This view arose to explain why pre-schoolers, in particular, failed at merging the shape of a rectangular room with landmarks, as well as to explain the interference of dual tasks (i.e., "verbal shadowing") on reorientation behaviour in adulthood (Hermer & Spelke, 1994; 1996; Hermer-Vazquez et al., 2001; 1999; Learmonth et al., 2002). However, evidence on nonhuman species unsupplied with language challenged the idea that verbal mechanisms would be needed (Tommasi et al., 2012), leading to refuse such a strictly modular approach.

Another view that has been proposed as an alternative to the modularity-plus-language was the "adaptive combination", which tried to better explain the growing amount of inconsistent data on the integrated use of geometry and landmarks (Newcombe & Huttenlocher, 2006; Ratliff & Newcombe, 2008). This approach claimed that the use of spatial geometry and landmarks would depend on weighted factors, such as “salience”, “usefulness”, and “experience”. From this perspective, optimal goal-oriented behaviours would be driven by multiple information, even if conflicting. Ratliff and Newcombe (2008) trained human adults both within a smaller and a larger room, requesting them to perform a conflict test within the opposite-sized room. It has been observed that the exposure to geometric and landmark cues did not have the same effects in large and small space: adults reoriented relying on the position of the landmark, irrespective of the room’s size. In other words, the experience acquired within the larger room improved landmark-use in the smaller one, whereas the experience acquired within the smaller room did not increase geometry-based reorientation in the larger one. These results emphasized the relevance of featural cues *per se* as well as the learning history of the organism during reorientation, posing in contrast with size-dependent effects found in other species (Vallortigara, 2009).

Studies with insects (Collett & Collett, 2002; Wystrach & Beugnon, 2009; Wystrach et al., 2011*a,b*), rats (Cheung et al., 2008; Stürzl et al., 2008), and birds (Pecchia & Vallortigara, 2010*a,b*), put forward the “image-matching” view, which do not distinguish between environmental geometry and landmarks. Compared to other approaches, it stated that disoriented animals would find their way through sequential panoramic images as a result of matching retinal representations (stored in memory) and current visual inputs of one target location. This view strongly supports the recruitment of sight-dependent mechanisms to regain heading or search for hidden objects.

A more recent view is the “modularity revised” (Lee & Spelke, 2010*a,b*; Spelke et al., 2010), which is consistent with the original ideas by Cheng (1986), Gallistel (1990), and Doeller and colleagues (2008). This approach suggests that spatial geometry, in terms of three-dimensional layouts or physical boundaries, and landmarks are dissociable systems in the brain of vertebrates. Starting from behavioural, neuroimaging, and neurophysiological evidence (Lee et al., 2017), it has been shown that whereas boundary-based orientation is associated with the hippocampal entorhinal cortex and subiculum, landmark-based orientation is instead associated with the striatal basal ganglia (Doeller et al., 2008; Keinath et al., 2017; Lee et al., 2018; Lever et al., 2009; Solstad et al., 2008).

Most of the studies on geometric reorientation was carried out by observing disoriented animals within rectangular and square arenas, in which the length of surfaces was the prevalent metric information. Although other kinds of shapes could provide metrics, thus becoming optimal spatial frameworks to assess reorientation behaviour, the use of different arenas than rectangular and square has been currently addressed mainly in humans.

In a study by Hupbach and Nadel (2005), rhombic environments were employed to understand if the angular magnitude (two acute angles of 60° and two obtuse ones of 120°) in relation to a landmark was differentially used within a manipulatory vs. locomotory space by children aged 4-6 y/o. It has been observed that in the "manipulatory space" (where direct movements were not allowed), only older children integrated the metric attribute with the landmark provided, while in the "locomotory space" (where direct movements were allowed), also younger children reoriented according to both information. Direct experiencing the physical space improved reorientation, as an important variable for the integration (see also: Learmonth et al., 2008).

The use of angular magnitude during disoriented navigation emerged also in adults (Lubyk et al., 2012). In this interesting study, virtual navigation experiments were run within arenas of different shapes (parallelogram, rhombic, rectangular, and reverse parallelogram), to control length, angular magnitude, and axis, in relation to left-right sense. Results showed a consistent effect of angular magnitude on reorientation, where such an attribute was mostly used when in conflict with the information provided by length and axis. Comparable behaviour has been observed in chickens (Tommasi & Polli, 2004) and pigeons (Lubyk, 2012; Lubyk et al., 2013).

Original evidence in humans was obtained within trapezoid-shaped arena, through which a new paradigm to evaluate the integrated use of spatial geometry and landmark has been developed (Twyman et al., 2018). The behavioural task there performed allowed to keep reorientation and searching for hidden objects apart, to explore the role of each process during disoriented navigation.

Net of findings on geometric spatial reorientation, a cohesive view that could consider all the aspects is hard to postulate. Most of evidence concurs on what animals were able to do to resolve geometric reorientation tasks (e.g., with or without landmarks, in small or large space, with or without experience), but not through what processes. Over the past twenty years, fish studies have supported the spatial geometry as a core concept of knowledge (Vallortigara, 2009; 2012), leading to original observations that cover several subdomains, such as the role of spontaneous vs. trained navigation, visual vs. extra-visual sensory systems, and salience of landmarks.

1.2.2 Geometric spatial reorientation in fish species

Fish species cover the 50% of extant vertebrates as a huge group of organisms that inhabit heterogeneous ecological niches. Fish usually face with challenging spatial problems, which required to plan and finalize goal-oriented movements with precision (Brown et al., 2011). The rich repertoire of spatial abilities embraces the use of multiple sensory sources of information, the implementation of task-dependent navigation strategies, and the capacity to store spatial memories within map-like representations. Since both egocentric (self-based) and allocentric (world-based) orientation have been observed in fish species, these organisms are considered as powerful animal models to look into neural mechanisms of spatial navigation (Rodríguez et al., 2021).

In the early Twenty's, Sovrano and colleagues (2002; 2003) started observing reorientation behaviour of *X. eiseni* within a rectangular white arena, in the presence or not of landmarks (a conspicuous blue wall or local panels at corners). In these two studies, a behavioural task through which train the fish to choose the geometrically correct corners was designed: the "rewarded exit task" requested the fish to enter a corridor embedded at the level of the arena's corners, which led to an enriched outer zone. For the first time in a fish species, the use of spatial geometry with and without landmarks was demonstrated, further supporting similar learning and memory representational skills even in the absence of verbal language formalisms.

In *C. auratus* as well, Vargas and colleagues (2004) found reorientation behaviour as a result of training procedures. One interesting aspect that differs from the previous studies relates to the behaviour of goldfish after the affine transformation test, where they preferred choosing in accord with landmarks. The behavioural assessment was a baseline for follow-up studies addressing the assumed independency of geometry- from landmark-use. In 2006, Vargas and colleagues observed that lateral pallium lesions impaired reorientation driven by geometry (place-learning), but non by landmarks (cue-learning). This evidence drew a parallel between the mammalian hippocampus and the fish lateral pallium as regards neural mechanisms underlying map-like navigation (Broglio et al., 2003; Rodríguez et al., 2021). However, it has also been suggested that an overlap between place- and cue-learning may occur during reorientation, since a corner position are representable either as a part of a global frame or as one single local cue (Vargas et al., 2011). Redundant processes on a same information cluster may explain why landmarks interfere with geometry-use in some circumstances (e.g., when the landmark is near to or far from the correct corner position).

Subsequent studies by Sovrano and colleagues (2005*a*; 2007) investigated reorientation behaviour of *X. eiseni* in large and small rectangular arenas, to test the effect of size in comparison with other vertebrate species (Vallortigara, 2009). Fish reoriented by spatial geometry if previously trained in a small space and then tested in a large one; conversely, by a conspicuous landmark (a blue wall) if trained in a large space and tested in a small one. The same behaviour emerged after the affine transformation test. Local view theories (Cheung et al., 2008; Sovrano & Vallortigara, 2006; Stürzl et al., 2008) can explain this dissociation: from the centre, the metric attribute of boundary length is detectable as a whole within small spaces only; within large spaces, a landmark becomes more reliable than geometry. Besides that, previous experience with one set of cues (e.g., training in small space: geometry) could have affected the subsequent approach to the other (Newcombe & Huttenlocher, 2006; Ratliff & Newcombe, 2007; 2008).

In another interesting study, Sovrano and colleagues (2005*b*) have shown that lateralized individuals of *X. eiseni* performed better at combining the spatial geometry of a rectangular arena and a blue wall, while both lateralized and nonlateralized fish learned to use local panels within a square arena. This evidence focused on the fact that reorientation behaviour with geometry and landmarks together may involve some sort of self-based information (e.g., long surface on my left/right), as it occurs when landmark distance is centred on who is reorienting and not on the corner position that must be reached.

The use of training procedures in geometric reorientation studies suggested that teleost fishes learn to use environmental geometry and landmarks to search for target locations, with a few exceptions. However, learning processes usually induce meaningful changes on “natural” behaviours of animals grew in the wild. From 2012 to 2020, a batch of original studies started adopting a new procedure to test spontaneous behaviour of fish within geometric contexts. The “social-cued memory task” evaluates short-term reorientation of untrained fish within one single session of test. Typically, one social companion is used to mark the correct corner position (i.e., one of the four corners), while allowing the experimental fish to observe it for a while. After removing the social cue, the subject is required to approach the target position with an accuracy above chance.

Lee and colleagues (2012*c*) reproduced the blue wall landmark experiment, by observing *X. eiseni* and *D. rerio*, also with the aim to draw a between-species comparison. Although untrained fish spontaneously reorient by the spatial geometry of a rectangular arena, several limitations were found at using both geometry and landmark information. One interesting aspect is that reorientation occurred only with the landmark near the correct location: conversely, fish made rotational errors in accord with geometry. Also, zebrafish behaviour mainly relied on geometry rather than the conspicuous landmark. To better understand how the landmark proximity could have interact with landmark-use in geometric environments, individuals of *X. eiseni* took part in several experiments, while varying the number (two, four) and proximity (near, far) of corner panel landmarks, the arena’s shape (rectangular, square), and the behavioural task (spontaneous, rewarded) (Sovrano et al., 2020*a*). It has been observed that landmark proximity interacted not only with geometry but also with the task, since untrained fish did not use distal landmarks in the absence of metric frame, while trained fish overcame that limit. However, reorientation behaviour of trained fish was biased by landmarks near the target position, acting as beacons to indicate one single exact location irrespective of the global frame.

The effect of beacons on spontaneous reorientation had been previously addressed by Lee and colleagues (2015*a*) in *D. rerio*, which was increasingly emerging as predominant model in neuroscience research (Levin & Cerutti, 2009; Lin et al., 2016). Untrained fish did not combine the rectangular geometry provided by transparent surfaces with several types of landmarks (a blue plastic sheet on the ground, a distal blue cylinder, a proximal light source), while showed boundary-mapping of opaque surfaces when the blue cylinder was either proximal to or distal from the correct corner position.

Transparent surfaces to build geometric layouts were introduced in 2013 by Lee and colleagues, to assess the use of isolated metric attributes in *D. rerio*. Distance (from centre to surfaces), corners (as meeting points of two tangent surfaces), and length (short or long surfaces) were set apart in a rectangular or square transparent arena. Zebrafish spontaneously used only the self-based distance to reorient, contrary to what most of reorientation evidence supported for long, that is, the use of boundary-length. Similar results had been obtained in children within rhombic arrays (Lee et al., 2012*a*).

The possibility to test fish reorientation within transparent, “nonvisual” environments led to investigate the role of other sensory modalities than sight in two hypogean species of fish (Sovrano et al., 2018*a*). The cavefish *Astyanax mexicanus* and *Phreatichthys andruzzii* underwent the rewarded exit task within a rectangular arena, with or without a conspicuous landmark (one plastic sheet with five embossed stripes) placed on the short wall. Despite the lack of sight, both the species learned to reorient by using spatial geometry alone and in conjunction with landmark. At that time, Gianni and colleagues (2018) was finding similar results in children aged 5-7 y/o during spontaneous reorientation within a rectangular room made of transparent glass surfaces. On these findings, the view that reorientation behaviour may be driven by haptic/tactile-like stimulation, overcoming visual constrains, was advanced.

One recent study focusing on reorientation behaviour of *D. rerio* over training is that by Baratti and colleagues (2020), which laid the foundations for most of the works reported in the present Thesis. Adult male zebrafish took part in the rewarded exit task and easily learned to reorient within a rectangular white arena, showing notable abilities (e.g., their performance improved over time) and resilience to generalisation test in conditions of response extinction. This evidence started challenging a common way of thinking about zebrafish cognition: the behavioural tasks put forward hitherto are not optimal enough to assess learning and memory mechanisms of this species (Bailey et al., 2015; Gerlai, 2011). However, the fact that spontaneous spatial abilities of zebrafish can be enhanced by experience (Arthur & Levin, 2001; Darland & Dowling, 2001; Sison & Gerlai, 2010; Williams et al., 2002) is a promising aspect, also in relation to a combinational approach (Grunwald & Eisen, 2002).

Overall, fish reorientation behaviour seems to be governed by two independent systems, one for the coding of geometric information and the other for nongeometric information, also considering the structural dissociation between lateral pallium and striatal regions, in homology with mammals, birds, and reptiles (Broglio et al., 2010; 2015; Durán et al., 2008; 2010; López et al., 2000*a*; Portavella & Vargas, 2005; Rodríguez et al., 2002; Salas et al., 1996*a,b*; Smeets et al., 2000; Solstad et al., 2008).

Even if a significant amount of data has been collected in fishes, several issues are still left unexplored. The present Thesis aimed at stressing the relationship between environmental geometry and landmark objects, the effect of task's demands, and the involvement of tactile-like sensory systems or motion patterns for reorienting. Besides fish, one reptile species has been observed: the Hermann tortoise (*T. hermanni*). The rationale behind that, first, was related to the opportunity to find geometry-based capacities in a sedentary ("non-nomadic") vertebrate; but secondly, to develop a fitting methodology to train these animals in semi-controlled settings.

1.3 Aim of the Thesis and overview of the studies

The present Thesis explored spatial reorientation behaviour of fishes and tortoises, addressing three issues: (1) the use of geometry and the integration of geometry with landmarks; (2) the role of behavioural tasks methodology; (3) the recruitment of extra-visual sensory channels and/or the implementation of motion patterns. Even if these three issues are interlaced, they will be dealt separately throughout the Thesis, to provide a coherent outline of the research project.

I investigated the use of geometry and the integration of geometry with landmarks in fish (*D. rerio*) and tortoises (*T. hermanni*), to expand the knowledge about these phenomena to two animal models of great interest. The view of two independent systems, one for spatial geometry and the other for landmarks, will be partially revised in the light of original evidence.

I investigated the role of two behavioural tasks in three fish species (*D. rerio*, *X. eiseni*, and *C. auratus*), with a focus on *D. rerio*, to understand to what degree the experimental procedure can affect reorientation behaviour with or without the influence of geometrically informative layouts. Tasks that depend on spatial reference memory (i.e., driven by learning) have been compared to those that depend on spatial working memory (i.e., driven by spontaneous behaviour), to stress the distinctive characteristics of each as regards the use of geometry.

I investigated the recruitment of extra-visual sensory channels and/or the implementation of motion patterns in *D. rerio*, to assess whether the use of environmental geometry in the case of non-visibility might be due to other sensory modalities than sight and/or motion patterns at reorienting within physical boundaries. The evaluation of motion patterns was performed also in the case of visibility, in *D. rerio* and *T. hermanni*.

The present Thesis embraces eight studies, which have been allocated to the three main issues just described. For clarity, an overview of the studies is provided in Table 1.

Main issue	**Study**	**Species**	**Scientific question**
1) Environmental geometry and landmarks	No. 1	*D. rerio*	Integrated use of environmental geometry and blue wall landmark
	No. 2	*D. rerio*	Landmark-use with or without the influence of informative environmental geometry
	No. 3	*T. hermanni*	Geometry-based reorientation
	No. 4	*T. hermanni*	Integrated use of environmental geometry and blue wall landmark

2) Spontaneous vs. acquired geometric spatial reorientation	No. 5	*D. rerio*, *X. eiseni*, *C. auratus*	Geometry-based reorientation through nonvisual, i.e., transparent, surfaces
	No. 6	*D. rerio*	Use of isolated geometric cues (distance, corners, length)
	No. 7	*D. rerio*	Integrated use of environmental geometry and 3D cylindrical blue landmark
3) Extra-visual sensory systems and motion patterns	No. 8	*D. rerio*	Geometry-based reorientation through nonvisual, i.e., transparent, surfaces after LL pharmacological ablation

Table 1. Overview of the eight studies included in the Thesis, as divided by main issues.

The present research project applied a comparative approach at different levels – across species, methods, and sensory channels – to broadly investigate the impact of environmental geometry on reorientation behaviour and spatial learning. To do so, two vertebrate groups were observed: three freshwater fish species and one terrestrial reptile species. All the fish came from laboratory breeding stocks or were provided by a local supplier (“Acquario G di Segatta Stefano”, Trento, Italy). Instead, the tortoises belonged to a preserved colony at the sanctuary “Sperimentarea” (Civic Museum Foundation of Rovereto, Trento, Italy). For each species, a brief description on ecology and a justification for their use have been provided in the following subsection.

1.4 Animal models

1.4.1 Zebrafish (*D. rerio*)

The zebrafish (Figure 3) is a cyprinid from Asia (India) and belongs to the order of Cypriniformes. In the wild, individuals are spread in the Ganges and Brahmaputra rivers, where they inhabit stagnant or shallow water basins rich in vegetation (e.g., canals, streams, and rice fields). Tropical monsoon climate is characterised by seasonal variations that affect the range of temperature: for zebrafish, the most suitable range covers approximately between 6° C in the wintertime and 38° C in summer (Spence et al., 2008).

Figure 3. A specimen of *D. rerio*.

Source: *Pixabay*.

Zebrafish live in large groups and exhibit patterns of collective motion (i.e., behaviours of schooling and shoaling; Kalueff et al., 2013; Miller & Gerlai, 2011; 2012); they feed on zooplankton, and their reproductive life highly depends on photoperiod, food cycles, and hormonal homeostasis (Abdollahpour et al., 2020; Pradhan & Olsson, 2015; Spence & Smith, 2006).

Sexual dimorphism is little accentuated between male and female specimens; sex determination and gonad differentiation are still unclear (Liew & Orbán, 2013). Adults typically range from 3 to 6 cm in body-length and show a striped blue-and-silver livery (sometimes blue-and-gold in males).

Using zebrafish as animal model is a common practice in biological sciences (Choi et al., 2021; Grunwald & Eisen, 2002; Levin & Cerutti, 2009; Luchiari et al., 2021), given some well-known characteristics of this species, such as sequenced genome and genetic manipulation, high embryos production, live imaging at early developmental stages. However, behavioural protocols for testing zebrafish cognitive abilities are reported to be lacking (Bailey et al., 2015; Gerlai, 2011; Meshalkina et al., 2017). Several studies have demonstrated to what extend this fish possess spatial knowledge (Arthur & Levin, 2001; Darland & Dowling, 2001; Sison & Gerlai, 2010; Williams et al., 2002) and remarkable precision in geometric reorientation tasks (Baratti et al., 2022), making zebrafish a model of great interest for multidomain combinational approaches in the understanding of reorientation behaviour.

1.4.2 Redtail splitfin fish (*X. eiseni*)

The redtail splitfin fish (Figure 4) is a goodeid from America (Mexico) and belongs to the order of Cyprinodontiformes. In the wild, individuals are spread in the Grande de Santiago and Balsas rivers, where they inhabit water basins poor in vegetation (Piller et al., 2015; Ritchie et al., 2007).

Figure 4. A male specimen of *X. eiseni*.

Source: *https://www.aquarist-classifieds.co.uk/php/image/574634.jpg*

Sexual dimorphism is marked well between male and female specimens, due to differences in the pigmentation of livery: males have an orange-coloured tail, the dorsal part of the head is darker while the area around gills can assume pink tones; by contrast, females show a monochromatic olive/brown colouration and a prominent abdomen (Currier, 2013). Adults typically range from 3 to 8 cm in body-length, are quite territorial with heterospecific individuals, and live in small groups.

Redtail splitfin fish has been used for long in geometric reorientation studies (Lee et al., 2012*c*; Sovrano et al., 2002; 2003; 2005*a*; 2007; 2020*a,b*; reviewed in Baratti et al., 2022), as well as for exploring other cognitive abilities of teleosts, such as social laterality (Sovrano et al., 1999; 2001), detour behaviour (Sovrano et al., 2018), visual discrimination learning and geometric illusions (Sovrano & Bisazza, 2008; 2009; Sovrano et al., 2015; 2016; 2022*a,b*; Stancher et al., 2013; Truppa et al., 2010), and numerical cognition (Stancher et al., 2013). The versatility of this species in managing and solving different kind of tasks is a relevant aspect, which has been considered when designing the comparative approach of the present Thesis project.

1.4.3 Goldfish (*C. auratus*)

The goldfish (Figure 5) is a cyprinid from Asia (mainly from China, Vietnam, and Korea) and belongs to the order of Cypriniformes. In the wild, individuals occur from the Iberian Peninsula to the Black Sea area, except for northern regions, such as Ireland and Scotland (Lelek 1987, Lorenzoni, 2007; Maitland, 2004). Hybrids are also observed in US waters, where they pose a threat to local ecosystems.

Figure 5. A specimen of *C. auratus*.

Source: *Pixabay*.

Goldfish are gregarious, but vulnerable to competition and predation (Piironen & Holopainen 1988, Paszkowski et al. 1990; Tonn et al. 1991). However, they can survive even under adverse environmental conditions, such as water pollution, high levels of turbidity, and sudden fluctuations in water temperature, salinity, and pH (Abramenko et al., 1997; Nico & Schofield, 2006; Spotila et al. 1979). High fecundity and omnivorous diet are other characteristics allowing the goldfish to grow easily in captivity (Lorenzoni, 2007).

Like zebrafish, goldfish is a limnophilic cyprinid whose reorientation behaviour has been widely investigated, even from a neurophysiological perspective (Vargas et al., 2004; 2006; 2011). Lesion studies in goldfish have led to draw a parallel between the mammalian hippocampus and the fish lateral pallium as regards neural mechanisms underlying the use of environmental geometry for goal-oriented navigation, making this fish an optimal model for behavioural and neurofunctional comparisons.

1.4.4 Hermann tortoise (*T. hermanni*)

The Hermann tortoise (Figure 6) is a reptile species from the order of Testudines, which commonly inhabits European regions with Mediterranean climate. Preferential habitats encompass evergreen oak forests, meadows, and coastal sand dunes (Stubbs, 1989). Two main subspecies can be found: while *T. h. hermanni* occurs in Western regions such as Italy, Spain, and France, *T. h. boettgeri* typically occupies Eastern and Balkan zones (van Dijk, 2004). Besides the range of distribution, these two subspecies also differ for morphological aspects, such as the features of carapace and plastron.

Figure 6. A specimen of *T. hermanni*.

Source: *Pixabay*.

Individuals of *T. hermanni* pass the late-autumn and winter months in hibernation; after awakening, they need a daily exposure to the sun to trigger metabolism off (exothermic reactions). As a sedentary species, they display a definite range of activity after hibernation (Stubbs & Swinglnad, 1985). Tortoises are mainly herbivorous, spending a lot of time in search of food within narrow territories, and lay eggs from May to July. About that, mating and breeding activities start with sexual maturity around 10 years and highly depend on several factors, such as the females' body size (Hailey, 1990). The visual and olfactory systems are developed well and involved in colour discrimination and social interactions (Galeotti et al., 2007; Pellitteri-Rosa et al., 2010).

Currently, Hermann tortoise cognition is less understood and described than fish cognition, most of all those aspects related to learning behaviour and spatial skills. In fact, orientation abilities have been described only in natural environments by observing spontaneous navigation routines (e.g., from displacement sites back to home sites: Chelazzi & Delfino, 1986; Chelazzi & Francisci, 1979). Also, since the use of environmental geometry has never been addressed in reptiles, Hermann tortoise could represent a promising animal model to explore the cognitive phenotype of sedentary vertebrates in the field of spatial behaviour.

1.5 Ethics statements

As regards investigations on fish, the research project was carried out in the Animal Cognition and Neuroscience Laboratory (ACN Lab) of the Center for Mind/Brain Sciences (CIMeC, University of Trento, Italy). Animal husbandry and experimental procedures complied with European Legislation for the Protection of Animals used for Scientific Purposes (Directive 2010/63/EU) and were previously authorized by the

University of Trento’s Ethics Committee for the Experiments on Living Organisms, and by the Italian Ministry of Health (auth. num. 1111/2015-PR; 848/2020-PR).

As regards investigations on tortoises, the research project was carried out at the natural estate of “Sperimentarea” (Civic Museum Foundation of Rovereto, Trento, Italy). Animal husbandry and experimental procedures complied with European Legislation for the Protection of Animals (Directive 2010/63/ EU) and were in accordance with the Italian and European Community laws on protected wild species (Art. 8/bis 150/92 all. A Reg. (CE) 338/97). The experimental protocol was authorized by the internal Ethics Committee of the Civic Museum Foundation of Rovereto. The number of animals was consistent with the alternative method of “Reduction”, which allows to observe the minimum number of animals to draw statistically valid results.

Chapter 2: Environmental Geometry and Landmarks

2.1 Study 1: Conspicuous landmark (blue wall) in zebrafish

2.1.1 Introduction

After Cheng (1986), the interest in using a coloured wall landmark in conjunction with rectangular frames started from behavioural observations in chickens (*Gallus gallus*, Vallortigara et al., 1990), pigeons (*Columba livia*, Kelly et al., 1998), primates (*Macaca mulatta*, Gouteaux et al., 2001), and humans (children and adults, Hermer & Spelke, 1994; 1996). While all these nonhuman species showed the simultaneous use of both information, human adults only resolved this reorientation task (choosing the correct corner position). However, children gave proof to reorient properly within a larger space (Learmonth et al. 2002; 2008) or in the presence of salient landmarks acting as beacons (Lee et al., 2006; Twyman et al. 2007; Shusterman et al. 2011; Wang et al., 1999). Thus, the process of incorporating geometric and nongeometric cues seems to interact with several factors, such as, the type of task and the memory system involved (spontaneous choice – working memory; rewarded training – reference memory), the experimental space's size, the opportunity to directly move within the space, the power of landmarks to be salient, and so forth. A debated issue also concerns the function of landmarks after disorientation, if they are used as directional cues to reorient (e.g., food in the corner near the landmark, on the left) or associative beacons during disoriented navigation (e.g., after boundary mapping, the landmark is used to choose the correct over the rotational corner).

Teleost fishes have been a promising animal model in geometric reorientation studies (Baratti et al., 2022). Among all, zebrafish has proven to possess notable spatial precision and memory, together with a natural disposition to spatial learning (Baratti et al., 2020; 2021; Haight & Schroeder, 2011; Lee et al., 2015*a*; 2013; 2012*c*; Spence et al., 2011; Sovrano et al., 2020*b*). Previous works showed that untrained zebrafish used the spatial geometry of a rectangular white arena (Lee et al., 2015*a*; 2012*b*), exhibiting the same behaviour when tested in extinction of response (Baratti et al., 2020). Furthermore, they spontaneously integrated the shape of that arena with a conspicuous landmark, but only if near a target location defined by a social companion. If far, the blue wall was not used to reorient, serving as an attractive local beacon (Lee et al., 2012*c*).

Study 1 investigated whether zebrafish could learn to combine the two information irrespective of landmark distance and length, also focusing on reorientation behaviour after blue wall removal (Test 1, also "geometric test", the landmark was removed, leaving available the geometric frame only) and after affine transformation (Test 2, the landmark was moved 90° right, putting into conflict metric and landmark information). If the failure at combining geometry and landmark depended on reorientation capacities, we would expected nor boundary mapping neither the use of landmark as a directional cue; if the landmark proximity was needed for the integration, zebrafish would not chose the correct corner position in relation to the far blue wall landmark; if learning mechanisms and experience could aid in overcoming the attractiveness of the landmark, we would expected the integrated use of spatial geometry and landmark over time, with a further validation in the affine transformation test.

2.1.2 Materials and Methods

Subjects were 18 adult male zebrafish (wild type), ranging from 3 to 5 cm in body-length. 9 fish took part in Experiment 1 (*Long blue wall landmark*) and 9 fish took part in Experiment 2 (*Short blue wall landmark*). The sample size was determined using G*Power (α = 0.05, power of .80) and is in line with several behavioural studies evaluating zebrafish cognitive abilities through operant conditioning procedure (Baratti et al., 2021; Gatto et al., 2021; Miletto Petrazzini et al., 2019; Santacà et al., 2021; Sovrano et al., 2022*b*). All the fish were reared within familiar home tanks (Wave Zen Artist, Amtra®, 35 × 28 × 30 cm, 27 L capacity), in which a hang-on-back filter (Niagara 250, WAVE) ensure the quality of water, and a 25 W heater (Newa Therm®, NEWA®) maintained the temperature of water around 26 ± 1°. The fish were kept in light-dark 14:10 photoperiod and, before starting the experiment, they were fed twice a day by administering a small amount of dry food (Vipan, sera®).

The apparatus was like that used by Sovrano and colleagues (2002; 2003; 2005*a*; 2007) to explore reorientation behaviour of another fish species, that is, the teleost *X. eiseni*. It consisted of a rectangular white arena (31 × 16 × 14 cm; area = 496 cm^2) made of polypropylene (Poliplak®), which was placed within a rectangular plastic tank (60 × 36 × 25 cm) enriched with polychromatic gravel and artificial plants. Fish underwent the geometric reorientation task within the rectangular white arena, while the surrounding tank provided the rewarding area where the experimental fish could find food and social companions. At the level of corners, the rectangular white arena had one small rectangular corridor (2 × 3 cm; 2.5 in length; 4.5 cm from the ground), through which the fish could leave the arena to access the external familiar zone. At the end of each corridor, there was a transparent acetate sheet (2.5 × 3.5 cm) that the fish could easily push to exit; the upper part of this sheet (2.5 × 2.5 cm) was covered with a pink-coloured (RGB: 255, 192, 203) plastic sheet. Only one corridor allowed the fish to leave the arena, while the other three ones were blocked from the outside through a green (RGB: 0, 128, 0) wire clip. Although the unrewarding corridors were blocked, three small holes (diameter: 0.5 cm) were carved in the lower transparent part of the sheet, to ensure a regular water flow. This apparatus had been previously validated by Baratti and colleagues (2020) to investigate the use of spatial geometry in trained zebrafish.

The blue wall landmark was created to perfectly fit the inner surfaces of the rectangular arena. To control the length of the wall as a variable, two landmarks were prepared: a long blue wall (31 × 16 cm) and a short blue wall (14 × 16 cm), RGB: 30, 144, 255. Figure 7.

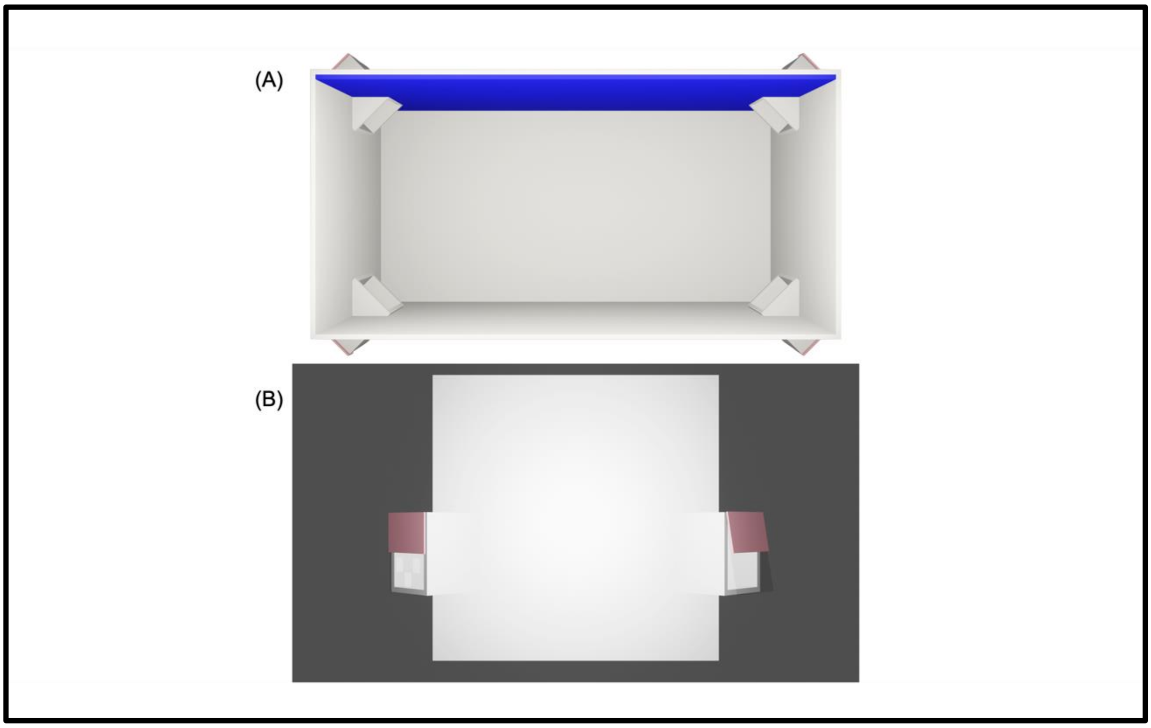


Figure 7. 3D reconstruction of the rectangular white arena with a conspicuous landmark. Top (A) and frontal (B) view, with a detail on the corridors. Here the long blue wall version is represented.

The experiment consisted of four phases: (1) Training, where the fish were required to resolve the geometric reorientation task, choosing more the correct corner; (2) Test

1, geometric test, where the blue wall was removed; (3) Re-training, where the fish had to meet the accuracy threshold ≥ 70% again, after the extinction induced by the geometric test; (4) Test 2, affine transformation, where the blue wall was moved 90° right but held the same position, to put the integration of geometry and landmark into conflict (the correct corner has the same metric properties, e.g., shorter wall on the right, longer wall on the left, but not the same sense properties, e.g., the longer blue wall on the left is now the shorter blue wall on the right). Figure 8.

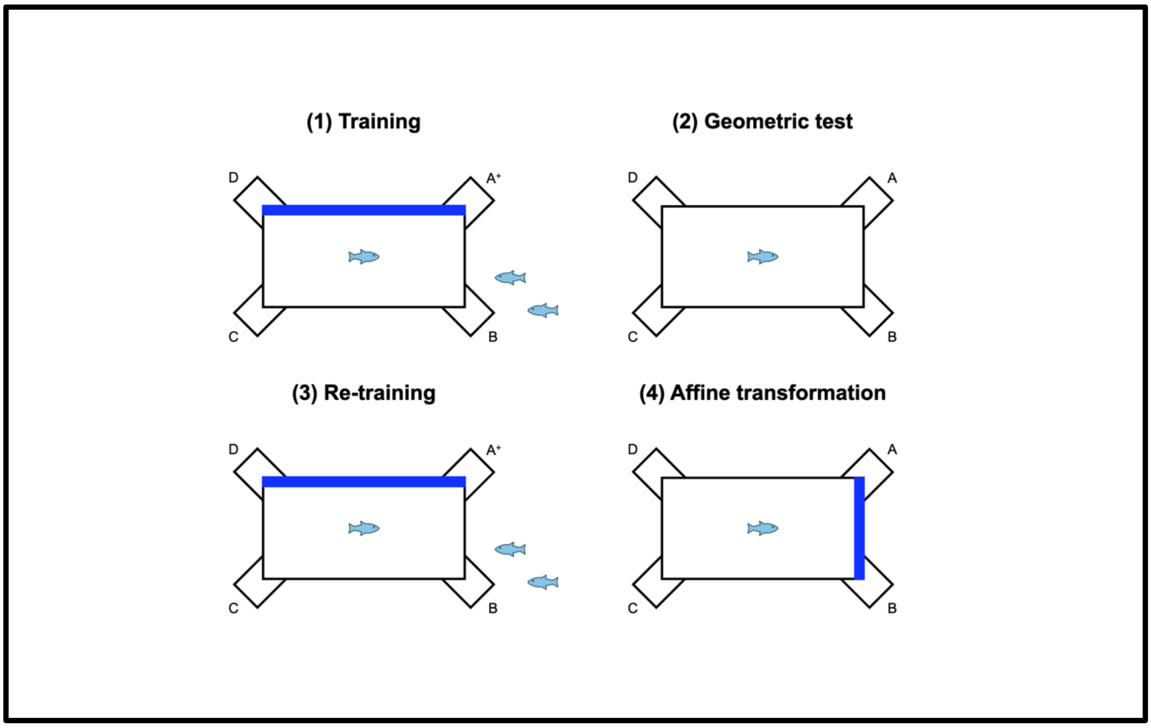


Figure 8. Schematic representation of the four experimental phases.

The procedure was similar as described by Baratti and colleagues (2020); each fish was individually observed from Monday to Friday.

Training and Re-training consisted of one session per day of 8 trials each, until the fish had met an accuracy threshold ≥ 70% for the correct corner (A^+) per two subsequent sessions of training. At the beginning of each trial, the experimental fish was hosted within a transparent cylinder (diameter: 6 cm; height: 8 cm) in the centre of the arena; after 30 s, the cylinder was slowly lifted, and the fish was free to move and explore the environment for a 10-min limit. During that time, all the choices made by the fish towards the four corners (i.e., getting into the corridors with the whole body until the tail moved as to force an exit) were sequentially scored. The four corners were labelled, for convenience: “A^+” (the correct corner); “B”, the incorrect corner near the correct; “C”, the incorrect corner on the diagonal (also, the rotational one); “D”, the incorrect corner far from the correct. A correction method (Caro et al., 1979) was applied: fish were allowed to choose the incorrect corners before leaving the arena through the correct one. Full reinforcement (only correct choices) consisted of the administration of food, plus a 6-min rest within the familiar zone, where two companions were free to join the experimental fish; partial reinforcement (also incorrect choices) consisted of a 2-min rest within the familiar zone, but where the two companions were hosted within a transparent jar. In the latter case, any physical exchange (i.e., contact, odour) were prevented. Fish were granted 25 sessions for learning.

As regards the tests (geometric test, affine transformation), they were performed when the fish had met the learning criterion ≥ 70% at training and re-training. Each test was carried out in two subsequent sessions of 5 trials, until collecting 10 valid test trials. Fish underwent the test in extinction of response, blocking all the four corridors and providing no differential reinforcement. To keep the fish motivation as high as possible, and to counteract extinction detriment, recall trials were run following this schema: three correct recalls, two test trials; two correct recalls, two test trials; one correct recall, one test trial. In the case of incorrect recalls, an accuracy ≥ 70% (both first and total choices) was needed before proceeding with the test. If this threshold was not achieved within 5 trials, the fish underwent a full training session as usual. Each test trial lasted 2 min but, if the fish was late in giving a response, that time could be extended until 10 min to have, at least, one valid choice. Intertrial interval (ITI) was 5 min.

An inter-observer reliability criterion (Caro et al., 1979) was applied in recoding different videos ($p < .001$, Pearson's correlation between the ratio calculated on the original coding and on *the novo* coding performed by an experimenter blind on the test condition of the experimental subject).

The evaluation of fish behaviour was based on the percentage [%] of first and total choices in the session of learning and during the two tests.

2.1.3 Results

A one-way ANOVA was performed to evaluate if the length of the blue wall landmark (long, short) and/or its distance (near, far) as for the correct corner A^+ affected the total number of trials [f] to meet the learning criterion ≥ 70%. The one-way ANOVA showed no significant effects (Wall Length: $F_{(1,16)} = .15$, $p = .71$; Wall Distance: $F_{(1,16)} = .06$, $p = .81$).

A repeated measures ANOVA was performed by considering the first and total choices [%] for the four corners in the session of learning (i.e., when the fish met the accuracy threshold ≥ 70%). Results are shown in Figure 9A. The ANOVA with Corner (A^+, B, C, D) as within-subject factor, Wall Length (Long, Short) and Wall Distance (Near, Far) as between-subject factors, showed the following results: there was a significant effect of Corner (first choices: $F_{(3,42)} = 260.2$, $p < .001$, $\eta_p^2 = .95$; total choices: $F_{(3,42)} = 265.56$, $p < .001$, $\eta_p^2 = .95$), while there were no significant effects of Corner ∗ Wall Length (first choices: $F_{(3,42)} = .17$, $p = .92$; total choices: $F_{(3,42)} = .79$, $p =$

.51), Corner ∗ Wall Distance (first choices: $F_{(3,42)} = 1.41$, $p = .25$; total choices: $F_{(3,42)} = 2.41$, $p = .08$), Corner ∗ Wall Length ∗ Wall Distance (first choices: $F_{(3,42)} = 1.13$, $p = .35$; total choices: $F_{(3,42)} = 1.09$, $p = .36$), Wall Length (first choices: $F_{(1,16)} = -1.502\text{e-}15$, $p = 1$; total choices: $F_{(1,14)} = 2.03$, $p = .18$), Wall Distance (first choices: $F_{(1,16)} = 5.971\text{e-}14$, $p = 1$; total choices: $F_{(1,14)} = 2.03$, $p = .18$), and Wall Length ∗ Wall Distance (total choices: $F_{(1,14)} = .03$, $p = .88$). A paired samples *t*-test was then applied to analyse the difference among corners. The *t*-test on the first choices showed a significant difference between A^+ and B (mean ± SEM: $A^+ = 81.81 \pm 2.28$; $B = 7.92 \pm 2.43$; $t_{(17)} = 18.87$, $p < .001$, 95% CI [2.89, 5.99]), A^+ and C (mean ± SEM: $A^+ = 81.81 \pm 2.28$; $C = 5.83 \pm 1.64$; $t_{(17)} = 22.42$, $p < .001$, 95% CI [3.46, 7.10]), A^+ and D (mean ± SEM: $A^+ = 81.81 \pm 2.28$; $D = 4.44 \pm 1.59$; $t_{(17)} = 24.23$, $p < .001$, 95% CI [3.75, 7.66]), but not between B and C (mean ± SEM: $B = 7.92 \pm 2.43$; $C = 5.83 \pm 1.64$; $t_{(17)} = .6$, $p = .56$), B and D (mean ± SEM: $B = 7.92 \pm 2.43$; $D = 4.44 \pm 1.59$; $t_{(17)} = .98$, $p = .34$), C and D (mean ± SEM: $C = 5.83 \pm 1.64$; $D = 4.44 \pm 1.59$; $t_{(17)} = .73$, $p = .47$). The *t*-test on the total choices showed a significant difference between A^+ and B (mean ± SEM: $A^+ = 81.74 \pm 2.42$; $B = 7.82 \pm 2.42$; $t_{(17)} = 18.79$, $p < .001$, 95% CI [2.88, 5.97]), A^+ and C (mean ± SEM: $A^+ = 81.74 \pm 2.42$; $C = 6.07 \pm 1.8$; $t_{(17)} = 20.44$, $p < .001$, 95% CI [3.14, 6.48]), A^+ and D (mean ± SEM: $A^+ = 81.74 \pm 2.42$; $D = 4.37 \pm 1.62$; $t_{(17)} = 22.54$, $p < .001$, 95% CI [3.48, 7.14]), but not between B and C (mean ± SEM: $B = 7.82 \pm 2.42$; $C = 6.07 \pm 1.8$; $t_{(17)} = .49$, $p = .63$), B and D (mean ± SEM: $B = 7.82 \pm 2.42$; $D = 4.37 \pm 1.62$; $t_{(17)} = .98$, $p = .34$), C and D (mean ± SEM: $C = 6.07 \pm 1.8$; $D = 4.37 \pm 1.62$; $t_{(17)} = .87$, $p = .4$).

A paired samples *t*-test was performed by considering the first and total choices [%] for the two diagonals in Test 1 (geometric test, after removing the blue wall). Results are shown in Figure 9B. The *t*-test showed a significance difference between AC and

BD (first choices: mean ± SEM: AC = 67.22 ± 3.41; BD = 32.78 ± 3.41; $t_{(17)}$ = 5.05, p < .001, 95% CI [.57, 1.79]; total choices: mean ± SEM: AC = 61.9 ± 1.6; BD = 38.1 ± 1.6; $t_{(17)}$ = 7.45, p < .001, 95% CI [1, 2.49]).

A repeated measures ANOVA was performed by considering the first and total choices [%] for the four corners in Test 2 (affine transformation, after moving the blue wall 90° right)[1]. Results are shown in Figure 9C. The ANOVA with Corner (A, B, C, D) as within-subject factor showed the following results: there was a significant effect of Corner (first choices: $F_{(2,32)}$ = 9.36, p < .001, η_p^2 = .37; total choices: $F_{(2,31)}$ = 11.55, p < .001, η_p^2 = .42). A paired samples *t*-test was then applied to analyse the difference among corners. The *t*-test on the first choices showed a significant difference between A and D (mean ± SEM: A = 28.82 ± 5.68; D = 5.29 ± 2.12; $t_{(16)}$ = 3.64, p = .002, 95% CI [.31, 1.44]), B and C (mean ± SEM: B = 45.29 ± 6.07; C = 20 ± 4.02; $t_{(16)}$ = 2.93, p = .01, 95% CI [.17, 1.24]), B and D (mean ± SEM: B = 45.29 ± 6.07; D = 5.29 ± 2.12; $t_{(16)}$ = 5.66, p < .001, 95% CI [.69, 2.03]), C and D (mean ± SEM: C = 20 ± 4.02; D = 5.29 ± 2.12; $t_{(16)}$ = 3.73, p = .002, 95% CI [.33, 1.46]), but not between A and B (mean ± SEM: A = 28.82 ± 5.68; B = 45.29 ± 6.07; $t_{(16)}$ = −1.54, p = .14), A and C (mean ± SEM: A = 28.82 ± 5.68; C = 20 ± 4.02; $t_{(16)}$ = 1.11, p = .28). The *t*-test on the total choices showed a significant difference between A and D (mean ± SEM: A = 30.85 ± 4.44; D = 10.93 ± 2.29; $t_{(16)}$ = 3.33, p = .004, 95% CI [.25, 1.35]), B and C (mean ±SEM: B = 39.25 ± 3.12; C = 18.97 ± 2.47; $t_{(16)}$ = 4.84, p < .001, 95% CI [.54, 1.79]), B and D (mean ± SEM: B = 39.25 ± 3.12; D = 10.93 ± 2.29; $t_{(16)}$ = 6.28, p < .001, 95% CI [.81, 2.22]), C and D (mean ± SEM: C = 18.97 ± 2.47; D = 10.93 ± 2.29; $t_{(16)}$ = 3.40, p = .004, 95% CI [.26, 1.37]), but not between A and B (mean ± SEM: A = 30.85 ± 4.44; B

---

[1] One fish did not take part to Test 2: it died of natural causes during the phase of re-training.

= 39.25 ± 3.12; $t_{(16)} = -1.3$, $p = .21$), A and C (mean ± SEM: A = 30.85 ± 4.44; C = 18.97 ± 2.47; $t_{(16)} = 1.83$, $p = .09$).

For clarity, all the statistic tests applied are reported as a supplementary material in a summarising table (“Supplementary Material” section, pp. 214-222, Suppl_1).

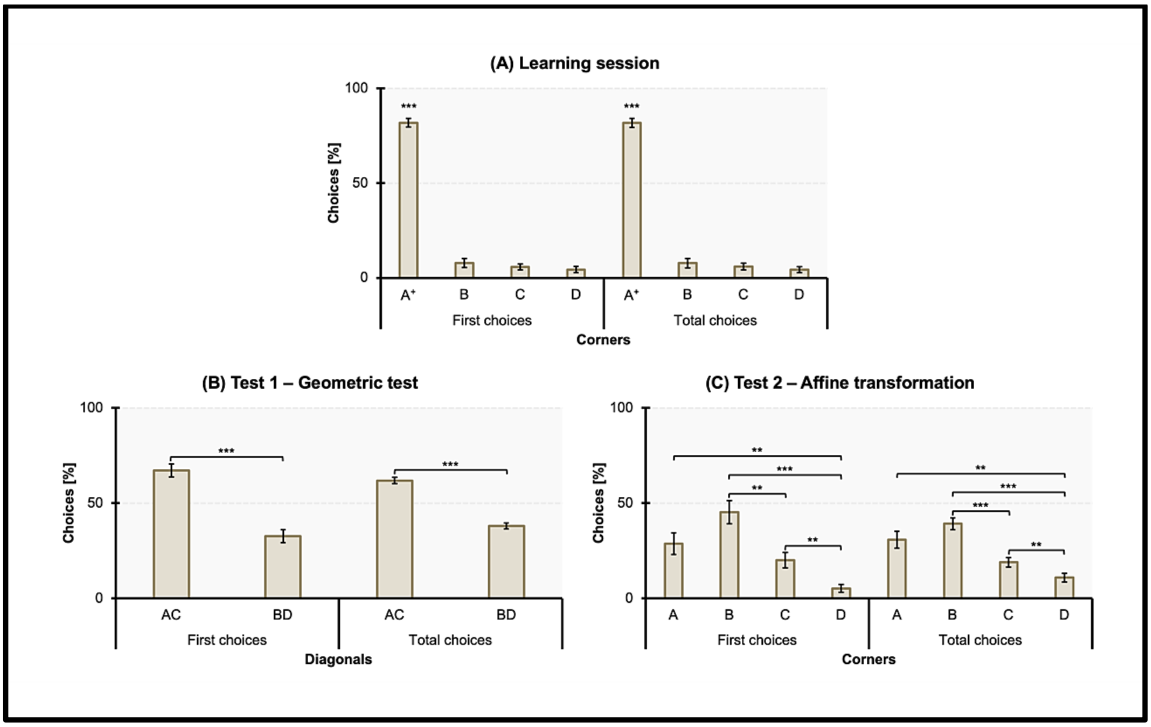


Figure 9. Results. (A) Learning session: first and total choices [%] towards the four corners (A^+, B, C, D). (B) Test 1, geometric test, after removing the blue wall: first and total choices [%] towards the two diagonals (geometrically correct AC, geometrically incorrect BD). (C) Test 2, affine transformation, after moving the blue wall 90° right: first and total choices towards the four corners (A, B, C, D). Mean ± SEM are shown. Significant *p* values are indicated with asterisks (* $p = .05$; ** $p = .001$; *** $p < .001$).

Results of Training showed that all the fish (18/18) learned to resolve the geometric reorientation task by integrating the spatial geometry with the blue wall landmark. Results of Test 1 (geometric test, with the blue wall removal) supported the residual use of geometry in the absence of the conspicuous landmark. Results of Test 2 (affine transformation, with the blue wall switch) showed that the fish reoriented following both geometry and the corner position having the conspicuous landmark on the left/right as they experienced at training.

2.1.4 Discussion

Study 1 explored the use of spatial geometry in conjunction with a conspicuous landmark in zebrafish, which were trained to reorient within a rectangular white arena equipped with a blue wall landmark. The length and proximity of the landmark were controlled as potential variables facilitating (or interfering with) the combined use of both information for reorientation.

Results of Training and Re-training (experimental phases 1 and 3) showed that all the fish learned to differentiate the correct corner position from the rotational one, irrespective of the landmark length and proximity. Trained zebrafish did show no attraction for the distal landmark as a beacon indicating one single nearby/exact location; however, they used that environmental object to reorient properly, overcoming the limits of spontaneous behaviour (Lee et al., 2012*c*).

Results of Test 1 (geometric test, with the blue wall removal) suggested that zebrafish used the global-shape parameters of the rectangular arena once the landmark was no longer present, strongly emphasising the deterministic role of spatial geometry for reorientation (Cheng et al., 2013; Vallortigara, 2018), in terms of metric properties staying constant when a conspicuous cue is moved or re-locate within a familiar geometric environment.

Results of Test 2 (affine transformation, with the blue wall switch) revealed that zebrafish had a preference towards both the spatial geometry, choosing more the two geometrically correct corners, and the corner position relying on the same left-right arrangement, but irrespective of surfaces length, as they experienced over training (e.g., blue wall landmark left).

Besides being consistent with a large body of literature (Tommasi et al., 2012; Vallortigara, 2009), these findings are like those obtained in another teleost fish (*X. eiseni*, Sovrano et al., 2002; 2003; 2005*a*). While the scientific question addressed in this study has not added theoretical complexity, the opportunity to test spatial learning abilities of zebrafish at selectively using more than one source of spatial information has a twofold advantage. First, to further explore the neural and molecular basis of geometry- and landmark-based spatial reorientation; second, to design relevant behavioural methodologies that could enhance the zebrafish natural predisposition to learn under certain conditions (e.g., providing an extensive training where motivational states are stressed). In agreement with Grunwald and Eisen (2002), the use of zebrafish requires «open-mindedness and tenacity by researchers with extraordinary vision».

2.2 Study 2: Local landmarks (corner panels) in zebrafish

2.2.1 Introduction

Landmark-based spatial reorientation is characterised by high variability across species, which may be due to several factors, such as cue competition, experience, task demands, or species specificity (Baratti et al., 2022; Cheng et al., 2013; Lee & Spelke, 2010*a,b*; Lee et al., 2015*a*; Twyman & Newcombe, 2010; Vallortigara, 2009). Despite that, a consistent body of evidence converges into the view of two independent systems underlying spontaneous goal-oriented navigation (Cheng, 1986;

Doeller et al., 2008; Gallistel, 1990; Keinath et al., 2017; Lee, 2017; Lee & Spelke, 2010*a,b*; Spelke et al., 2010), one for extended three-dimensional surfaces (boundary mapping) and the other for environmental objects (perceptual landmark coding). According to this approach, learning-dependent mechanisms through regular reinforcement would enhance the association between geometric terrain layout and featural landmarks, to identify one target location.

Sovrano and colleagues (2020*a*) explored landmark-based spatial reorientation in *X. eiseni*, focusing on the role of experience to reorient with or without the influence of informative geometry (i.e., rectangular vs. square arena). Interestingly, it has been found landmark-use in conjunction with geometry both in untrained and trained fish, although untrained fish did not fully integrate distal landmarks. By contrast, within the square arena, trained fish as well showed biases at using distal landmarks as directional cues to locate the correct corner position: reorientation behaviour was biased by near landmarks, which partially deflected the attention paid by the fish.

Since differences have been observed between *X. eiseni* and *D. rerio* in landmark-use during spontaneous reorientation (Lee et al., 2012*c*), the current interest was exploring landmark-use over training in *D. rerio*, with and without informative geometry (i.e., rectangular vs. square arena). Study 2 embraced four experiments to evaluate whether zebrafish: (1) learned to use local landmarks in conjunction with informative geometry, generalising after changes in the spatial arrangement of landmarks; (2) showed landmark-use over time without the influence of geometry, and if such a behaviour was somehow affected by the arena's size and the landmarks' salience. In fact, one debated issue proper relates to visual discrimination abilities of zebrafish, which seem to depend either more on methodological aspects (Agrillo et al., 2012;

Colwill et al., 2005; Gatto et al., 2020; Oliveira et al., 2015; Santacà et al., 2021; Sovrano et al., 2022*b*) than cognitive constraints.

2.2.2 Materials and Methods

Subjects were 34 adult male zebrafish (wild type), ranging from 3 to 5 cm in body-length. 9 fish took part in Experiment 1 (*Landmark-use in a rectangular opaque arena*); 8 fish took part in Experiment 2 (*Landmark-use in a large square opaque arena*); 8 fish took part in Experiment 3 (*Landmark-use in a small square opaque arena*); 9 fish took part in Experiment 4 (*Landmark-use in a large square transparent arena*). Housing conditions were the same as described above (p. 31).

A rectangular opaque arena was used in Experiment 1 (Figure 10A). This arena was the same as described by Baratti and colleagues (2020) and employed in Study 1.

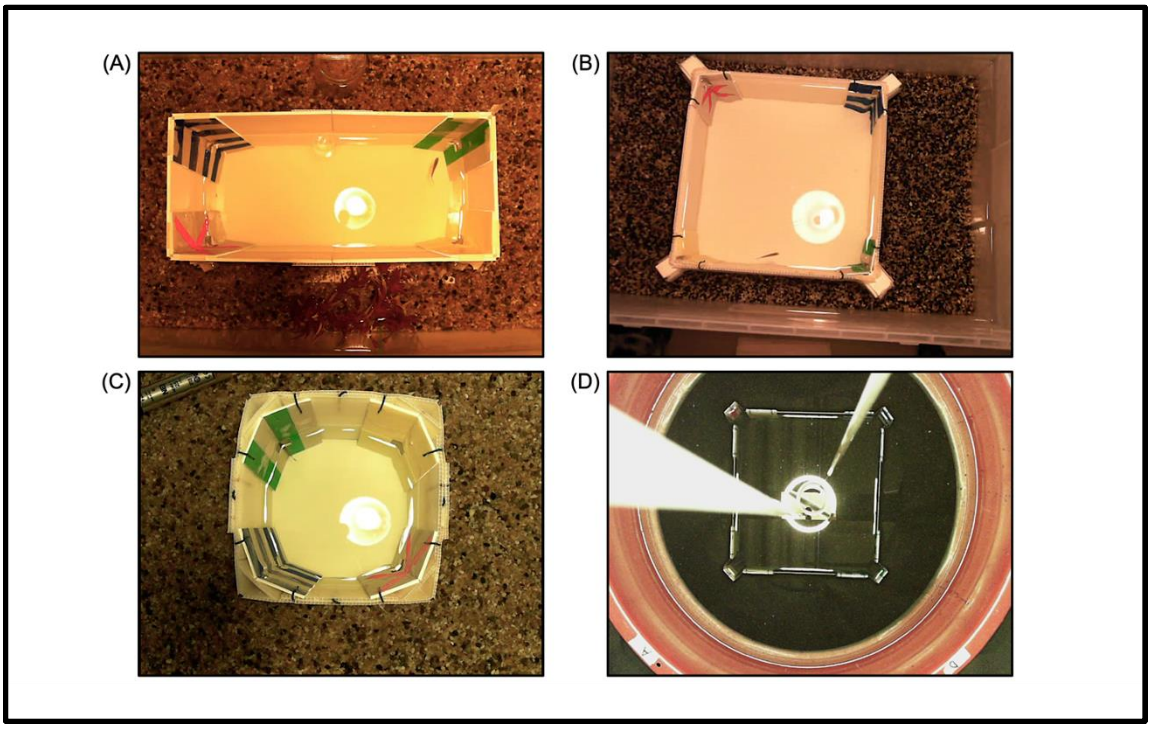


Figure 10. Photographs of the four experimental arenas. (A) Rectangular opaque arena. (B) Large square opaque arena. (C) Small square opaque arena. (D) Large square transparent arena.

The landmarks were four corner panels (8 × 16 cm) made of polypropylene (Poliplak®), which had unique patterns (Figure 11). The first panel was uniformly yellow (RGB: 255, 255, 0); the second panel had two vertical green (RGB: 34, 139, 34) and two vertical grey (RGB: 211, 211, 211) stripes (2 × 16 cm); the third panel had four horizontal blue (RGB: 30, 144, 255) and four horizontal grey (RGB: 211, 211, 211) stripes (8 × 2 cm); the fourth panel had a red “X” (stripes’ size: 1 × 11.5 cm; RGB: 255, 0, 0) in the upper part of a grey (RGB: 211, 211, 211) background. Each panel had a hole (2 × 3 cm, 5.3 cm from the ground) to fit the corridor’s entrance.

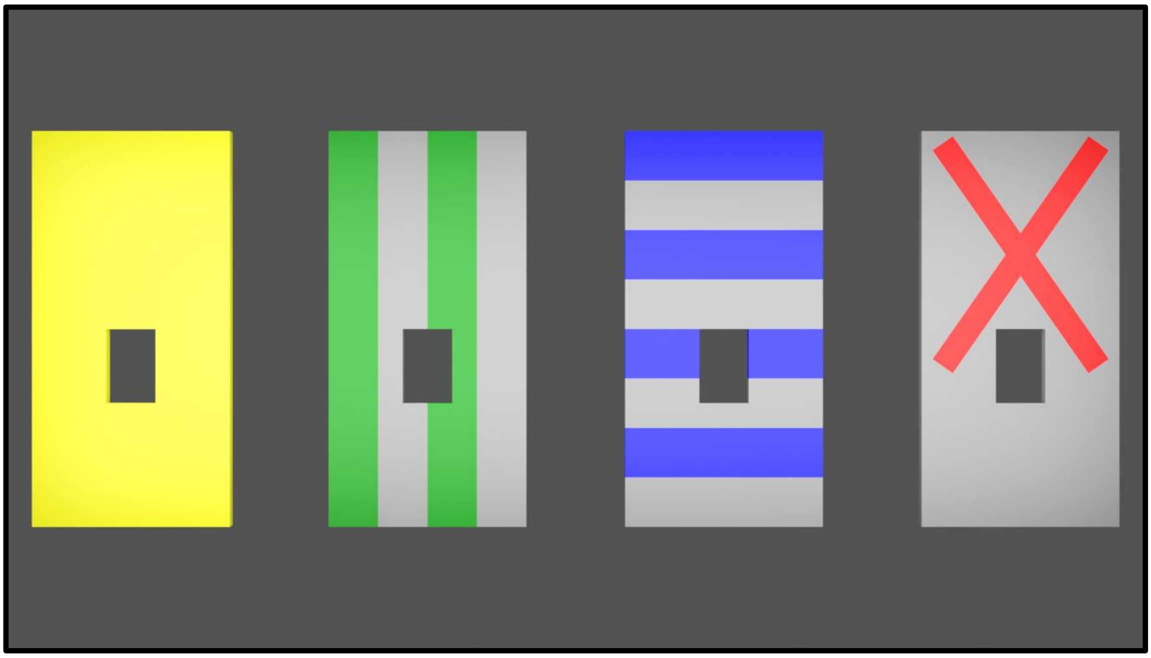


Figure 11. 3D reconstruction of the corner panel landmarks.

A small square opaque arena was used in Experiment 2 (Figure 10B) This arena was the same as used in several studies that assess visual discrimination learning abilities in *X. eiseni* (Albertazzi et al., 2017; Sovrano & Bisazza, 2008; 2009; Sovrano et al., 2015; 2016; 2022*a*; Truppa et al., 2010) and *D. rerio* (Sovrano et al., 2022*b*). It was an octagon (oblique segment: 4 × 15 cm; straight segment: 9 × 15 cm) inscribed into a square (15 × 15 × 15 cm; area = 225 cm^2), made of white polypropylene (Poliplak®), and hosted within a rectangular tank (57 × 38 × 18 cm) to create an enriched outer zone with gravel and artificial plants. The fish could leave the arena swimming through two corridors diagonally placed (3 × 4.5 cm; 2.5 cm in length; 2.5 cm from the ground), at the end of which there was a transparent acetate sheet (3 × 4.5 cm) with a pink-coloured upper part (2.5 × 4.5 cm; RGB: 255, 192, 203). Only one of the two corridors allowed the fish exiting, while the other was clipped from the outside with a green (RGB: 0, 128, 0) wire clip.

The landmarks were the same corner panels as described above and used in Experiment 1.

A large square opaque arena was used in Experiment 3 (Figure 10C). This arena was the same as reported by Sovrano and colleagues (2020*a*) to explore the effect of learning and spatial geometry on landmark-based reorientation behaviour in *X. eiseni*. It was made of white polypropylene (Poliplak®, 25 × 25 × 10 cm; area = 625 cm^2) and surrounded by a rectangular tank (57 × 38 × 18 cm) to have a familiar outer zone as usual. At the level of corners, the arena had one corridor each (2 × 3 cm; 2.5 in length; 6 cm from the ground), through which the fish could go into the familiar rewarding area. Like the arenas described above, there was a transparent acetate sheet (2.5 × 3.5 cm) with a pink-coloured part (2.5 × 2.5 cm; RGB: 255, 192, 203) at the end of each corridor.

The landmarks were like those previously used, but their size (8 × 10 cm) was adapted to fit the arena and keep balanced proportions. The corner panels were made of polypropylene (Poliplak®) and had the same unique patterns as above, with slight variations due to their lower height (10 instead of 16 cm). The blue-grey panel had three horizontal blue (RGB: 30, 144, 255) and two horizontal grey (RGB: 211, 211, 211) stripes (8 × 2 cm); the red-X panel had a red “X” (stripes’ size: 1 × 7.2 cm; RGB: 255, 0, 0) in the lower part of a grey (RGB: 211, 211, 211) background. Each panel had a hole (2 × 3 cm, 6 cm from the ground) to fit the corridor’s entrance.

A large square transparent arena was used in Experiment 4 (Figure 10D). This arena was the same as used in Study 6 and by Baratti and colleagues (2021) to test the use of geometric spatial attributes in *D. rerio*. It was a glass enclosure (30 × 30 × 8 cm; area = 900 cm^2) composed of four surfaces of equal size (26 × 26 × 11 cm), which were installed into single-track supports, placed on a PVC basement (50 × 50 cm), and covered with a layer of dark gravel (3 cm in depth). At the meeting point of each pair of tangent surfaces, four transparent corridors were placed at 2.5 cm from the corners, facing outward. Each corridor consisted of one glass sheet (3 × 11 cm) and two acetate sheets (2.5 × 9 cm): the two acetate sheets were perpendicularly glued on the glass sheet to create a C-shape design and could have a different pattern of vertical slits (Figure 12). The rewarding corridor (i.e., through which the fish could leave the arena) had a thick central slit (1 × 7.43 cm) and two thin lateral slits (0.2 × 7.43 cm). Conversely, the unrewarding corridor (i.e., through which the fish could not leave the arena) had a 3 × 3 matrix of thin slits (upper and lower series: 0.3 × 2.5 cm; central series: 0.3 × 2 cm). The overall perimeter of the slits of rewarding and unrewarding corridors was balanced (47.4 cm), to exclude any hydrodynamic effects detectable by tactile-like systems, such as the lateral line (Dijkgraaf, 1963; 1964; 1989;

Sovrano et al., 2018*a*; 2020*b*). The arena was placed within a circular amaranth tank (diameter × height: 175 × 27 cm), which was surrounded by a circular black curtain fixed on a wood-and-metal frame. It was homogeneously lit from above (height: 100 cm) through a 24-watt fluorescent white light tube (Lumilux, Osram GmbH, D), since the apparatus was hosted in a darkened room. To create perceptive continuity without light reflections, the arena was submerged (0.5 cm gap in height). The water temperature was kept around 26 ± 1 °C by a 50-watt heater (NEWA Therm®, NEWA), while a filter (NEWA Duetto®, NEWA) made sure good water quality.

Figure 12. 3D reconstruction of the transparent arena's corridors.

Since the landmarks had to be equipped on the C-shape corridors, getting visible on the adjacent surfaces, these corner panels were composed of three parts, one central (3 × 10 cm) and two laterals (6 × 10 cm). The first panel was uniformly yellow (RGB: 255, 255, 0); the second panel had five vertical green (RGB: 34, 139, 34) and five vertical grey (RGB: 211, 211, 211) stripes (1.5 × 10 cm); the third panel had three horizontal blue (RGB: 30, 144, 255) and two horizontal grey (RGB: 211, 211, 211) stripes (15 × 2 cm); the fourth panel had a red cross (short vertical stripe: 1 × 8.5 cm; long horizontal stripe: 1 × 14.4 cm; RGB: 255, 0, 0) in the central part of a grey (RGB: 211, 211, 211) background.

Experiment 1 was divided into two main blocks: the phase of training, through the rewarded exit task, and the phase of test, where four generalization tests were scheduled. Each test was preceded by a learning performance ≥ 70% achieved by the fish towards the correct panel (A^+). The first test was the affine transformation: all the corner panels were moved 90° right, putting into conflict the information provided by metric and landmarks (A^+ has the correct geometry but the incorrect panel). The second test was the diagonal transposition: the training panel (A^+) was moved to the rotational corner (C), on the diagonal. The third test was the partial removal of panels: the two panels on the geometrically correct diagonal (A^+ and C) were taken off. The fourth test was the total removal of panels (i.e., geometric test): all the panels were taken off, leaving the rectangular geometric framework only (Figure 13). The procedure was the same as described above for Study 1 (pp. 33-35).

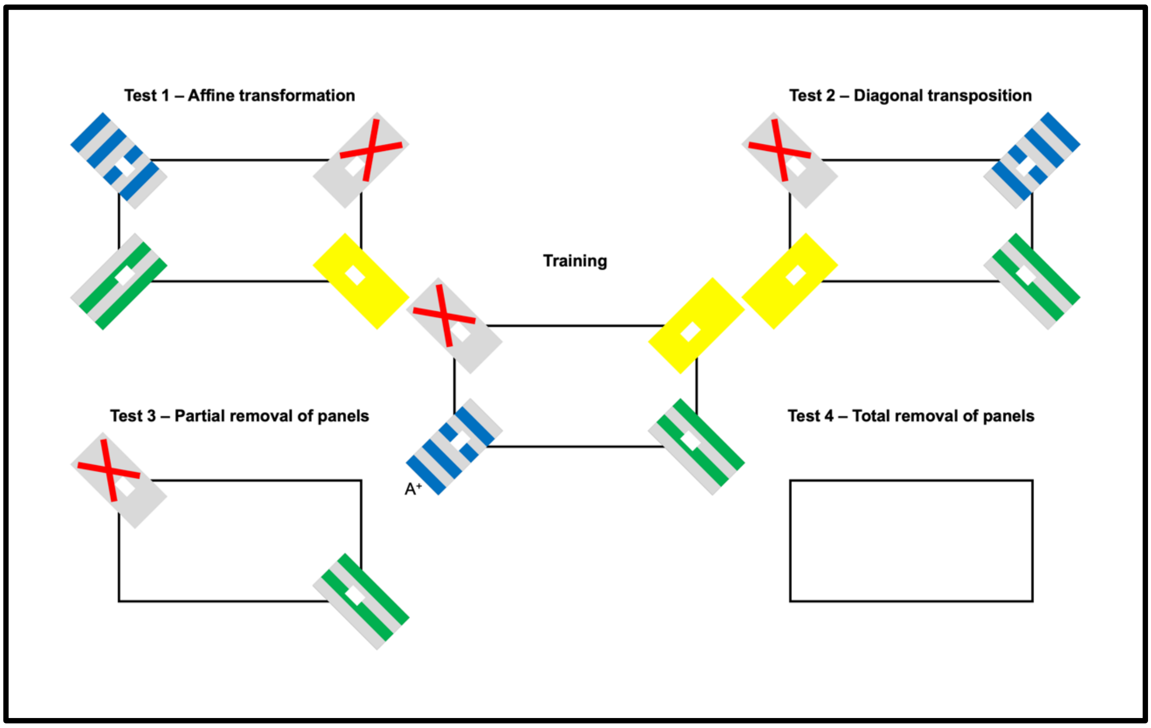


Figure 13. Schematic representation of the experimental design. Each test was preceded by a phase of training.

Experiments 2, 3, and 4 required the phase of training, but without testing since the geometric framework was a square. While Experiments 2 and 3 were performed over 25 sessions of training, Experiment 4 lasted 10 sessions to conform to the learning procedure as described for transparent arenas in previous works (Baratti et al., 2021; Sovrano et al., 2020*b*).

2.2.3 Results

Experiment 1: Landmark-use in a rectangular opaque arena

All the fish (9/9) reached a learning performance ≥ 70% in 74.89 ± 11.87 trials (≈ 11 training sessions).

A repeated measures ANOVA was performed by considering the first and total choices [%] for the four corners in the learning session (i.e., when the fish met the accuracy threshold ≥ 70%). Results are shown in Figure 14. The ANOVA with Corner (A^+, B, C, D) as within-subject factor and Panel (Yellow, Green-grey, Blue-grey, Red-X) as between-subject factor showed the following results: there was a significant effect of Corner (first choices: $F_{(3,15)} = 108.25$, $p < .001$, $\eta_p^2 = .96$; total choices: $F_{(3,15)} = 137.22$, $p < .001$, $\eta_p^2 = .94$), while there were no significant effects of Corner ∗ Panel (first choices: $F_{(9,15)} = 1.51$, $p = .23$; total choices: $F_{(9,15)} = 1.26$, $p = .33$) and Panel (first choices: $F_{(3,5)} = -3.951\text{e-}16$, $p = 1$; total choices: $F_{(3,5)} = 1.22$, $p = .39$). A paired samples *t*-test was then applied to analyse the difference among corners. The *t*-test on the first choices showed a significant difference between A^+ and B (mean ± SEM: $A^+ = 80.59 \pm 4.1$; $B = 5.12 \pm 2.04$; $t_{(8)} = 15.31$, $p < .001$, 95% CI [2.57, 7.63]), A^+ and C (mean ± SEM: $A^+ = 80.59 \pm 4.1$; $C = 7.22 \pm 4.01$; $t_{(8)} = 10.54$, $p < .001$, 95% CI [1.69, 5.31]), A^+ and D (mean ± SEM: $A^+ = 80.59 \pm 4.1$; $D = 7.07 \pm 2.96$; $t_{(8)} = 11.61$, $p < .001$, 95% CI [1.89, 5.83]), but not between B and C (mean ± SEM: $B = 5.12 \pm 2.04$; $C = 7.22 \pm 4.01$; $t_{(8)} = -.39$, $p = .71$), B and D (mean ± SEM: $B = 5.12 \pm 2.04$; $D = 7.07 \pm 2.96$; $t_{(8)} = -.66$, $p = .53$), C and D (mean ± SEM: $C = 7.22 \pm 4.01$; $D = 7.07 \pm 2.96$; $t_{(8)} = .03$, $p = .98$). The *t*-test on the total choices showed a significant difference between A^+ and B (mean ± SEM: $A^+ = 81.57 \pm 4.05$; $B = 4.69 \pm 1.94$; $t_{(8)} = 15.54$, $p < .001$, 95% CI [2.61, 7.75]), A^+ and C (mean ± SEM: $A^+ = 81.57 \pm 4.05$; $C = 6.84 \pm 2.96$; $t_{(8)} = 12.12$, $p < .001$, 95% CI [1.99, 6.08]), A^+ and D (mean ± SEM: $A^+ = 81.57 \pm 4.05$; $D = 6.9 \pm 2.3$; $t_{(8)} = 12.25$, $p < .001$, 95% CI [2.01, 6.14]), but not between B and C (mean ± SEM: $B = 4.69 \pm 1.94$; $C = 6.84 \pm 2.96$; $t_{(8)} = -.5$, $p = .63$), B and D (mean ± SEM: $B = 4.69 \pm 1.94$; $D = 6.9 \pm 2.3$; $t_{(8)} = -.91$, $p = .39$), C and D (mean ± SEM: $C = 6.84 \pm 2.96$; $D = 6.9 \pm 2.3$; $t_{(8)} = .02$, $p = .99$).

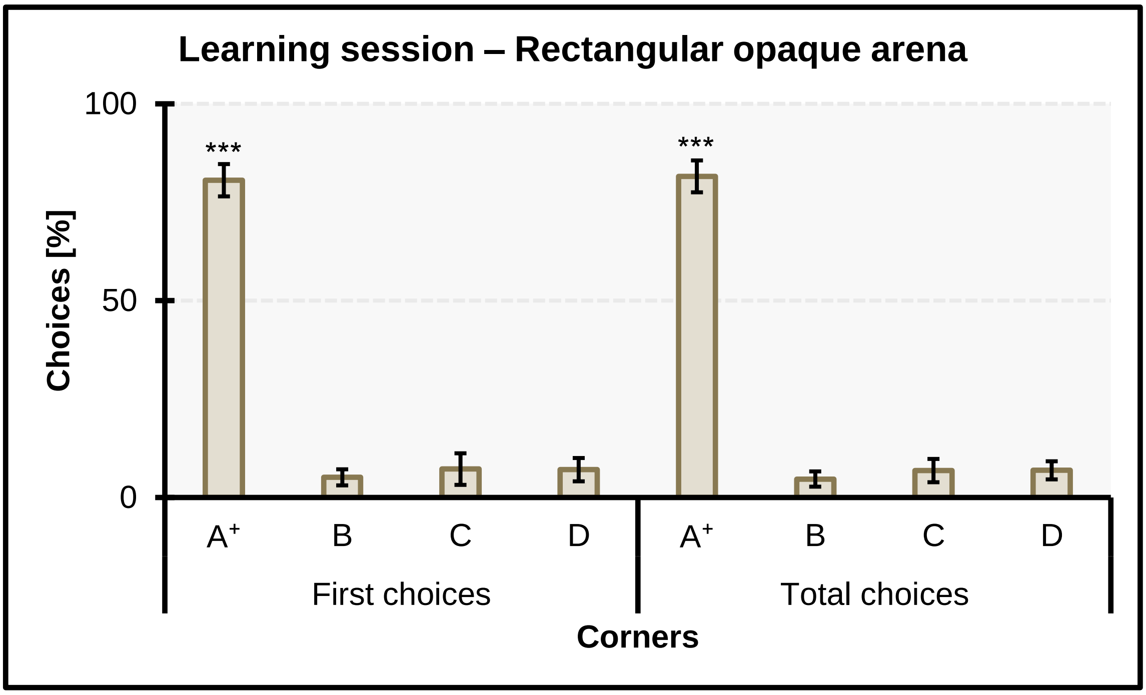


Figure 14. Results of the learning session. First and total choices [%] towards the four corners (A^+, B, C, D). Mean ± SEM are shown. Significant *p* values are indicated with asterisks (* $p = .05$; ** $p = .001$; *** $p < .001$).

A repeated measures ANOVA was performed by considering the first and total choices [%] for the four corners in Test 1 (affine transformation). Results are shown in Figure 15A. The ANOVA with Corner (A, B, C, D) as within-subject factor showed the following results: there was a significant effect of Corner (first choices: $F_{(3,24)} = 124.83$, $p < .001$, $\eta_p^2 = .94$; total choices: $F_{(3,24)} = 16$, $p < .001$, $\eta_p^2 = .67$). A paired samples *t*-test was then applied to analyse the difference among corners. The *t*-test on the first choices showed a significant difference between A and B (mean ± SEM: A = 20 ± 4.08; B = 54.44 ± 6.69; $t_{(8)} = -3.44$, $p = .009$, 95% CI [−1.98, −.27]), A and D (mean ± SEM: A = 20 ± 4.08; D = 5.56 ± 3.38; $t_{(8)} = 2.73$, $p = .03$, 95% CI [.1, 1.68]), B and C (mean ± SEM: B = 54.44 ± 6.69; C = 20 ± 4.08; $t_{(8)} = 3.54$, $p = .008$, 95% CI [.29, 2.03]), B and D (mean ± SEM: B = 54.44 ± 6.69 ± 3.38; D = 5.56 ± 3.38; $t_{(8)} = 5.70$, $p < .001$, 95% CI [.76, 3.01]), C and D (mean ± SEM: A = 20 ± 4.08; D = 5.56 ± 3.38; $t_{(8)} = 2.49$, $p = .04$, 95% CI [.05, 1.58]), but not between A and C (mean ± SEM: A = 20 ± 4.08; C = 20 ± 4.08; $t_{(8)} = 0$, $p = 1$). The *t*-test on the total choices showed a significant difference between A and B (mean ± SEM: A = 26.03 ± 2.1; B = 38.67 ± 2.25; $t_{(8)} = -2.64$, $p = .03$, 95% CI [−1.64, −.08]), A and D (mean ± SEM: A = 26.03 ± 2.1; D = 11.29 ± 2.19; $t_{(8)} = 3.84$, $p = .005$, 95% CI [.36, 2.16]), B and C (mean ± SEM: B = 38.67 ± 2.25; C = 24.01 ± 2.18; $t_{(8)} = 5.60$, $p < .001$, 95% CI [.74, 2.96]), B and D (mean ± SEM: B = 38.67 ± 2.25; D = 11.29 ± 2.19; $t_{(8)} = 7.32$, $p < .001$, 95% CI [1.08, 3.77]), C and D (mean ± SEM: C = 24.01 ± 2.18; D = 11.29 ± 2.19; $t_{(8)} = 3.41$, $p = .009$, 95% CI [.27, 1.97]), but not between A and C (mean ± SEM: A = 26.03 ± 2.1; C = 24.01 ± 2.18; $t_{(8)} = 0.43$, $p = .68$).

A repeated measures ANOVA was performed by considering the first and total choices [%] for the four corners in Test 2 (diagonal transposition). Results are shown in Figure 15B. The ANOVA with Corner (A, B, C, D) as within-subject factor showed the following results: there was a significant effect of Corner (first choices: $F_{(3,24)} = 13.41$, $p < .001$, $\eta_p^2 = .63$; total choices: $F_{(3,24)} = 9.15$, $p < .001$, $\eta_p^2 = .53$). A paired samples *t*-test was then applied to analyse the difference among corners. The *t*-test on the first choices showed a significant difference between A and C (mean ± SEM: A = 23.33 ± 5.77; B = 8.89 ± 2.61; $t_{(8)} = -2.8$, $p = .02$, 95% CI [−1.71, −.12]), B and C (mean ± SEM: B = 8.89 ± 2.61; C = 52.22 ± 5.47; $t_{(8)} = -6.71$, $p < .001$, 95% CI [−3.48, −.96]), C and D (mean ± SEM: C = 52.22 ± 5.47; D = 15.56 ± 3.38; $t_{(8)} = 5.05$, $p < .001$, 95% CI [.62, 2.70]), but not between A and B (mean ± SEM: A = 23.33 ± 5.77; B = 8.89 ± 2.61; $t_{(8)} = 2.04$, $p = .08$), A and D (mean ± SEM: A = 23.33 ± 5.77; D = 15.56 ± 3.38; $t_{(8)} = 1.02$, $p = .34$), B and D (mean ± SEM: B = 8.89 ± 2.61; D = 15.56 ± 3.38; $t_{(8)} = -1.63$, $p = .14$). The *t*-test on the total choices showed a significant difference between A and C (mean ± SEM: A = 21.98 ± 3.47; C = 37.76 ± 2.14; $t_{(8)} = -2.93$, p = .02, 95% CI [−1.76, −.15]), B and C (mean ± SEM: B = 20.67 ± 1.39; C = 37.76 ± 2.14; $t_{(8)} = -5.92$, $p < .001$, 95% CI [−3.11, −.80]), C and D (mean ± SEM: C = 37.76 ± 2.14; D = 19.6 ± 2.35; $t_{(8)} = 8.20$, $p < .001$, 95% CI [1.25, 4.19]), but not between A and B (mean ± SEM: A = 21.98 ± 3.47; B = 20.67 ± 1.39; $t_{(8)} = .37$, p = .72), A and D (mean ± SEM: A = 21.98 ± 3.47; D = 19.6 ± 2.35; $t_{(8)} = .43$, p = .68), B and D (mean ± SEM: B = 20.67 ± 1.39; mean D = 19.6 ± 2.35; $t_{(8)} = .33$, p = .75).

A paired samples *t*-test was performed by considering the first and total choices [%] for the two diagonals in Test 3 (partial removal of panels). Results are shown in Figure 15C. The *t*-test showed no difference between AC and BD (first choices: mean ± SEM: AC = 60 ± 7.82; BD = 40 ± 7.82; $t_{(8)} = 1.28$, p = .24; total choices: mean ± SEM: AC = 51.02 ± 2.73; BD = 48.98 ± 2.73; $t_{(8)} = .37$, p = .72).

A paired samples *t*-test was performed by considering the first and total choices [%] for the two diagonals in Test 4 (total removal of panels). Results are shown in Figure 15D. The *t*-test showed a significant difference between AC and BD in the total choices only (first choices: mean ± SEM: AC = 62.22 ± 7.22; BD = 37.78 ± 7.22; $t_{(8)} = 1.69$, p = .13; total choices: mean ± SEM: AC = 58.82 ± 2.94; BD = 41.18 ± 2.94; $t_{(8)} = 2.1$, p = .02).

For clarity, all the statistic tests applied are reported as a supplementary material in a summarising table (“Supplementary Material” section, pp. 214-222, Suppl_1).

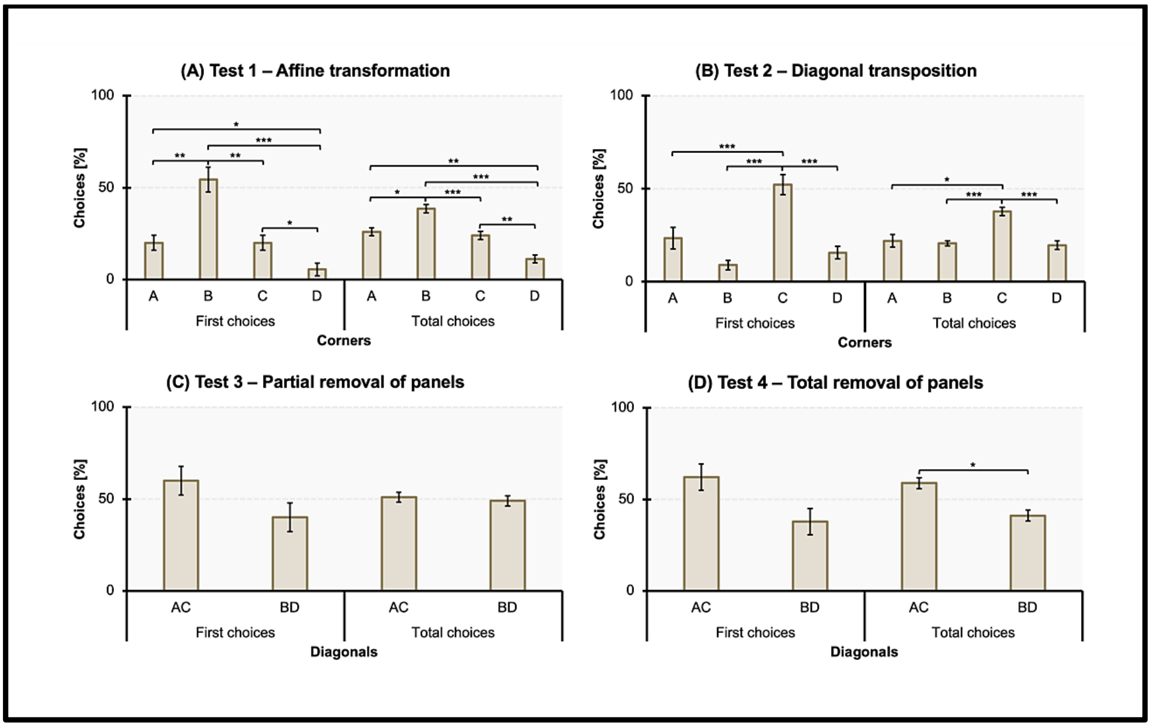


Figure 15. Results of tests. (A) Affine transformation: first and total choices [%] towards the four corners (A, B, C, D). (B) Diagonal transposition: first and total choices [%] towards the four corners (A, B, C, D). (C) Partial removal of panels: first and total choices towards the two diagonals (geometrically correct AC, geometrically incorrect BD). (D) Total removal of panels: first and total choices towards the two diagonals (geometrically correct AC, geometrically incorrect BD). Mean ± SEM are shown. Significant *p* values are indicated with asterisks (* *p* = .05; ** *p* = .001; *** *p* < .001).

Results revealed that all the fish (9/9) learned to choose the corner marked by the panel landmark, with no differences among the training panels. Results of Test 1 (affine transformation) revealed that corner B, at test the corner with the correct training panel but incorrect geometry, was chosen more than A, C, and D. Fish chose according to the landmark irrespective of changes in geometric information. Results of Test 2 (diagonal transposition) revealed that corner C, at test the corner with the training panel on the rotational position, was chosen more than A, B, and D. Fish preferred the landmark that, changing position, was no longer consistent with geometric information. Results of Test 3 (partial removal of panels) revealed that fish chose AC and BD at random, not using the residual presence of other landmarks. Results of Test 4 (total removal of panels) revealed that fish chose more the correct diagonal AC, reorienting in accord with the spatial geometry, in the total choices only.

Overall, these results seem to indicate a strong salience of the local landmark, but also the use of spatial geometry (Test 4) even if not necessary, since over training the information provided by the corner panels were enough to find the correct corner position. However, the use of geometry did not interfere with corner panel transformations (Test 1-3).

Experiment 2: Landmark-use in a large square opaque arena

Almost all the fish (7/8) did not reach a learning performance ≥ 70% within the 25 training sessions provided. 1/8 fish (trained with the red-X panel) learned to choose the corner marked by the landmark after 139 trials (≈ 17 training sessions).

Without the influence of a distinctive geometry, in a large square opaque arena, the panel landmark was not used to find the correct corner position.

Experiment 3: Landmark-use in a small square opaque arena

Almost all the fish (7/8) did not reach a learning performance ≥ 70% within the 25 training sessions provided. 1/8 fish (trained with the green-grey panel) learned to choose the corner marked by the landmark after 82 trials (≈ 10 training sessions).

Likewise Experiment 2, in a small square opaque arena, the panel landmark was not used to find the correct corner position. Therefore, the arena’s size (i.e., the distance among the panels) did not affect reorientation.

Experiment 4: Landmark-use in a large square transparent arena

6/9 fish reached a learning performance ≥ 70% in 40 ± 6.49 trials (≈ 6 training sessions), while 3/9 fish did not learn to choose the corner marked by the landmark within the 10 training sessions provided. Individual learning curves are shown in Figure 16.

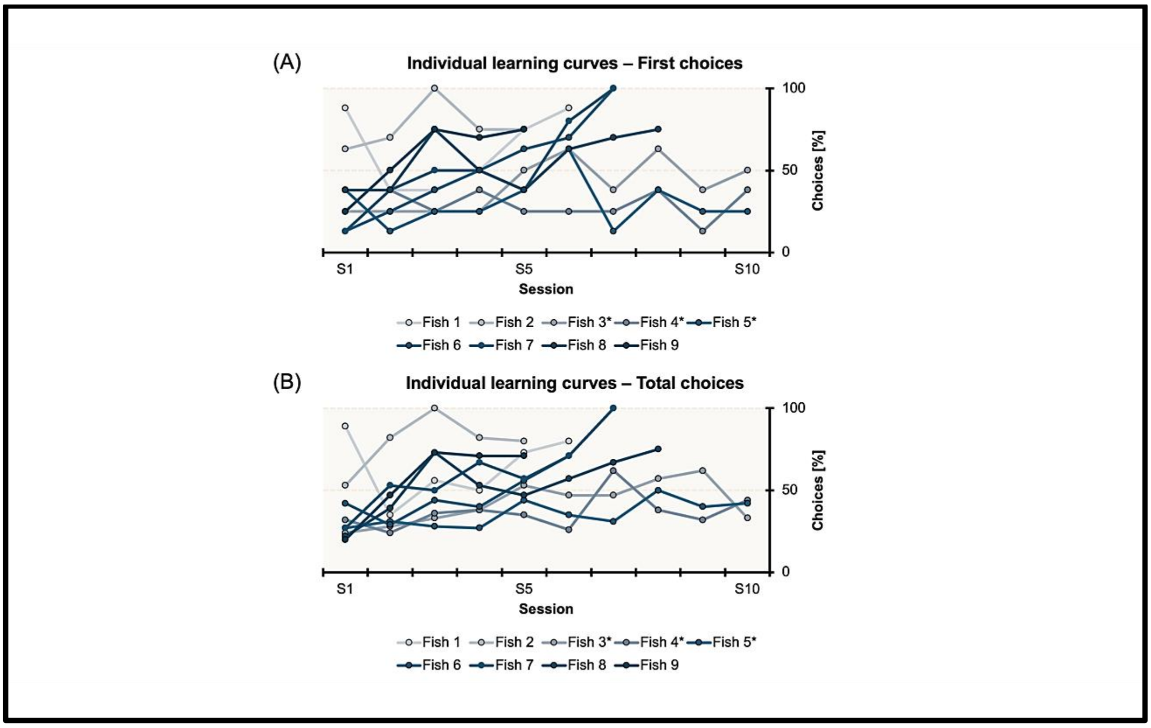


Figure 16. Individual learning curves. First (A) and total (B) choices [%] towards the correct corner A$^+$. Fish #3, #4, and #5 did not meet the accuracy threshold ≥ 70%.

As regards successful fish, a repeated measures ANOVA was performed by considering the first and total choices [%] for the four corners in the session of learning (i.e., when the fish met the accuracy threshold ≥ 70%). Results are shown in Figure 17. The ANOVA with Corner (A$^+$, B, C, D) as within-subject factor and Panel (Yellow, Green-grey, Blue-grey, Red-X) as between-subject factor showed the following results: there was a significant effect of Corner (first choices: $F_{(3,9)} = 43.37$, $p < .001$,

$\eta_p^2 = .94$; total choices: $F_{(3,9)} = 61.06$, $p < .001$, $\eta_p^2 = .95$), while there were no significant effects of Corner $*$ Panel (first choices: $F_{(6,9)} = .38$, $p = .88$; total choices: $F_{(6,9)} = .85$, $p = .56$) and Panel (first choices: $F_{(2,3)} = 3.228\text{e-}13$, $p = 1$; total choices: $F_{(2,3)} = 1$, $p = .47$). A paired samples *t*-test was then applied to analyse the difference among corners. The *t*-test on the first choices showed a significant difference between A^+ and B (mean ± SEM: $A^+ = 73.33 \pm 1.67$; $B = 7.92 \pm 4.1$; $t_{(8)} = 15.56$, $p < .001$, 95% CI [2.49, 10.25]), A^+ and C (mean ± SEM: $A^+ = 73.33 \pm 1.67$; $C = 8.75 \pm 3.15$; $t_{(8)} = 17.57$, $p < .001$, 95% CI [2.84, 11.56]), A^+ and D (mean ± SEM: $A^+ = 73.33 \pm 1.67$; $D = 10 \pm 3.65$; $t_{(8)} = 13.27$, $p < .001$, 95% CI [2.09, 8.76]), but not between B and C (mean ± SEM: $B = 7.92 \pm 4.1$; $C = 8.75 \pm 3.15$; $t_{(8)} = -.13$, $p = .91$), B and D (mean ± SEM: $B = 7.92 \pm 4.1$; $D = 10 \pm 3.65$; $t_{(8)} = -.3$, $p = .78$), C and D (mean ± SEM: $C = 8.75 \pm 3.15$; $D = 10 \pm 3.65$; $t_{(8)} = -.25$, $p = .81$). The *t*-test on the total choices showed a significant difference between A^+ and B (mean ± SEM: $A^+ = 72.89$; $B = 10.86$; $t_{(8)} = 13.47$, $p < .001$, 95% CI [2.13, 8.89]), A^+ and C (mean ± SEM: $A = 72.89$; $C = 7.31$; $t_{(8)} = 15.2$, $p < .001$, 95% CI [2.43, 10.01]), A^+ and D (mean ± SEM: $A = 72.89$; $D = 8.94$; $t_{(8)} = 15.58$, $p < .001$, 95% CI [2.49, 10.26]), but not between B and C (mean ± SEM: $B = 10.86$; $C = 7.31$; $t_{(8)} = .64$, $p = .55$), B and D (mean ± SEM: $B = 10.86$; $D = 8.94$; $t_{(8)} = .26$, $p = .80$), C and D (mean ± SEM: $C = 7.31$; $D = 8.94$; $t_{(8)} = -.42$, $p = .69$).

For clarity, all the statistic tests applied are reported as a supplementary material in a summarising table (“Supplementary Material” section, pp. 215-22, Suppl_1).

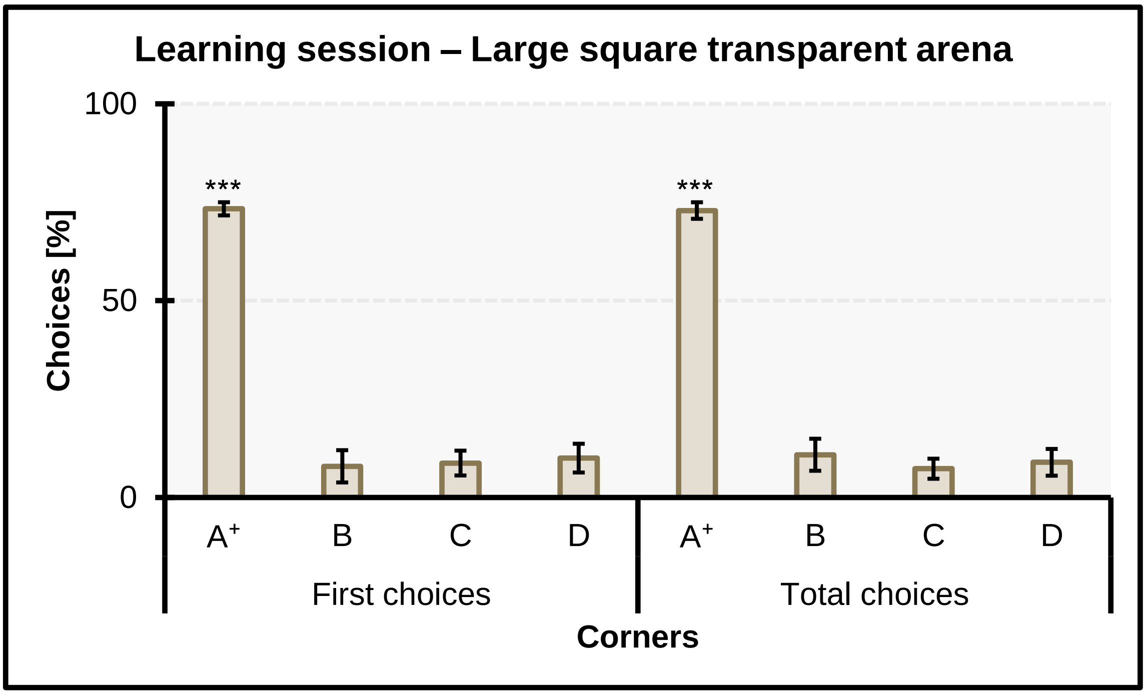


Figure 17. Results of the learning session. first and total choices [%] towards the four corners (A^+, B, C, D). Mean ± SEM are shown. Significant *p* values are indicated with asterisks (* *p* = .05; ** *p* = .001; *** *p* < .001).

Results revealed that, in a large square transparent arena with local panels at the corners, 6/9 fish learned to choose the corner marked by the landmark, with no differences among the training panels. Probably, without the visual continuity provided by opaque surfaces, single local landmarks acquired salience to find the correct corner position.

2.2.4 Discussion

Study 2 explored landmark-use in zebrafish during reorientation behaviour over training, while changing the geometric frame of the experimental space (rectangular or square arena) and, vicariously, the effect of arena's size and landmarks' salience.

Results of Experiment 1 (*Landmark-use in a rectangular opaque arena*) showed that all the fish learned to choose the correct corner position that was marked by one specific featural cue (i.e., one corner panel landmark with a distinctive visual pattern). After learning, each fish underwent four transformation tests, with the aim to analyse the choices for the landmarks while varying their spatial arrangement in relation to the geometric frame.

Zebrafish reorientation behaviour after the affine transformation (Test 1) provides important insights about the “strength” of geometric vs. nongeometric information in fish species. In fact, a robust preference for the corner panel landmark experienced at training came out, thus denoting a primacy effect of featural cues. Such a finding acquires relevance within a wider comparative framework: the teleost fishes *X. eiseni* and *C. auratus* did not choose according to the panel landmark after putting into conflict the two sources of information (Sovrano et al., 2003; Vargas et al., 2004), while both zebrafish and redtail splitfin fish preferred the panel landmark when transposed into the rotational corner position (Test 2). Currently, only in bird species had this tendency already been observed (Kelly et al., 1998; Vallortigara et al., 1990), where this difference had been explained in terms of salience of visual local cues in animals that use sight as a primary sensory channel. However, landmark-use in zebrafish might also depend on methodological aspects. Visual discrimination studies in this species do not converge into a consistent body of evidence as regards several attributes of the experimental visual stimuli, such as, size, colour, thickness, pixel density, salience, and so forth (Agrillo et al., 2012; Colwill et al., 2005; Gatto et al., 2020; Oliveira et al., 2015; Santacà et al., 2021; Sovrano et al., 2022*b*). It is possible that the behavioural methods used to test zebrafish visual discrimination affect the performance, irrespective of their attentional abilities (Echevarria et al., 2011).

As well, the total removal of panels (Test 4) seems to support landmark-use over geometry, at least in part. After removing the four corner panel landmarks, thus leaving available the geometric frame only, zebrafish approached the four corners at random as a first choice, while they approached the two geometrically correct corners as total choices. At that point, it is unclear whether spatial geometry could have provided an informative cue for reorientation, also considering that fish did not reorient through distal landmarks once the training landmark had been removed (Test 3).

However, results of Experiment 2 (*Landmark-use in a large square opaque arena*) and Experiment 3 (*Landmark-use in a small square opaque arena*) revealed that zebrafish did not learn to use one distinctive landmark to reorient, within a larger or smaller opaque experimental space, without the influence of informative geometry. The need to adjust the corner landmark panels to fit the arena's size could have produced different levels of salience, since within the larger arena the visual stimuli appear less incorporated into the frame than within the smaller one (Figure 10). Despite that, two fish learned to resolve the task, one per experiment, leading to exclude this difference as due to a covarying variable.

Knowing this, results of Experiment 4 (*Landmark-use in a large square transparent arena*) provide interesting food for thought. Two third of the fish learned to associate a distinctive landmark with the correct corner position, reorienting in the absence of informative geometry. Moreover, in this experiment, the square arena was composed of transparent surfaces where the landmarks took on the role of freestanding environmental objects. The transparency of the global context may have accentuated the salience of the only visible cues and, above all, of that cue associated with the reward. The fact that one third of the fish did not learn (2/3 fish were trained with the yellow panel) could be explained in two ways: the number of sessions provided for learning was not enough or, within a large transparent environment where visible objects were salient, the uniformly yellow pattern was the less attractive for zebrafish (Avdesh et al., 2010; 2012).

Contrary to Sovrano and colleagues (2020*a*) and Study 1, Study 2 did not focus on landmarks' proximity but, rather, on the comparison among apparatus, to explore if other variables could have interacted with acquired landmark-use. The present study adds a piece of knowledge about relational and associative spatial learning in zebrafish, supporting it as a tool to widen further research on the neuro-molecular correlates of geometry- and landmark-based reorientation.

2.3 Study 3: Environmental geometry in tortoises

2.3.1 Introduction

The use of environmental geometry in spontaneous and rewarded spatial tasks has been investigated in several vertebrate species (Baratti et al., 2022; Cheng, 2008; Cheng & Newcombe, 2005; Tommasi et al., 2012; Vallortigara, 2009), but it has never been addressed in reptiles. In this regard, the Hermann tortoise is an excellent reptile model: apart from being little known from the behavioural perspective, individuals do not need to accomplish substantial movements within their natural habitat. One reason may hide in tortoises' spontaneous proneness to be sedentary and display modest activities after hibernation (Stubbs & Swingland, 1985). The possibility that also a non-nomadic species takes advantage of environmental geometry as a tool during navigation would stress even more the adaptive strength of a geometry-grounded cognitive equipment.

Despite the sedentary nature of this species, Chelazzi and Francisci (1979) observed that *T. hermanni* showed efficient homing behaviours when passively displaced far from nest at regular temporal intervals. Chelazzi and Delfino (1986) also found an impairment of homing behaviours after induced anosmia and suggested a recruitment of olfaction for navigating back to home sites. It is so possible that geometric attributes can play a part in tortoises' orientation routines, for nourishment and breeding, although these animals are awake a few months per year due to hibernation.

Study 3 aimed at exploring whether *T. hermanni* could learn to use the spatial geometry of a rectangular arena under a process of learning over time, to reach a correctness threshold ≥ 70% for two training sessions in a row. We were further interested in investigating if tortoises could develop a systematic behavioural pattern to choose the geometrically correct diagonal, in terms of motion strategy (approach from perimeter, "wall-following", or diagonal, "centre-to-corner") and motion direction (approach from left or right), together with a preference to spend more time within the perimetrical, diagonal, or neutral zone of the rectangular arena.

The focus on motion patterns stems from comparative studies with insects (Wystrach, 2009; Wystrach & Beugnon, 2009), chickens (Pecchia & Vallortigara, 2010*a,b*; 2012), and evolved agents (Dawson et al., 2010; Miglino & Lund, 2001; Ponticorvo & Miglino, 2010), which have indicated the use of ego-centred mechanisms instead of geo-centred representations in reorientation behaviours.

2.3.2 Materials and Methods

This study was carried out during summer 2019 at the natural estate of "Sperimentarea", which is a unique site of the Civic Museum Foundation of Rovereto

(Trento, Italy; Figure 18A), in collaboration with the ACN Lab of the CIMeC (University of Trento). For this work, semi-naturalistic observations were conducted.

Subjects were 6 adult males (*T. h. hermanni*), ranging from 15 to 20 cm in body-length. The tortoises were kept within comfortable open-air fences (Figure 18B) and individually observed from Monday to Friday, until they learned to resolve the geometric task. Fresh lettuce or fruit was provided only during the training, while fresh water was daily replaced.

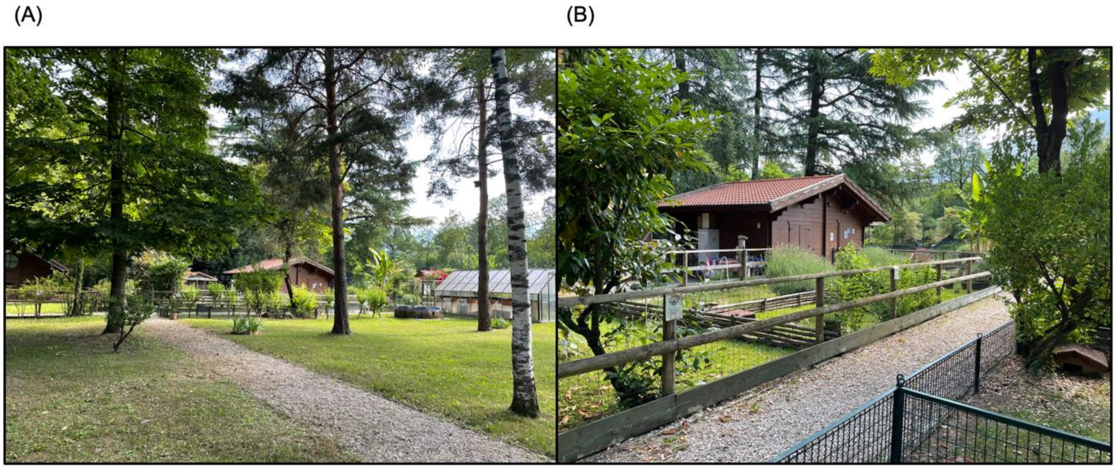


Figure 18. Photographs taken at the natural site of Sperimentarea. (A) Outdoor spaces. (B) Open-air fences for hosting the tortoises under training.

The apparatus was in an indoor space of Sperimentarea and consisted of a rectangular white arena made of wood (160 × 80 × 40 cm), where the floor was covered by a layer of pellet fuels to make the tortoise's movements easier. At the level of corners, four holes (20 × 20 × 20 cm) were present: the tortoise could cross a hole by pushing an up-and-over door with its forelegs. The four doors were visually the same, but only those placed at the level of the two geometrically correct corners could be pushed, while the other two were blocked from the outside through a stone that the animal could not see from the inside. Beyond the hole, a wooden feeder box was located (130 × 40 × 30 cm). The arena was centrally lit from above (105 cm) through a 400-W halogen lamp and surrounded by a circular dark curtain fixed on a metal gazebo, to eliminate any outer cues (Figure 19). The behaviour of tortoises was videotaped from the top by means of a webcam (Microsoft LifeCam Studio), which was fixed on a wooden tripod.

Figure 19. Photograph of the apparatus and detail of the up-and-over door. At the level of corners, the rectangular white arena had four holes supplied with doors that the tortoise could push to reach the external feeder box.

The experiment was performed within the two windows of time in which the tortoises were most active (9:30-12:30, 14:30-17:30). The procedure consisted of a rewarded training to meet a correctness threshold ≥ 70% relative to total choices towards the geometrically correct diagonal $C_{1,2}$ for two consecutive sessions (learning and validation). Each tortoise did one session of 8 trials per day and up to 30 training session were given for learning. At the beginning of each trial, the tortoise was slowly turned in place and then laid down in the centre of the arena, providing it a 15-minute limit to move and approach the doors. The tortoise was fed with lettuce or fruit in the case of correct choices for $C_{1,2}$ (i.e., the two rewarded symmetric corners, geometrically correct), but not in the case of incorrect choices for $X_{1,2}$ (i.e., the opposite symmetric corners, geometrically incorrect). In the preliminary phases of training, within the rectangular arena, all the tortoises underwent a daily shaping procedure to get them progressively used to push the door and cross the hole. The shaping could last two or more days, depending on emotional reactions and willingness to cooperate of each tortoise.

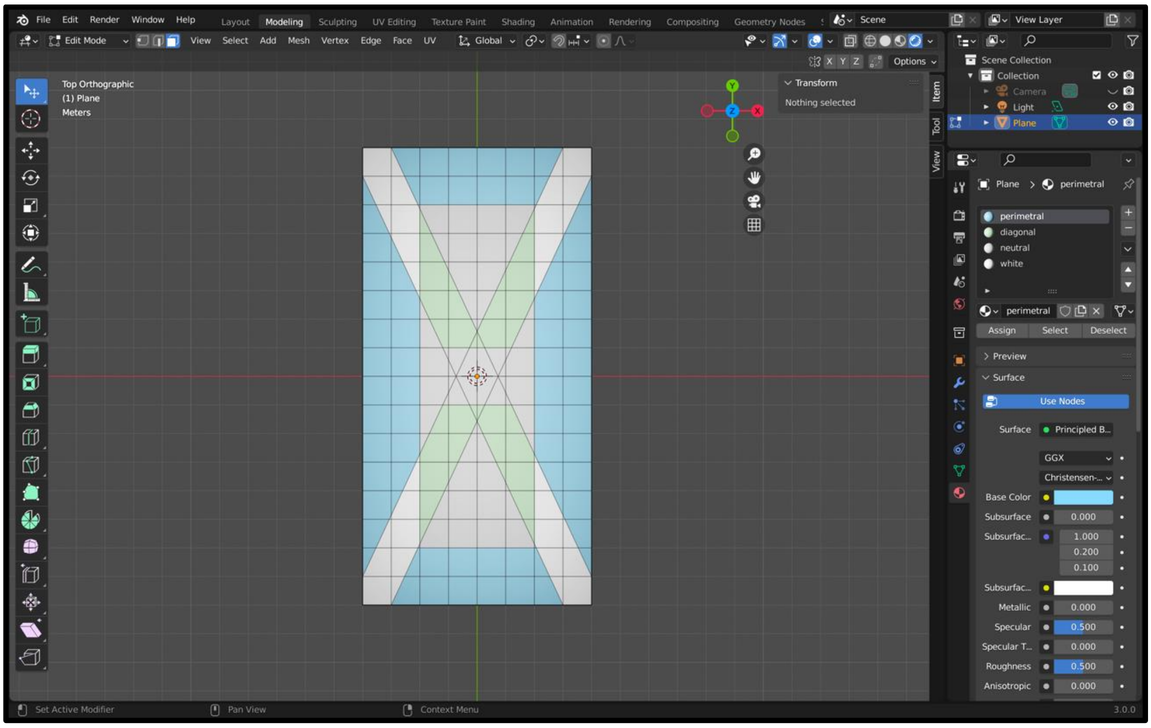
The total choices made by the tortoises towards the two diagonals (Correct $C_{1,2}$ vs. Incorrect $X_{1,2}$) were analysed, while motion strategy (Wall-following vs. Centre-to-corner) and motion direction (Left vs. Right) were computed on the total choices for the Correct $C_{1,2}$. In fact, motion patterns considered only the correct choices, where a behavioural consistency might be expected. Motion strategy and motion direction were calculated as proportion indexes through the formulas:

$$Motion\ Strategy\ Index = \frac{C_{1,2}Wf}{(C_{1,2}Wf\ +\ C_{1,2}Cc\ )}$$

$$Motion\ Direction\ Index = \frac{C_{1,2}WfL}{(C_{1,2}WfL\ +\ C_{1,2}WfR\ )}$$

“Wf” is “Wall-following”, “Cc” is “Centre-to-corner”; “WfL” is “Wall-following Left”, “WfR” is “Wall-following Right”.

Further, the time spent by the tortoises within three zones of the arena (Perimetrical vs. Diagonal vs. Neutral) was analysed. To assess this last variable, a matrix of 16 × 8 cells was designed with Blender 2.93 and used to codify the tortoises’ motion patterns. This grid was applied offline, on the video recordings of each trial, to score the temporal intervals among cells (Figure 20). Since the Perimetrical was the widest zone, the semi-perimeter was computed to balance the number of cells in common with the other two zones. An inter-observer reliability criterion was applied (Caro et al., 1979; p. 35). Paired Student *t*-test and repeated measures ANOVA were performed with Jasp 0.15, after having collapsed the sessions of learning and validation.



Surface Principled B...

GGX

Christensen-...

Base Color

Subsurface 0.000

Subsurfac... 1.000 0.200 0.100

Subsurfac...

Metallic 0.000

Specular 0.500

Specular T... 0.000

Roughness 0.500

Anisotropic 0.000

Figure 20. 3-zone grid. A matrix of 16 × 8 cells was designed with Blender 2.93 and used to score the position of the tortoise within the rectangular arena. Blue cells marked the Perimetrical zone; green cells marked the Diagonal zone; grey cells

marked the Neutral zone, which included the starting position in the centre. Overlapping cells of Perimetrical and Diagonal zone (white cells) fell outside the computation. The grid was applied offline, on the videos of each trial: the temporal intervals in which the tortoise moved from one zone to another was scored in seconds, from the beginning to the end of the recording.

2.3.3 Results

A *post-hoc* analysis showed that the sample (N = 6) was large enough to detect a moderate effect size (0.786).

Results are shown in Figure 21.

6/6 tortoises learned to choose the geometrically correct diagonal $C_{1,2}$ in 57 ± 9 trials (≈ 8 training sessions). A paired samples *t*-test applied on the total choices showed a significant preference towards the Correct $C_{1,2}$ ($t_{(5)} = 15.14$, $p < .001$, 95% CI [2.42, 9.98]). A paired samples *t*-test applied on motion strategy and motion direction showed no significant differences for Wall-following vs. Centre-to-corner ($t_{(5)} = 1.93$, $p = 0.11$) and Left vs. Right ($t_{(5)} = -.05$, $p = .96$). A repeated measures ANOVA applied on the time spent showed a significant difference among zones ($F_{(1,5)} = 32.95$, $p = .002$, $\eta_p^2 = .87$). A paired samples *t*-test better defined this difference, revealing a significant preference towards the Perimetrical zone (Perimetrical vs. Diagonal: $t_{(5)} = 7.34$, $p < .001$, 95% CI [1.02, 4.95]; Perimetrical vs. Neutral: $t_{(5)} = 4.81$, $p = .005$, 95% CI [.52, 3.37]; Diagonal vs. Neutral: $t_{(5)} = -2.3$, $p = .07$).

About individual motion strategy: 4/6 tortoises showed a preference for Wall-following (one-sample *t*-test: $t_{(3)} = 4.24$, $p = .01$, 95% CI [.46, ∞]), 1/6 for Centre-to-corner, 1/6 neither. About individual motion direction, which was calculated on wall-following tortoises: 2/4 tortoises showed a preference for Left, 1/4 for Right, 1/4 neither.

For clarity, all the statistic tests applied are reported as a supplementary material in a summarising table (“Supplementary Material” section, pp. 214-222, Suppl_1).

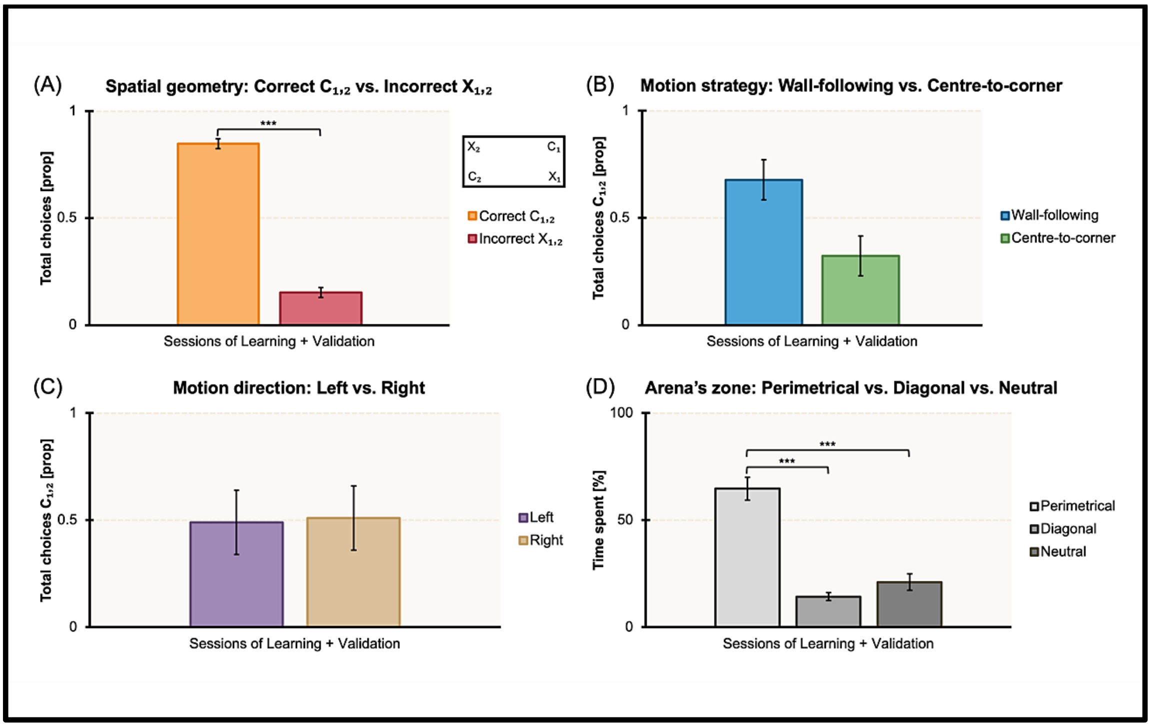


Figure 21. Results. (A) Spatial geometry during reorientation: Correct $C_{1,2}$ vs. Incorrect $X_{1,2}$ choices [proportion]. (B) Motion strategy during reorientation: Wall-following vs. Centre-to-corner strategy [proportion index]. (C) Motion direction during reorientation: Left vs. Right choices [proportion index][2]. (D) Permanence within the three zones of the rectangular arena: Perimetrical vs. Diagonal vs. Neutral [percentage of time spent]. Mean ± SEM are shown. Significant *p* values are indicated with asterisks (* *p* = .05; ** *p* = .001; *** *p* < .001).

---

[2] Two tortoises were not included in the analysis: their motion strategy was not Wall-following.

2.3.4 Discussion

Reorientation behaviour of *T. hermanni* was tested within a rectangular arena in which the tortoises were trained to choose the geometrically correct diagonal through a food incentive. Materials and methods from rewarded reorientation tasks with teleosts (Baratti et al., 2020; 2021; Sovrano et al., 2002; 2003; 2018; 2020*a,b*) were ingeniously readapted and applied to a phylogenetically remote reptile. Semi-naturalistic observations were carried out in the tortoise sanctuary Sperimentarea.

All the tortoises learned to reorient according to the geometric attribute of length (short/long) in connection with sense (left/right), as it occurs in other vertebrate species (Baratti et al., 2022; Cheng, 2008; Cheng & Newcombe, 2005; Tommasi et al., 2012; Vallortigara, 2009).

If considering the discrete choices as a measure of motion strategy (Wall-following vs. Centre-to-corner), the tortoises exhibited individual solutions rather than a systematic navigation pattern at the population level to approach the geometrically correct diagonal. However, when evaluating the time spent within three zones of the arena (Perimetrical vs. Diagonal vs. Neutral), all the tortoises showed a strong tendency to explore the geometric layout by moving close to the physical perimeter instead of visiting both the diagonal and neutral zone. No differences in terms of motion direction (Left vs. Right) were observed, even if *T. hermanni* had displayed lateralised behaviours in other contexts, such as social recognition and righting behaviour (Sovrano et al., 2018*b*; Stancher et al., 2006).

Although comparative studies with insects (Wystrach, 2009; Wystrach & Beugnon, 2009), chickens (Pecchia & Vallortigara, 2010*a,b*; 2012), and evolved agents (Dawson et al., 2010; Miglino & Lund, 2001; Ponticorvo & Miglino, 2010) suggested the role of panoramic or local view-matching mechanisms rather than coding of geometric layouts, the results here indicate that interindividual differences could mask potential effects of the processes involved in reorientation, as it occurs in chickens (Pecchia & Vallortigara, 2010*b*), thus increasing the debate's complexity.

For the first time, spatial learning abilities of non-nomadic tortoises at resolving a geometric reorientation task were explored in semi-controlled conditions, highlighting their predisposition to use environmental geometry to find a meaningful place for daily purposes, such as seeking out food. The challenge of testing boundary mapping in *T. hermanni*, whose metabolism highly depends on unpredictable variables such as climate, further enhances the tortoises' success at learning. It becomes increasingly evident that environmental characteristics are crucial for navigation and orientation habits, and to survive. Moreover, the chance to investigate geometry-based reorientation in relation to landmarks may highlight the capacity of this species to integrate different source of spatial information, that is, the global shape of living environments and direct cues to location.

Until now, studies on orientation abilities in tortoises focused on natural environments and spontaneous navigation (Chelazzi & Delfino, 1986; Chelazzi & Francisci; 1979): the present study propounds the Hermann tortoise as a crucial animal model to explore the cognitive phenotype of sedentary vertebrates in the field of spatial behaviour.

Concepts of rudimental geometry (Vallortigara, 2012) were found in a non-nomadic reptile species, therefore providing ecological meanings to environmental geometry even as regards animals that are little prone to accomplish substantial movements within their niches. This evidence follows the idea that a strongly adaptive coding of spatial geometry exists in the animals' brain, carrying over a long history of adaptation in response to exogenous pressures. Moreover, it supports the view that basic geometric intuitions can occur without formal education, symbolic formalisms, and a language rich of geometrical terms (Dehaene et al., 2006). Although the verbal language is an inborn endowment of humans, continuous interactions between the animals' nervous system (which coordinates the implementation of the most proper behaviours), and environmental stimuli, still remain a key-equipment shared across species.

2.4 Study 4: Conspicuous landmark (blue wall) in tortoises

2.4.1 Introduction

Study 3 has revealed that adult male tortoises of *T. hermanni* learned to reorient within a rectangular white arena in accord with informative geometry (metric length and relational sense). Moreover, it has validated a good method to trigger spatial learning abilities of these animals in a semi-naturalistic testing environment. Starting from these encouraging observations, we decided to "raise the bar", introducing a conspicuous landmark (a blue wall) to assess the combined used of both information, also in terms of landmark distance (i.e., proximal vs. distal as regards the correct corner position).

Although such a scientific question has been addressed for long among vertebrate species (Baratti et al., 2022; Cheng, 2008; Cheng et al., 2013; Cheng & Newcombe, 2005; Lee et al., 2017; Tommasi et al., 2012), the originality of this work lies in terrestrial tortoises as the animal model used, together with the possibility to find reorientation behaviour based on two concurrent sources of spatial information in a reptile species.

As for Study 3, in Study 4, motion patterns (strategy and direction) of tortoises were observed, to understand if the trend found towards the wall-fallowing motion strategy could become more consistent or, by contrast, not manifest at all. It was also possible that locating one target corner instead of two might be supported by motion approaches from the arena's perimeter, where the distinctive landmark was on the left or right dependently on motion directions of tortoises during reorientation (Dawson et al., 2010; Miglino & Lund, 2001; Ponticorvo & Miglino, 2010).

2.4.2 Materials and Methods

Study 4 was carried out during summer 2020 and 2021 at the natural estate of Sperimentarea (Civic Museum Foundation of Rovereto) in collaboration with the ACN Lab of the CIMeC (University of Trento).

Subjects were 16 adult males (*T. h. hermanni*), ranging from 15 to 20 cm in body-length. Housing conditions were the same as reported above (pp. 61-62). 8 tortoises took part in Experiment 1 (*Blue wall landmark near the correct corner*) and were observed during summer 2020; 8 tortoises took part in Experiment 2 (*Blue wall landmark far from the correct corner*) and were observed during summer 2021.

The blue wall landmark was a sheet of polypropylene (Poliplak®) that fitted the short surface of the rectangular white wooden arena (80 × 40 cm; RGB: 30, 144, 255). The apparatus, training procedure, and data handling were the same as described above (pp. 62-65). However, the time spent [s] by the tortoises within the three zones of the arena (Perimetrical vs. Diagonal vs. Neutral) was not analysed, since a wall-following motion strategy had already become consistent at learning. The time spent has been calculated only in Study 3, in the case of spatial geometry alone, because the wall-following strategy was a trend; thus, to have an additional index of perimetrical explorations.

2.4.3 Results

A one-way ANOVA was performed to evaluate if the distance of the blue wall landmark (near, far) as for the correct corner A^+ affected the total number of trials [f] to meet the learning criterion ≥ 70%. The one-way ANOVA showed no significant effect of Wall Distance ($F_{(1,14)} = .25$, $p = .62$). 16/16 tortoises learned to choose the correct corner A^+ in 84 ± 16.30 trials (≈ 13 training sessions).

A repeated measures ANOVA was performed by considering the proportion of total choices for the four corners, after having collapsed the sessions of learning and validation. Results are shown in Figure 22A. The ANOVA with Corner (A^+, B, C, D) as within-subject factor and Wall Distance (Near, Far) as between-subject factor, showed the following results: there was a significant effect of Corner ($F_{(3,42)} = 942.7$, $p < .001$, $\eta_p^2 = .99$), while there were no significant effects of Corner $*$ Wall Distance ($F_{(3,42)} = 2.3$, $p = .09$), and Wall Distance ($F_{(1,14)} = 2.33$, $p = .15$). A paired samples t-test was then applied to analyse the difference among corners. The t-test on the total choices showed a significant difference between A^+ and B (mean ± SEM: $A^+ = .87 \pm .02$; $B = .02 \pm .01$; $t_{(15)} = 35.31$, $p < .001$, 95% CI [5.66, 11.88]), A^+ and C (mean ± SEM: $A^+ = .87 \pm .02$; $C = .09 \pm .01$; $t_{(15)} = 32.32$, $p < .001$, 95% CI [5.18, 10.88]), A^+ and D (mean ± SEM: $A^+ = .87 \pm .02$; $D = .02 \pm .01$; $t_{(15)} = 36.17$, $p < .001$, 95% CI [5.8, 12.16]), B and C (mean ± SEM: $B = .02 \pm .01$; $C = .09 \pm .01$; $t_{(15)} = -3.62$, $p = .003$, 95% CI [−1.48, −.31]), C and D (mean ± SEM: $C = .09 \pm .01$; $D = .02 \pm .01$; $t_{(15)} = 4.13$, $p < .001$, 95% CI [.41, 1.63]), but not between B and D (mean ± SEM: $B = .02 \pm .01$; $D = .02 \pm .01$; $t_{(15)} = 31$, $p = .76$).

A repeated measures ANOVA was performed to evaluate the use of motion strategies, by considering the proportion of total choices for the correct corner A^+. Results are shown in Figure 22B (left). The ANOVA with Strategy (Wall-following, Centre-to-corner) as within-subject factors, and Wall Distance (Near, Far) as between-subject factor, showed the following results: there was a significant effect of Strategy ($F_{(1,14)} = 25.34$, $p < .001$, $\eta_p^2 = .64$) and Strategy $*$ Wall Distance ($F_{(1,14)} = 5.86$, $p = .03$, $\eta_p^2 = .3$), while there was no a significant effect of Wall Distance ($F_{(1,14)} = 1$, $p = .33$).

A repeated measures ANOVA was performed to evaluate the use of motion directions, by considering the proportion of total choices for the correct corner A^+. Results are shown in Figure 22B (right). The ANOVA with Direction (Left, Right) as within-subject factors, and Wall Distance (Near, Far) as between-subject factor, showed the following results: there were no significant effects of Direction ($F_{(1,11)} = .02$, $p = .89$), Direction $*$ Wall Distance ($F_{(1,11)} = .10$, $p = .75$), and Wall Distance ($F_{(1,11)} = .56$, $p = .47$)[3].

About individual motion strategy: 13/16 tortoises showed a preference for Wall-following (one-sample t-test: $t_{(13)} = 7.63$, $p < .001$, 95% CI [1.25, ∞]), 1/6 for Centre-to-corner, 2/6 neither. About individual motion direction, which was calculated on wall-following tortoises: 5/13 tortoises showed a preference for Left (one-sample t-test: $t_{(4)} = 4.25$, $p = .007$, 95% CI [.55, ∞]), 4/13 for Right (one-sample t-test: $t_{(3)} = 3.62$, $p = .02$, 95% CI [.32, ∞]), 4/13 neither.

For clarity, all the statistic tests applied are reported as a supplementary material in a summarising table (“Supplementary Material” section, pp. 214-222, Suppl_1).

---

[3] Three tortoises were not included in the analysis: their motion strategy was not Wall-following.

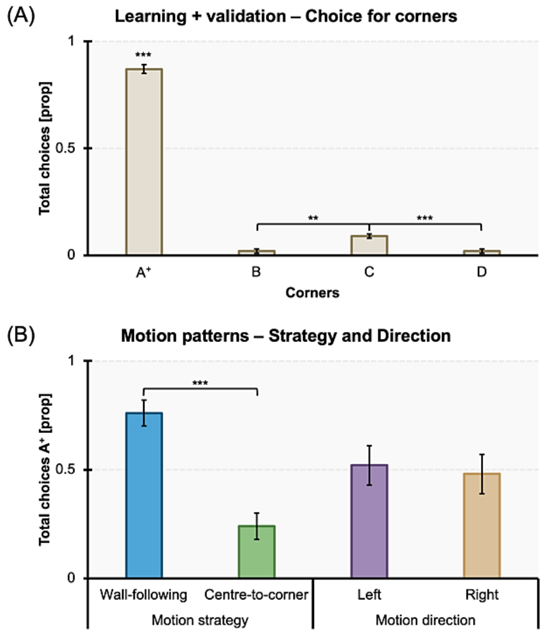


Figure 22. Results. (A) Total choices [proportion] for the arena's corners (A^+, B, C, D). (B) Total choices [proportion index] for the correct corner A^+ in relation to the use of motion patterns (strategy, on the left; direction, on the right). Mean ± SEM are shown. Significant *p* values are indicated with asterisks (* $p = .05$; ** $p = .001$; *** $p < .001$).

Results showed that all the tortoises (16/16) learned to resolve the geometric reorientation task by integrating the spatial geometry with the conspicuous blue wall landmark. Interestingly enough, the rotational corner was chosen more than the two geometrically incorrect corners, strongly denoting the concurrent use of spatial geometry. At the population level, a wall-following motion strategy emerged, while no lateralized reorientation in terms of motion direction. Left-right behavioural laterality emerged at the individual level.

2.4.4 Discussion

Study 4 explored reorientation behaviour driven by spatial geometry in conjunction with a conspicuous landmark in *T. hermanni*, and was a prosecution of Study 3, in which geometry-based reorientation had been observed for the first time in sedentary tortoises. The use of spatial geometry itself was considered a precondition for conjoining. The proximity of the blue wall landmark to the correct corner position was balanced over the two-year period of data acquisition (Experiment 1*, Blue wall landmark near the correct corner*; Experiment 2*, Blue wall landmark far from the correct corner*). The behavioural analyses focused on the tortoises' capacity to reorient following an integrated source of spatial information (rectangular shape + blue wall landmark), and on motion patterns adopted to explore the target location.

Results revealed that the tortoises learned to choose the correct corner position over the other three with precision and good accuracy. Moreover, they showed a preference for the rotational corner position as well, proving to be highly sensitive to the arena's geometry. Such a finding also supports what it was observed in Study 3, that is, the capacity to distinguish between two opposite geometric instances (e.g., short surface left, long surface right). Unlikely Study 3, where the use of the wall-following strategy of motion was a trend but not a consistent pattern at the population level (maybe due to the sample size), here almost all the tortoises approached the target location moving close to the perimeter but with no preferential left-right direction. Even if at the individual level most of the wall-following tortoises was lateralised, their direction of motion was not associated with the position of the blue wall landmark experienced at training. During their explorative routines, the tortoises found the distinctive cue on the right, either when near (Experiment 1, in that case, near the correct corner position) and far (Experiment 2, by contrast, near the rotational corner position). In individual asymmetries, the direction of the asymmetry of a lateralized behaviour has a random distribution, although that behaviour may be exhibited by all the individuals of a population. In population asymmetries, however, the percentage of a lateralized behaviour exceeds the 50% and keeps the same direction within that population (e.g., human people are 90% right-handed; Rogers et al., 2013).

Altogether, the results of the present study do not allow to determine if motor action sequences (Dawson et al., 2010; Miglino & Lund, 2001; Ponticorvo & Miglino, 2010) were crucially used by disoriented tortoises to conjoin geometric with featural representations of the target location. However, we suggest that a combined “coding” of visual and tactile stimulation may occur, and that this intelligent solution does not depend on highly-sophisticated linguistic formalisms (Dehaene et al., 2006) but, rather, on the ecological value of environmental geometry.

Chapter 3: Spontaneous vs. Acquired Geometric Spatial Reorientation

3.1 Study 5: Nonvisual environmental geometry in zebrafish, redtail splitfin fish, and goldfish[4]

3.1.1 Introduction

Evidence in insects (Wystrach, 2009; Wystrach & Beugnon, 2009; Wystrach et al., 2011*a,b*) and birds (Pecchia & Vallortigara, 2010*a,b*; 2012), has led to advance that geometric reorientation would require some sort of view-based coding of spatial information. However, further studies have shown that boundary mapping can occur even in situations of non-visibility. Children aged 5-7 y/o reoriented within a transparent rectangular room (Gianni et al., 2018), and cavefishes (*Astyanax mexicanus* and *Phreatichthys andruzzii*) learned to use the spatial geometry of an opaque arena in the absence of sight (Sovrano et al., 2018*a*). While the role of visual geometric information is sufficiently understood, specially thanks to human studies (Dilks et al., 2013; Doeller & Burgess, 2008; Epstein, 2005; 2008; Epstein & Kanwisher, 1998; Grill-Spector, 2003; Huttenlocher & Lourenco, 2007; Julian et al., 2016; Lee et al., 2012*a*; Lourenco et al., 2009; Maguire, 2001; Park et al., 2011; Park & Chun, 2009), similar investigations with nonhuman animals have not been performed yet.

---

[4] The present Study has been published on 15th May on *Scientific Reports* journal. To avoid self-plagiarism, it will be summarized to provide essential information, findings, and keys to the reading. In some cases, the figures have been readjusted.

Fish are excellent models to address reorientation behaviour driven by nonview-based mechanisms, since they have one particular tactile-like system, the “lateral line” (description below, pp. 131-132), which works in synch with touch, hearing, and chemoreception during navigation (Putland et al., 2018; Von der Hemde & Bleckmann, 1994; Windsor et al., 2010). Moreover, the opportunity to test both spontaneous and acquired reorientation behaviour would ensure to investigate whether the use of transparent surfaces is “natural” or depend on finer capacities built over time.

Study 5 aimed at exploring reorientation behaviour of three species of fish (*D. rerio*, *X. eiseni*, *C. auratus*) within a rectangular transparent arena, in which each group of fish took part in one experiment only. Three experiments were performed, by handling the type of experience that fish could acquire (no vs. short vs. extensive experience) within the arena, in relation to the target location (i.e., two geometrically correct corners).

3.1.2 Materials and Methods

Subjects were 58 adult fish from three species: 20 *D. rerio*[5], 19 *X. eiseni*, and 19 *C. auratus* (averaged body-length: 4 cm). 6 zebrafish took part in Experiment 1, 6 in Experiment 2, 8 in Experiment 3; 5 redtail splitfin fish took part in Experiment 1, 6 in Experiment 2, 8 in Experiment 3; 5 goldfish took part in Experiment 1, 7 in Experiment 2, 7 in Experiment 3. Social companions were used to incentivise the experimental fish. Housing conditions were the same as described above (p. 31).

For this study, a rectangular transparent arena was built. It was like the square transparent arena used in Study 2 (pp. 45-46) but consisting of a glass enclosure (30

---

[5] The experiments with zebrafish had been already presented for the master’s degree that I achieved in October 2017 at the University of Trento.

× 20 × 8 cm) with two long walls (26 × 11 cm) and two short walls (16 × 11 cm). The corridors were the same, with slight variations for the goldfish, which had a major body-length (4-6 cm instead of 2-5 cm). In such a case, the two acetate sheets were 3.5 × 9 cm; the central slit of the rewarding corridor was 1.5 × 7.43 cm, while the slits of the unrewarding corridor were 0.36 × 2.5 cm (upper and lower series) and 0.36 × 2 cm (central series); the overall slits' perimeter was 48.4 cm. The corridors were placed at 3 cm from the corners and were used for Experiment 3 only. As regards Experiment 1 and *2*, the corridors were removed and replaced by four water-filled glass jars (diameter: 6 cm; height: 8 cm), which were placed inside the arena at the level of corners.

The experimental procedure of Experiment 3 was the same as described above (pp. 32-35). The geometric test was an adaptation of that developed by Baratti and colleagues (2020), in which new corridors without slits were built to reproduce the extinction procedure and bear out that learning had been driven by spatial geometry.

Conversely, Experiment 1 and *2* applied a spontaneous choice task (also, "social-cued memory task": Lee et al., 2015*a*; 2013; 2012; Sovrano et al., 2020*a)* to evaluate the use of nonvisual spatial geometry in the absence of training, providing (Experiment 2) or not (Experiment 1) a short period of experience. Experiment 1 had three phases: observation (120 s), disorientation (30 s), test (120 s). Experiment 2 had four phases: acclimation (30 s), exploration (120 s), disorientation (30 s), test (120 s). During observation, the experimental fish was kept confined within a transparent jar in the centre of the arena, where it could observe one social companion hosted within one of the four jars at corners. During disorientation, the jar with the subject in was covered through a blue plastic cylinder (diameter: 6.5 cm; height: 8.5 cm; RGB: 30, 144, 255), gently removed from the arena and slowly turned 360° clockwise/anticlockwise;

meanwhile, also the jar hosting the social companion was removed and replaced with a water-filled jar (as the other three already present). During test, the subject was gently poured into a glass cylinder in the centre of the arena; after that, the cylinder was perfectly lifted upward by means of a metal pulling mechanism. Experiment 2 differed in the phase of acclimation and exploration. During acclimation, the subject stayed in the jar for the minimum time needed to be accustomed to the environment. During exploration, the subject was free to move and approach the arena, but also, to experience the goal-position (i.e., the corner where the jar hosted the social companion) in relation to spatial geometry. Before each trial starting, the apparatus was turned 90° clockwise to remove any extra-tank cues.

Although the phase of test lasted 120 s, the first 30 s only were coded and analysed. Video recordings were coded by superimposing a transparent grid on the computer monitor: this grid traced the boundaries of the arena and jars out, also showing two concentric rings around the jars to set the choice area at 1 cm from them.

The following variables were measured: for Experiment 1 and 2, the first and total choices [f] towards each diagonal (geometrically correct C-R vs. incorrect N-F) during the 30 s of test in the five sessions; for Experiment 3, the mean number of trials [f] to meet the learning criterion ≥ 70%, the first and total choices [f] towards each diagonal (geometrically correct $C_{1,2}$ vs. incorrect $X_{1,2}$) in the first five sessions of training, in the learning day, and in the geometric test.

A repeated measures ANOVA was performed to compare the use of spatial geometry (correct vs. incorrect) over time (1-5 sessions) and among species (*D. rerio*, *X. eiseni*, *C. auratus*). To compare the two corners on the same diagonal (C vs. R; N vs. F; C_1 vs. C_2; X_1 vs. X_2), the Wilcoxon test was applied on the first choices and the Student’s *t*-test on the total choices. Nonparametric statistics were run on first choices only, in which a finite number of observations (one single choice per trial = 8) were obtained. The Shapiro-Wilk test was performed to assess normality, whereas the Levene's test of equality of error variances and the Mauchly's sphericity test were performed to assess homoscedasticity. For clarity, all the statistic tests applied are reported as a supplementary material in a summarising table ("Supplementary Material" section, pp. 214-222, Suppl_1). To estimate the effect size of significant data analysis, we reported η_p^2 as an index for ANOVA and 95% confidence intervals as an index for Student's *t*-test. Statistical analyses were performed with IBM® SPSS Statistic 20 software package.

3.1.3 Results

Experiment 1: Social-cued memory task with no experience

Results are shown in Figure 23A.

A repeated measures ANOVA was performed by considering the first choices in frequencies [f]. The ANOVA with Time (1-5 training sessions) and Geometry (C-R, N-F) as within-subject factors, and Species (*D. rerio*, *X. eiseni*, *C. auratus*) as between-subject factor, showed the following results: there were no significant effects of Time ($F_{(4,52)}$ = .79, p = .53), Geometry ($F_{(1,13)}$ = .06, p = .81), Time × Geometry ($F_{(4,52)}$ = .37, p = .83), Species ($F_{(2,13)}$ = 1.14, p = .35), Time × Species ($F_{(8,52)}$ = 1.06, p = .40), Geometry × Species ($F_{(2,13)}$ = .11, p = .89), and Time × Geometry × Species ($F_{(8,52)}$ = .58, p = .79). A repeated measures ANOVA was then performed by considering the total choices in frequencies [f] made by fish in 30 s. The ANOVA with Time (1-5 training sessions) and Geometry (C-R, N-F) as within-subject factors, and Species (*D. rerio*, *X. eiseni*, *C. auratus*) as between-subject factor, showed the following results:

there was a significant effect of Species ($F_{(2,13)}$ = 6.97, p = .01, η_p^2 = .52), while there were no significant effects of Time ($F_{(4,52)}$ = 1.91, p = .12), Geometry ($F_{(1,13)}$ = .44, p = .52), Time × Geometry ($F_{(4,52)}$ = 1.67, p = .17), Time × Species ($F_{(8,52)}$ = .33, p = .95), Geometry × Species ($F_{(2,13)}$ = .03, p = .97), Time × Geometry × Species ($F_{(8,52)}$ = 1.51, p = .18). The main effect of Species was due to a higher number of choices made by zebrafish (*D. rerio*: C-R = 263.5 ± 49.22, N-F = 260.83 ± 49.66; *X. eiseni*: C-R = 86.8 ± 10.75, N-F = 85.8 ± 9.66; *C. auratus*: C-R = 90 ± 40.67, N-F = 87.2 ± 39.09).

Results revealed that fish, irrespective of the species, did not reorient following the spatial geometry of the transparent rectangular arena, when subjected to a social-cued memory task with no experience.

Experiment 2: Social-cued memory task with short experience

Results are shown in Figure 23B.

A repeated measures ANOVA was performed by considering the first choices in frequencies [f]. The ANOVA with Time (1-5 training sessions) and Geometry (C-R, N-F) as within-subject factors, and Species (*D. rerio*, *X. eiseni*, *C. auratus*) as between-subject factor, showed the following results: there was a significant effect of Time × Geometry ($F_{(4,64)}$ = 2.52, p = .05, η_p^2 = .14), while there were no significant effects of Time ($F_{(4,64)}$ = 2.41, p = .06), Geometry ($F_{(1,16)}$ = .39, p = .54), Species ($F_{(2,16)}$ = .87, p = .44), Time × Species ($F_{(8,64)}$ = .53, p = .83), Geometry × Species ($F_{(2,16)}$ = .42, p = .66), Time × Geometry × Species ($F_{(8,64)}$ = .59, p = .78). A paired samples Wilcoxon test was applied over the five training sessions separately, to analyse the Time × Geometry interaction. The Wilcoxon test showed a significant effect of Geometry in session 2 (Z = −2.24, p = .03), but not in the other three (session 1: Z = −1.91, p = .06; session 3: Z = −.08, p = .93; session 4: Z = −.48, p = .63; session 5: Z = −.41, p = .68).

A repeated measures ANOVA was then performed by considering the total choices in frequencies [f] made by fish in 30 s. The ANOVA with Time (1-5 training sessions) and Geometry (C-R, N-F) as within-subject factors, and Species (*D. rerio*, *X. eiseni*, *C. auratus*) as between-subject factor, showed the following results: there was a significant effect of Species ($F_{(2,16)} = 8.76$, $p = .003$, $\eta_p^2 = .52$), while there were no significant effects of Time ($F_{(4,64)} = 1.77$, $p = .14$), Geometry ($F_{(1,16)} = .81$, $p = .38$), Time × Geometry ($F_{(4,64)} = 2.26$, $p = .07$; Time × Species ($F_{(8,64)} = .44$, $p = .89$), Geometry × Species ($F_{(2,16)} = .03$, $p = .97$), Time × Geometry × Species ($F_{(8,64)} = 1.05$, $p = .41$). As for Experiment 1, the main effect of Species was due to a higher number of choices made by zebrafish (*D. rerio*: C-R = 149.3 ± 32, N-F = 147.3 ± 36.63; *X. eiseni*: C-R = 67 ± 12.36, N-F = 63.2 ± 13.88; *C. auratus*: C-R = 33.14 ± 8.32, N-F = 29.43 ± 6.09).

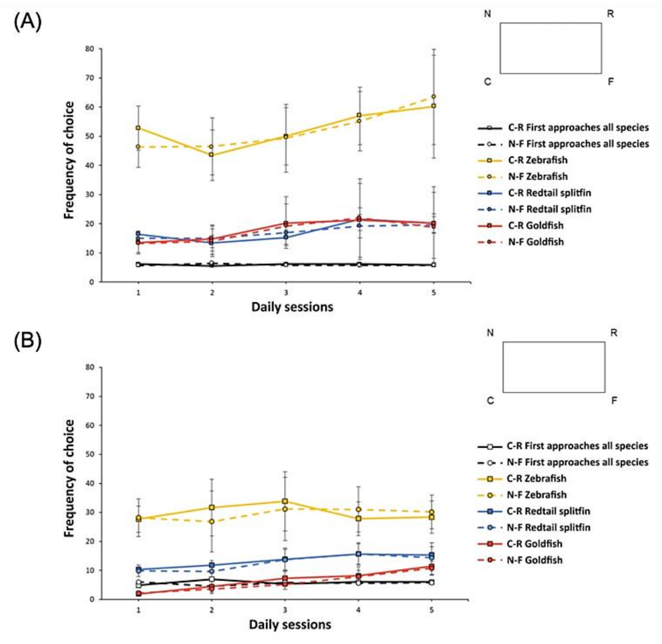


Figure 23[6]. Results of Experiment 1 (A) and Experiment 2 (B). First and total choices [f] towards the geometrically correct corners C-R vs. incorrect N-F over the first five

---

[6] Charts by Sovrano and colleagues (2020*b*) were rearranged.

training sessions. Total choices are reported for each species, due to a difference among them. Mean ± SEM are shown.

Results revealed that fish, irrespective of the species, did not reorient following the spatial geometry of the transparent rectangular arena, when subjected to a social-cued memory task with short experience.

Experiment 3: Rewarded exit task with extensive experience

Results are shown in Figure 24.

A one-way ANOVA was performed to evaluate if the total number of trials [f] to meet the learning criterion ≥ 70% was affected by the species (*D. rerio*, *X. eiseni*, *C. auratus*). The one-way ANOVA showed a significant effect of Species ($F_{(2,20)} = 29.34$, $p < .001$, $\eta^2_p = .75$; *D. rerio*: mean ± SEM = 28.6 ± 3.4; *X. eiseni*: mean ± SEM = 36.6 ± 4.8; *C. auratus*: mean ± SEM = 101.3 ± 11.9).

A repeated measures ANOVA was performed by considering the first and total choices in frequencies [f] in the learning session (i.e., when the fish met the accuracy threshold ≥ 70%). The ANOVA with Geometry (C-R, N-F) as within-subject factor and Species (*D. rerio*, *X. eiseni*, *C. auratus*) as between-subject factor, showed the following results: on the first choices, there was a significant effect of Geometry ($F_{(1,20)} = 387,21$, $p < .001$, $\eta^2_p = .95$), while there were no significant effects of Species ($F_{(2,20)} = .21$, $p = .81$) and Geometry × Species ($F_{(2,20)} = .34$, $p = .72$). A paired samples Wilcoxon test was applied to analyse the difference between the geometrically correct corners (C_1, C_2) and between the geometrically incorrect corners (X_1, X_2). The Wilcoxon test showed no significant effects (C_1 vs. C_2: $Z = -1.11$, $p = .26$; X_1 vs. X_2: $Z = -.45$, $p = .72$). The ANOVA with Geometry (C-R, N-F) as within-subject factor and

Species (*D. rerio*, *X. eiseni*, *C. auratus*) as between-subject factor, showed the following results: on the total choices, there was a significant effect of Geometry ($F_{(1,20)} = 563,52$, $p < .001$, $\eta_p^2 = .97$), while there were no significant effects of Species ($F_{(2,20)} = 1.62$, $p = .22$) and Geometry × Species ($F_{(2,20)} = 3.1$, $p = .07$). A paired samples *t*-test was applied to analyse the difference between the geometrically correct corners (C_1, C_2) and between the geometrically incorrect corners (X_1, X_2). The *t*-test showed no significant effects (C_1 vs. C_2: $t_{(22)} = .83$, $p = .42$; X_1 vs. X_2: $t_{(22)} = –.12$, $p = .91$).

A repeated measures ANOVA was performed by considering the first and total choices in frequencies [f] in the geometric test. The ANOVA with Geometry (C-R, N-F) as within-subject factor and Species (*D. rerio*, *X. eiseni*, *C. auratus*) as between-subject factor, showed the following results: on the first choices, there was a significant effect of Geometry ($F_{(1,20)} = 101.75$, $p < .001$, $\eta_p^2 = .84$), while there were no significant effects of Species ($F_{(2,19)} = .21$, $p = .81$) and Geometry × Species ($F_{(2,19)} = .86$, $p = .44$). A paired samples Wilcoxon test was applied to analyse the difference between the geometrically correct corners (C_1, C_2) and between the geometrically incorrect corners (X_1, X_2). The Wilcoxon test showed no significant effects (C_1 vs. C_2: $Z = -.75$, $p = .45$; X_1 vs. X_2: $Z = -.87$, $p = .39$). The ANOVA with Geometry (C-R, N-F) as within-subject factor and Species (*D. rerio*, *X. eiseni*, *C. auratus*) as between-subject factor, showed the following results: on the total choices, there was a significant effect of Geometry ($F_{(1,19)} = 40.17$, $p < .001$, $\eta_p^2 = .68$) and Species ($F_{(2,19)} = 12$, $p < .001$, $\eta_p^2 = .56$), while there was not a significant effect of Geometry × Species ($F_{(2,19)} = .95$, $p = .4$). The main effect of Species was due to a higher number of choices made by goldfish (*D. rerio*: C-R = 14.5 ± 2.83, N-F = 9.38 ± 2.46; *X. eiseni*: C-R = 16.25 ± 5.61, N-F = 7.6 ± 3.7; *C. auratus*: C-R = 44 ± 6.24, N-F = 37 ± 6.81). A paired samples *t*-test was applied to analyse the difference between the geometrically correct corners (C_1, C_2) and between the geometrically incorrect corners (X_1, X_2). The *t*-test showed no significant effects (C_1 vs. C_2: $t_{(21)} = .23$, $p = .82$; X_1 vs. X_2: $t_{(21)} = 2.03$, $p = .06$).

To compare the fish performance over the first five sessions of training, as done for Experiment 1 and 2, a repeated measures ANOVA was performed by considering the first and total choices in frequencies [f]. The ANOVA with Time (1-5 training sessions) and Geometry (C-R, N-F) as within-subject factors, and Species (*D. rerio*, *X. eiseni*, *C. auratus*) as between-subject factor, showed the following results: on the first choices, there was a significant effect of Time ($F_{(4,80)} = 2.69$, $p = .04$, $\eta_p^2 = .12$), Geometry ($F_{(1,20)} = 18.44$, $p < .001$, $\eta_p^2 = .48$), and Time × Geometry ($F_{(4,80)} = 3.04$, $p = .02$, $\eta_p^2 = .13$), while there were no significant effects of Species ($F_{(2,20)} = .06$, $p = .94$), Time × Species ($F_{(4,80)} = .88$, $p = .53$), Geometry × Species ($F_{(2,20)} = 3.12$, $p = .07$), and Time × Geometry × Species ($F_{(8,80)} = 1.78$, $p = .92$). The ANOVA with Time (1-5 training sessions) and Geometry (C-R, N-F) as within-subject factors, and Species (*D. rerio*, *X. eiseni*, *C. auratus*) as between-subject factor, showed the following results: on the total choices, there was a significant effect of Time ($F_{(4,80)} = 7.19$, $p < .001$, $\eta_p^2 = .27$), Geometry ($F_{(1,20)} = 24.86$, $p < .001$, $\eta_p^2 = .55$), and Time × Species ($F_{(8,80)} = 3.94$, $p < .001$, $\eta_p^2 = .28$), while there were no significant effects of Time × Geometry ($F_{(4,80)} = .74$, $p = .57$), Species ($F_{(2,20)} = 2.25$, $p = .13$), Geometry × Species ($F_{(2,20)} = 1.78$, $p = .19$), and Time × Geometry × Species ($F_{(8,80)} = .52$, $p = .84$).

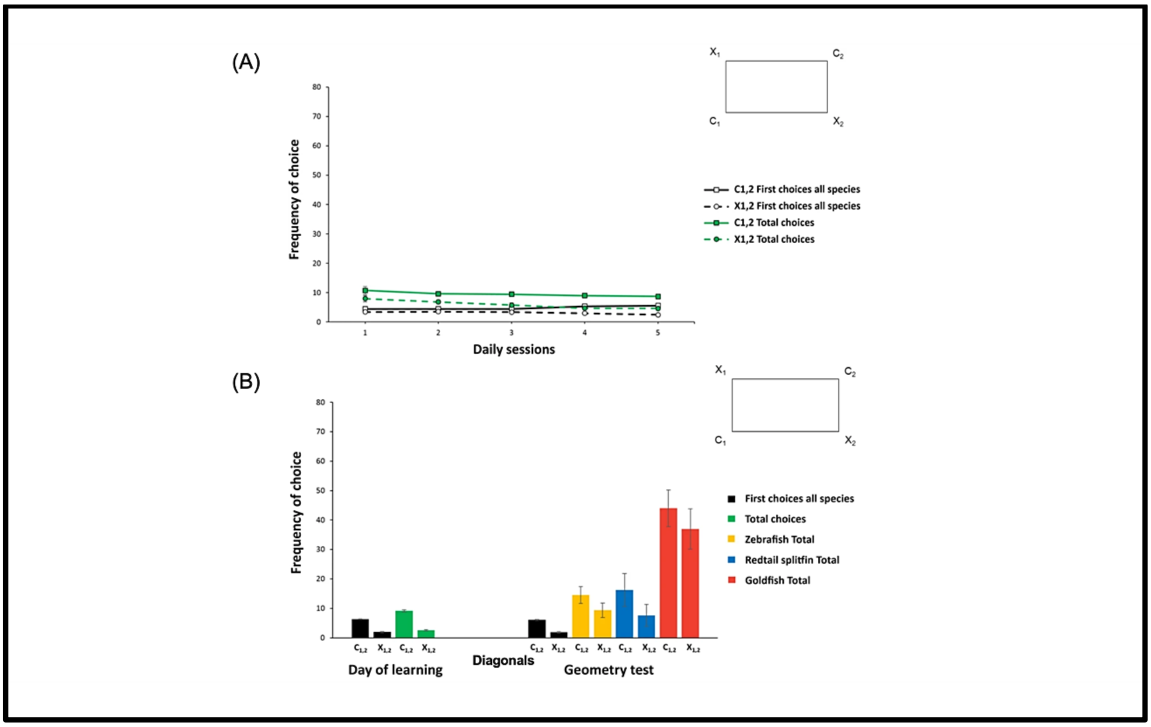


Figure 24[7]. Results of Experiment 3. (A) First and total choices [f] towards the geometrically correct corners $C_{1,2}$ vs. incorrect $X_{1,2}$ over the first five training sessions. (B) First and total choices [f] towards the geometrically correct corners $C_{1,2}$ vs. incorrect $X_{1,2}$ in the learning session (left) and geometric test (right). At test, total choices are reported for each species, due to a difference among them. Mean ± SEM are shown.

Results of training revealed that fish, irrespective of the species, learned to reorient following the spatial geometry of the transparent rectangular arena, when subjected to a rewarded exit task with extensive experience. Moreover, results of geometric test confirmed that learning had been driven by spatial geometry.

---

[7] Charts by Sovrano and colleagues (2020*b*) were rearranged.

3.1.4 Discussion

Study 5 investigated whether three eyed species of fish (*D. rerio*, *X. eiseni*, *C. auratus*) used the nonvisual spatial geometry of a rectangular transparent arena to reorient, during their search behaviour for an object (i.e., one social companion or food). The type of geometric task was varied among three experiments: Experiment 1 and Experiment 2 requested the fish to face a spontaneous social-cued memory task, where also the time-period for experiencing the arena was inspected (no vs. short experience); by contrast, Experiment 3 requested the fish to face a rewarded exit task over time, thus providing an extensive experience until learning. These two behavioural tasks have been validated well in fish species (Baratti et al., 2022).

Results of Experiment 1 and 2 revealed that fish did not reorient properly, choosing the four corners (i.e., entering the arena's corridors) at random, irrespective of no or short experience. A difference among species was found in the frequency of total choices towards the corners, showing that zebrafish took a higher number of attempts (but this was probably due to their typical swim activity under stressful or novelty contexts: Kalueff et al., 2013; Vernier et al., 2012; Wong et al., 2010). In other words, fish could not spontaneously take advantage of the transparent surfaces for reorienting, contrary to what had been observed in human children (Gianni et al., 2018).

Results of Experiment 3 revealed that fish reoriented properly over a period of training in which differential reinforcements were directly associated to correct responses. The opportunity to gain experience within the arena, probably, allowed fish to consolidate the global-shape parameters in relation to the two symmetric target locations (i.e., the geometrically correct corners). Really intriguing was the view that fish could have used other sensory systems than sight, thus supporting previous results in hypogean cavefish (Sovrano et al., 2018*a*) and, more recently, in congenitally blind mice (Normandin et al., 2022).

In Experiment 3, a geometric test in response extinction (i.e., replacing the two rewarding corridors with two unrewarding ones) was performed: in such a way, fish were required to choose the four corner positions without differential reinforcement. The aim was to verify that learning had been achieved through geometric information rather than thanks to not considered hydrodynamic differences. Fish kept the preference at choosing more the geometrically correct vs. incorrect corners, sustaining the validity of the test.

Differences among species were found in two situations: the goldfish (1) took a higher number of trials (more than double) to meet the learning criterion; (2) made more choice attempts at test. As regards the first issue, learning times are consistent with those previously reported by Vargas and colleagues (2004). The second issue could be explained following the law of effect (Thorndike, 1927), by which the more rewarding trials the goldfish took, the stronger associative connections they formed (Bitterman et al., 1970; Mackintosh, 1971). On the other hand, the zebrafish and redtail splitfin fish showed improvements over time, especially in the first five training sessions.

Overall results suggested that reorientation within nonvisual geometrically informative frames does not spontaneously occur, but can be acquired over exploratory experience, at least in fish. Even if sight mechanisms are reported to be interlaced with geometry-based spatial reorientation (Cheng, 2008; Nardini et al., 2009; Pecchia & Vallortigara, 2010*a,b*; 2012; Stürzl et al., 2008; Wystrach & Beugnon, 2009), fish species may perceive transparent surfaces thanks to a dissociation of vision from other-than-vision channels. The lateral line (Bleckmann & Zelick, 2009), as well as fins (Aiello et al., 2018), ensure mechanoreception through detecting relevant tactile-like environmental stimuli, likely working in synch. Therefore, targeted studies are needed to understand if (and to what extent) these systems are crucial for reorienting, also by employing pharmacological and/or surgery techniques (see Study 8, pp. 130-152, for a pharmacological approach).

3.2 Study 6: Isolated environmental geometric cues in zebrafish[8]

3.2.1 Introduction

Currently, there is a lack of spatial reorientation studies in which differences between spontaneous vs. acquired use of spatial geometry and landmarks have been stressed. In mammals (Cheng, 1986; Cheng & Gallistel, 1984; Lee et al., 2015*b*), chickens (Lee et al., 2012*b*; Pecchia & Vallortigara 2010*a,b*; 2012), and fish species (Baratti et al., 2022; Study 5, pp. 77-90), such a distinction involved two methodological ways to assess spatial working vs. reference memory, respectively. However, the body of evidence converges into the view of learning-independent mechanisms for boundary mapping and dependent ones for landmark-use.

One issue relates to what metric attributes are needed to reorient, since boundary length, distance, and corners are usually enclosed within an interconnected polygon. Lee and colleagues (2012*a*) tested the influence of informative metric attributes in children aged 2-3 y/o, observing that their spontaneous reorientation behaviour relied on boundary distance only (e.g., target location left/right the nearer/farther surface), while not on boundary length. Likewise, neither freestanding objects (e.g., rectangular

---

[8] The present study has been published on 5th July 2021 on *Animals* journal. To avoid self-plagiarism, it will be summarized to provide essential information, findings, and keys to the reading. In some cases, the figures have been readjusted.

arrays of four cylindrical columns) nor fragmented corners were used by children to reorient (Gouteux & Spelke, 2001; Lee & Spelke, 2008; 2010; 2011). Lee and colleagues (2013) have reported similar results in zebrafish, showing that untrained zebrafish behaved as human children, not taking advantage of length and corners to reorient.

Study 6 went into the question, with the aim to investigate if zebrafish could learn to use boundary length and fragmented corners, once isolated from the global layout. Since boundary mapping has been suggested to depend on learning-independent mechanisms, zebrafish unsuccess to spontaneously reorient through the metric attributes of length and corners is not fully clear. Moreover, if both the cues were salient information for disoriented fish, we would expect reorientation over training.

3.2.1 Materials and Methods

Subjects were 30 adult male zebrafish (wild type), ranging from 3 to 5 cm in body-length. 8 fish took part in Experiment 1, 8 in Experiment 2, 8 in Experiment 3, while 6 in Experiment 4. Each fish was observed only once. Two female companions were used as social stimuli in rewarding the experimental fish. Housing conditions were the same as described above (p. 31).

The apparatus was the same as described above (square transparent arena: pp. 45-46; rectangular transparent arena: pp. 78-79). Three experiments were designed to investigate whether zebrafish could learn to use the geometric spatial attribute of distance (Experiment 1), corners (Experiment 2), and length (Experiment 3), when singularly presented within fragmented layouts. One control experiment (Experiment 4) was scheduled to validate the square transparent arena built *ex-novo* and replicate the experimental conditions by Lee and colleagues (2013). Distance, corners, and length were provided by visual cues made of white polypropylene (Poliplak®) and equipped along the arenas’ perimeter. Distance attribute was designed through four panels equal in length (15 × 10 cm) arranged on the rectangular transparent arena; corners attribute through four fragmented corners composed of three panels (central panel: 3 × 10 cm; lateral panels: 6 × 10 cm) on the rectangular transparent arena; length attribute through four panels of ≈ 2:1 ratio in length (long panels: 20.4 × 10 cm; short panels: 10.6 × 10 cm) on the square transparent arena (Figure 25).

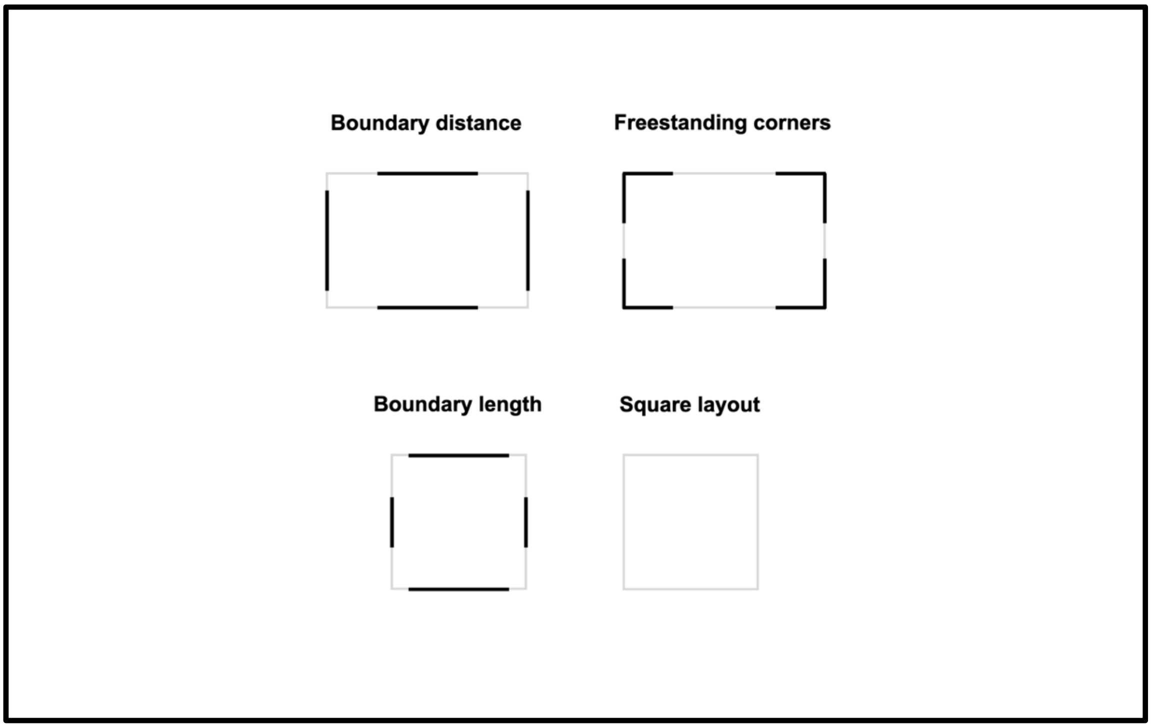


Figure 25. Schematic representation of the four geometric layouts.

The procedure was the same as described above (pp. 33-35).

The following variables were measured: for Experiment 1, 2, and 3, the mean number of trials [f] to meet the accuracy threshold ≥ 70% in two subsequent training sessions (learning and validation), the first and total choices [%] towards each diagonal (geometrically correct $C_{1,2}$ vs. incorrect $X_{1,2}$); for Experiment 4, the first and total choices [%] towards each diagonal over the 10 sessions scheduled to run out the training.

As regards Experiment 1, 2, and 3, a Student's t-test was performed to compare the use of spatial geometry (correct vs. incorrect) in the sessions of learning and validation (i.e., when the fish met and confirmed an accuracy ≥ 70%), and the two corners on the same diagonal (C_1 vs. C_2; X_1 vs. X_2). As regards Experiment 4, a repeated measures ANOVA was performed to compare the use of spatial geometry (correct vs. incorrect) over time (1-10 sessions). The Shapiro-Wilk test was performed to assess normality, whereas the Levene's test of equality of error variances and the Mauchly's sphericity test were performed to assess homoscedasticity. For clarity, all the statistic tests applied are reported as a supplementary material in a summarising table ("Supplementary Material" section, pp. 214-222, Suppl_1). To estimate the effect size of significant data analysis, we reported η_p^2 as an index for ANOVA and 95% confidence intervals as an index for Student's *t*-test. Statistical analyses were performed with IBM® SPSS Statistic 27 software package.

3.2.2 Results

Experiment 1: Use of boundary distance

An unpaired samples *t*-test was performed to evaluate if the total number of trials [f] to meet the learning criterion ≥ 70% was affected by the local position of the geometrically correct corners $C_{1,2}$ (diagonal 1 or 2). The *t*-test did not show a significant effect ($t_{(6)} = .93$, $p = .39$). Fish learned to resolve the geometric reorientation task in 39.63 ± 6.28 trials (≈ 6 training sessions). Results are shown in Figure 26A.

A paired samples *t*-test was applied on the first and the total choices [%] towards $C_{1,2}$ vs. $X_{1,2}$ in the learning session (i.e., when the fish met the accuracy threshold ≥ 70%). The *t*-test showed a significant effect of Geometry (first choices: $t_{(7)} = 14.15$, $p < .001$, 95% CI [51.36, 71.97]; total choices: $t_{(7)} = 11.47$, $p < .001$, 95% CI [41.49, 63.03]). A paired samples *t*-test was applied to analyse the difference between the geometrically correct corners (C_1, C_2) and between the geometrically incorrect corners (X_1, X_2). The *t*-test showed no significant effects (C_1 vs. C_2: first choices: $t_{(7)} = .24$, $p = .82$; total choices: $t_{(7)} = -.25$, $p = .81$; X_1 vs. X_2: first choices: $t_{(7)} = 2.17$, $p = .07$; total choices: $t_{(7)} = 2.06$, $p = .08$).

A paired samples *t*-test was then applied on the first and the total choices [%] towards $C_{1,2}$ vs. $X_{1,2}$ in the validation session (i.e., the session after learning in which the fish kept an accuracy threshold ≥ 70%). The *t*-test showed a significant effect of Geometry (first choices: $t_{(7)} = 6.77$, $p < .001$, 95% CI [39.04, 80.96]; total choices: $t_{(7)} = 8.37$, $p < .001$, 95% CI [43.32, 77.47]). A paired samples *t*-test was applied to analyse the difference between the geometrically correct corners (C_1, C_2) and between the geometrically incorrect corners (X_1, X_2). The *t*-test showed no significant effects (C_1 vs. C_2: first choices: $t_{(7)} = .35$, $p = .74$; total choices: $t_{(7)} = .06$, $p = .96$; X_1 vs. X_2: first choices: $t_{(7)} = -.50$, $p = .63$; total choices: $t_{(7)} = .23$, $p = .83$).

Results revealed that all the fish (8/8) learned to resolve the geometric reorientation task by using the geometric attribute of distance.

Experiment 2: Use of freestanding corners

An unpaired samples *t*-test was performed to evaluate if the total number of trials [f] to meet the learning criterion ≥ 70% was affected by the local position of the geometrically correct corners $C_{1,2}$ (diagonal 1 or 2). The *t*-test did not show a significant effect ($t_{(6)} = -1.69$, $p = .14$). Fish learned to resolve the geometric reorientation task in 60.38 ± 5.57 trials (≈ 8 training sessions). Results are shown in Figure 26A.

A paired samples *t*-test was applied on the first and the total choices [%] towards $C_{1,2}$ vs. $X_{1,2}$ in the learning session (i.e., when the fish met the accuracy threshold ≥ 70%). The *t*-test showed a significant effect of Geometry (first choices: $t_{(7)} = 14.71$, $p < .001$, 95% CI [43.53, 60.22]; total choices: $t_{(7)} = 18.77$, $p < .001$, 95% CI [45.81, 59.02]). A paired samples *t*-test was applied to analyse the difference between the geometrically correct corners (C_1, C_2) and between the geometrically incorrect corners (X_1, X_2). The *t*-test showed no significant effects (C_1 vs. C_2: first choices: $t_{(7)} = -.47$, $p = .65$; total choices: $t_{(7)} = .13$, $p = .90$; X_1 vs. X_2: first choices: $t_{(7)} = -.06$, $p = .95$; total choices: $t_{(7)} = -.31$, $p = .77$).

A paired samples *t*-test was then applied on the first and the total choices [%] towards $C_{1,2}$ vs. $X_{1,2}$ in the validation session (i.e., the session after learning in which the fish kept an accuracy threshold ≥ 70%). The *t*-test showed a significant effect of Geometry (first choices: $t_{(7)} = 8.36$, $p < .001$, 95% CI [45.81, 80.99]; total choices: $t_{(7)} = 10.01$, $p < .001$, 95% CI [50.21, 81.26]). A paired samples *t*-test was applied to analyse the difference between the geometrically correct corners (C_1, C_2) and between the geometrically incorrect corners (X_1, X_2). The *t*-test showed no significant effects (C_1 vs. C_2: first choices: $t_{(7)} = 1.39$, $p = .21$; total choices: $t_{(7)} = 2.13$, $p = .07$; X_1 vs. X_2: first choices: $t_{(7)} = .14$, $p = .9$; total choices: $t_{(7)} = .75$, $p = .48$).

Results revealed that all the fish (8/8) learned to resolve the geometric reorientation task by using the geometric attribute of corners.

Experiment 3: Use of boundary length

An unpaired samples *t*-test was performed to evaluate if the total number of trials [*f*] to meet the learning criterion ≥ 70% was affected by the local position of the geometrically correct corners $C_{1,2}$ (diagonal 1 or 2). The *t*-test did not show a significant effect ($t_{(6)} = 1.09$, $p = .32$). The fish learned to resolve the geometric reorientation task in 48.75 ± 10.01 trials (≈ 7 training sessions). Results are shown in Figure 26A.

A paired samples *t*-test was applied on the first and the total choices [%] towards $C_{1,2}$ vs. $X_{1,2}$ in the learning session (i.e., when the fish met the accuracy threshold ≥ 70%). The *t*-test showed a significant effect of Geometry (first choices: $t_{(7)} = 13.9$, $p < .001$, 95% CI [44.61, 62.9]; total choices: $t_{(7)} = 15.92$, $p < .001$, 95% CI [49.23, 66.41]). A paired samples *t*-test was applied to analyse the difference between the geometrically correct corners (C_1, C_2) and between the geometrically incorrect corners (X_1, X_2). The *t*-test showed no significant effects (C_1 vs. C_2: first choices: $t_{(7)} = -1.19$, $p = .27$; total choices: $t_{(7)} = -.7$, $p = .51$; X_1 vs. X_2: first choices: $t_{(7)} = .17$, $p = .87$; total choices: $t_{(7)} = -.39$, $p = .71$).

A paired samples *t*-test was then applied on the first and the total choices [%] towards $C_{1,2}$ vs. $X_{1,2}$ in the validation session (i.e., the session after learning in which the fish kept an accuracy threshold ≥ 70%). The *t*-test showed a significant effect of Geometry (first choices: $t_{(7)} = 9.03$, $p < .001$, 95% CI [43.83, 74.93]; total choices: $t_{(7)} = 9.09$, $p < .001$, 95% CI [45.83, 78.05]). A paired samples *t*-test was applied to analyse the difference between the geometrically correct corners (C_1, C_2) and between the geometrically incorrect corners (X_1, X_2). The *t*-test showed no significant effects (C_1 vs. C_2: first choices: $t_{(7)} = .34$, $p = .74$; total choices: $t_{(7)} = .08$, $p = .94$; X_1 vs. X_2: first choices: $t_{(7)} = .81$, $p = .44$; total choices: $t_{(7)} = -1.01$, $p = .34$).

Results revealed that all the fish (8/8) learned to resolve the geometric reorientation task by using the geometric attribute of length.

Experiment 4: Control

No fish (0/6) learned to resolve the geometric reorientation task within the 10 training sessions provided for learning. Results are shown in Figure 26B.

A repeated measures ANOVA was performed by considering the first and total choices [%] towards $C_{1,2}$ vs. $X_{1,2}$ over training (1-10 sessions). The ANOVA with Geometry ($C_{1,2}$, $X_{1,2}$) and Session (1-10) as within-subject factors, and Diagonal (1, 2) as between-subject factor, showed the following results: there were no significant effects of Geometry (first choices: $F_{(1,4)} = 1.23$, $p = .33$; total choices: $F_{(1,4)} = 5.59$, $p = .08$), Geometry × Diagonal (first choices: $F_{(1,4)} = 1.96$, $p = .23$; total choices: $F_{(1,4)} = 2.44$, $p = .19$), Session (first choices: $F_{(9,36)} = .95$, $p = .5$; total choices: $F_{(9,36)} = .95$, $p = .49$), Session × Diagonal (first choices: $F_{(9,36)} = .95$, $p = .49$; total choices: $F_{(9,36)} = .95$, $p = .49$), Geometry × Session (first choices: $F_{(9,36)} = .69$, $p = .71$; total choices: $F_{(9,36)} = 1.1$, $p = .39$), Geometry × Session × Diagonal (first choices: $F_{(9,36)} = .37$, $p = .94$; total choices: $F_{(9,36)} = .59$, $p = .8$), and Diagonal (first choices: $F_{(1,4)} = 1$, $p = .37$; total choices: $F_{(1,4)} = 1$, $p = .37$).

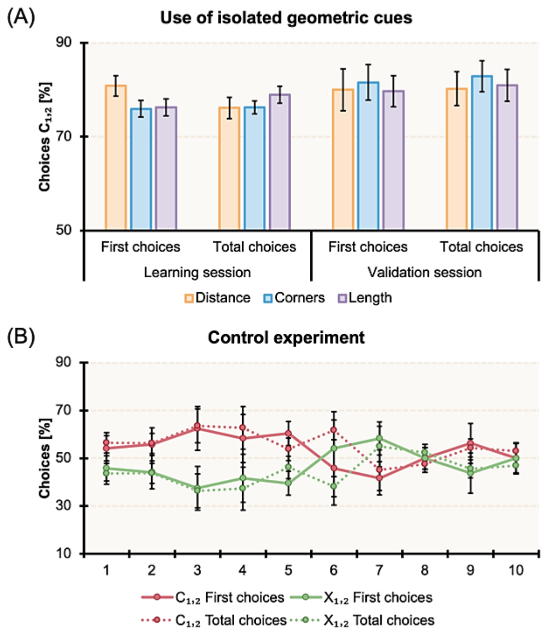


Figure 26[9]. Results. (A) Use of geometric cues during reorientation: first and total choices [%] towards the geometrically correct corners $C_{1,2}$ in relation to boundary distance, corners, and length. All the values significantly exceed the learning criterion ≥ 70%. (B) Control experiment: first and total choices [%] towards the geometrically correct corners $C_{1,2}$ vs. incorrect $X_{1,2}$ over 10 training sessions within the square transparent arena.

Results revealed that no fish (0/6) learned to resolve the reorientation task within the square transparent arena, in the absence of both visual geometric attributes and nonvisual (i.e., physical) metric layout, thus ensuring that reorientation behaviour under the previous experimental conditions (Experiments 1-3) was really associated with geometric attributes.

---

[9] The figure is a modified version of those presented by Baratti and colleagues (2021).

3.2.3 Discussion

Study 6 explored the use of metric cues (boundary distance, freestanding corners, and boundary length) in relation to left-right sense by zebrafish that underwent a rewarded exit task over time. As usual, fish were required to distinguish the geometrically correct corners but, in this case, under the influence of one geometric cue at a time. The aim was to determine the role of each parameter and to compare the learning performance with spontaneous behaviour of zebrafish under the same experimental conditions (Lee et al., 2013). Four experiments were performed, two within a rectangular transparent arena (Experiment 1 and 2) and two within a square transparent arena (Experiment 3 and 4), where the geometric attributes were provided by opaque visual cues equipped along the perimeter.

Results of Experiment 1 (*Use of boundary distance*), 2 (*Use of freestanding corners*), and 3 (*Use of boundary length*) showed that fish learned to reorient in accord with all the three parameters, contrary to what it has been observed under spontaneous behaviour. While the use of distance emerged in both the behavioural task (i.e., social-cued memory task and rewarded exit task), corners and length did not provide a crucial cue without training. Such evidence applies to both zebrafish (Lee et al., 2013) and human children (Gouteux & Spelke, 2001; Lee & Spelke, 2008; 2010*a*; 2011). It is likely that the opportunity to be exposed over time to each geometric attribute could have enhanced the capacity of zebrafish to represent them in long-term memory.

Results of Experiment 4 (*Control*) revealed that no reorientation occurred within a geometrically uninformative frame composed of equal-length transparent surfaces. Also, this finding acquires relevance for methodological aspects related to reorientation behaviour of fish under a condition of transparency, by validating the apparatus and procedure here introduced. In fact, the zebrafish chose the four corner positions at random: they could not behave otherwise, unless some other factors (e.g., self-based coordinates built on unexpected intra- and/or extra-tank visual cues) had interfered during disoriented navigation.

Overall results suggested that in zebrafish there is an acquired use of several parameters of geometric environments, that is, boundary distance (where we confirm the spontaneous preference reported by Lee et al., 2013), freestanding corners (probably through some sort of “amodal reconstruction” of the perimeter around the corners; Michotte et al., 1991; Nanay et al., 2018), and boundary length (where our findings conflict with previous literature, most of all in humans; Gouteux & Spelke, 2001; Lee & Spelke, 2008; 2010*a*; 2011). Moreover, the present study led to focus on the deterministic role of motivational factors (food and companions) for goal-oriented navigation, at least in zebrafish (Daggett et al., 2019; Kalueff et al., 2014; Sison & Gerlai, 2010). Lastly, spatial memory as independent systems (short- and long-term) strongly affects how the fish use metric attributes when they are not enclosed within an interconnected polygon. To fix distance, corners, and length elements, it seems essential experiencing spatial geometry for a purpose that animals may learn to expect over time.

3.3 Study 7: 3D outside landmark (blue cylinder) in zebrafish

3.3.1 Introduction

Previous studies have shown that zebrafish reoriented within a rectangular white arena, both in situations of spontaneous (Lee et al., 2012*c*; 2013) and acquired (Baratti et al., 2020) navigation. Moreover, zebrafish learned to resolve the geometric reorientation task if observed, under training, within a rectangular transparent arena (Study 5, pp. 77-90; Sovrano et al., 2020*a*). Lee and colleagues (2012*c*) found that zebrafish spontaneously integrated the shape of a rectangular white arena with a blue wall landmark, and we have observed that they accomplished all the generalisation tests to assess the role of geometry and landmarks apart (Study 1, pp. 29-40; Study 2, pp. 40-60).

Also, it has been shown that zebrafish did not integrate the shape of a rectangular transparent arena with a single outside blue cylinder, which provided a 3D shape information, in the absence of training (Lee et al., 2015*a*). In details, this landmark was placed outside in the centre of one side of the transparent arena, by handling its proximity to the target corner in relation to the length of surfaces (short vs. long side). Zebrafish failed at reorienting when: (1) the landmark was near the target corner on the long side of the arena – proximal long condition; (2) the landmark was far from the target corner on the short side of the arena – distal short condition; (3) the landmark was far from the target corner on the long side of the arena – distal long condition. Only when the landmark was near the target corner on the short side of the arena – proximal short condition – zebrafish exhibited a preference for the target corner, but even showing an attractiveness-bias for the closest corner position. Such a behaviour has been explained in terms of landmark’s perceptive salience, where a primacy of the visual cue prevented the use of spatial geometry.

However, another interpretation may be grounded on the role of behavioural tasks on zebrafish reorientation abilities, as it occurred in previous studies (Baratti et al., 2021; Sovrano et al., 2020*b*) and reported above (Study 5, pp. 77-90; Study 6, pp. 90-100). With the aim to understand if a rewarded training over time would have aided zebrafish in overpassing the attractiveness-bias, leading to effective reorientation irrespective of landmark's proximity and side's length, in Study 7 we reproduced the experimental condition described above, but training the fish through the rewarded exit task as usual (Baratti et al., 2020; 2021; Sovrano et al., 2002; 2003; 2005*a,b*; 2007; 2018; 2020*a,b*).

If proximity and length affect the process of integration, we will expect an increasing range of difficulty at learning, where proximal short > proximal long > distal short > distal long. Also, if a repeated experience enhances reorientation behaviour at the expense of salience-driven biases, we will expect the use of spatial geometry in the proximal short condition.

3.3.2 Materials and Methods

Subjects were 16 adult male zebrafish (wild type), ranging from 3 to 5 cm in body-length. Housing conditions were the same as described above (p. 31).

The apparatus was that used in previous studies described above (pp. 78-79), as well as the procedure (pp. 33-35). The landmark was a cylinder (diameter: 9.5 cm; height: 14 cm) externally covered with a blue plastic sheet (RGB: 30, 144, 255).

Four experimental conditions were designed (N = 4 each), while varying the landmark position in relation to the rectangular transparent arena: in the Proximal Short condition (PS), the landmark was placed near the Correct corner on the short side of the arena; in the Proximal Long condition (PL), the landmark was placed near the Correct corner on the long side of the arena; in the Distal Short condition (DS), the landmark was placed far from the Correct corner on the short side of the arena; in the Distal Long condition (DL), the landmark was placed far from the Correct corner on the long side of the arena. Figure 27.

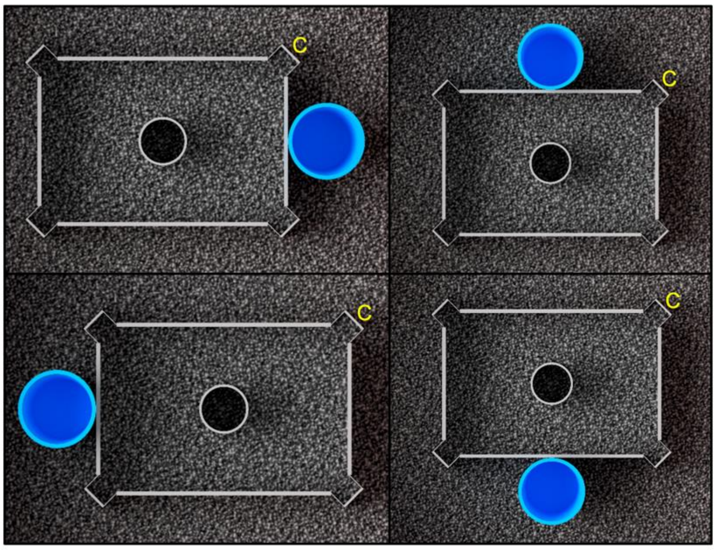


Figure 27. Experimental design. For each condition, the position of the cylindrical blue landmark was changed. Proximal landmark on the short wall (top left); Proximal landmark on the long wall (top right); Distal landmark on the short wall (bottom left); Distal landmark on the long wall (bottom right). The correct corner position is highlighted.

The following dependent variables were measured: the mean number of trials [f] for the experimental conditions in which fish met the learning criterion ≥ 70% towards the Correct corner; the frequencies of total choices towards the four corners of the experimental arena; the frequencies of total choices towards the Correct corner with respect to motion strategy (Wall-following vs. Centre-to-corner) and motion direction (Left vs. Right); the latency times in seconds before going out, in the first and last sessions of training.

A repeated measures ANOVA was applied to compare the total choices towards the four corners (Correct “C”, Near “N”, Rotational “R”, Far “F”) and the two diagonals

(Correct “CR”, Incorrect “NF”), depending on the experimental condition (Proximal Short “PS”, Proximal Long “PL”, Distal Short “DS”, Distal Long “DL”) and session. A repeated measures ANOVA was also applied on motion strategy, motion direction, and latency times. A student’s *t*-test for paired samples was applied to compare differences among the four corners and between the two diagonals.

The Shapiro-Wilk test was performed to assess normality, whereas the Levene’s test of equality of error variances and the Mauchly’s sphericity test were performed to assess homoscedasticity. For clarity, all the statistic tests applied are reported as a supplementary material in a summarising table (“Supplementary Material” section, pp. 214-222, Suppl_1). To estimate the effect size of significant data analysis, we reported η_p^2 as an index for ANOVA and 95% confidence intervals as an index for Student’s *t*-test. Statistical analyses were performed with IBM® SPSS Statistic 27 software package.

3.3.3 Results

A *post-hoc* analysis showed that the sample (N = 16, 4 fish per group) was large enough to detect a moderate effect size (.77), with a power of .72. A similar sample size was used by Sovrano and colleagues (2020*a*) to assess the role of learning and spatial geometry on fish landmark-based reorientation.

Condition 1: Proximal Short (PS)

Results are shown in Figure 28, considering the first and total choices [%] towards the Correct corner. 4/4 fish met the learning criterion ≥ 70%.

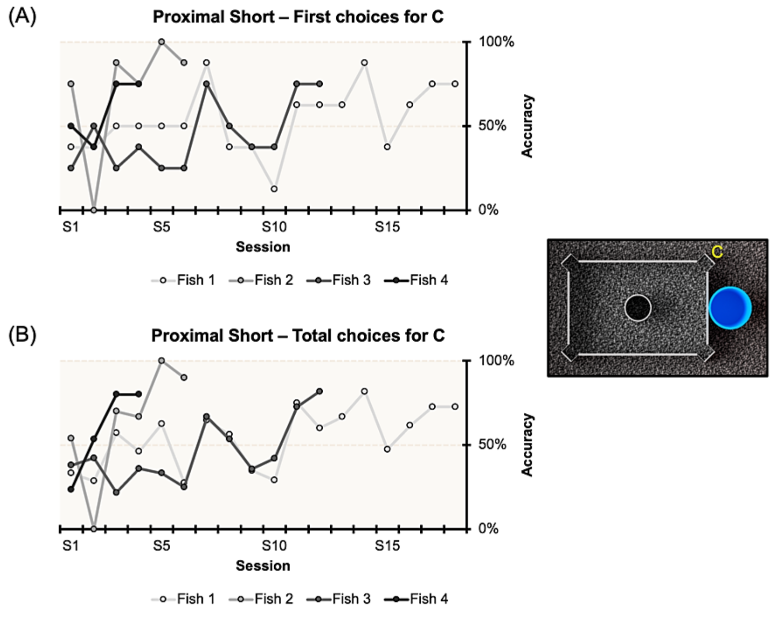


Figure 28. Results. First (A) and total (B) choices towards the Correct corner for PS (Proximal Short): individual curves. 4/4 fish met the learning criterion ≥ 70%.

Condition 2: Proximal Long (PL)

Results are shown in Figure 29, considering the first and total choices [%] towards the Correct corner. 3/4 fish met the learning criterion ≥ 70%.

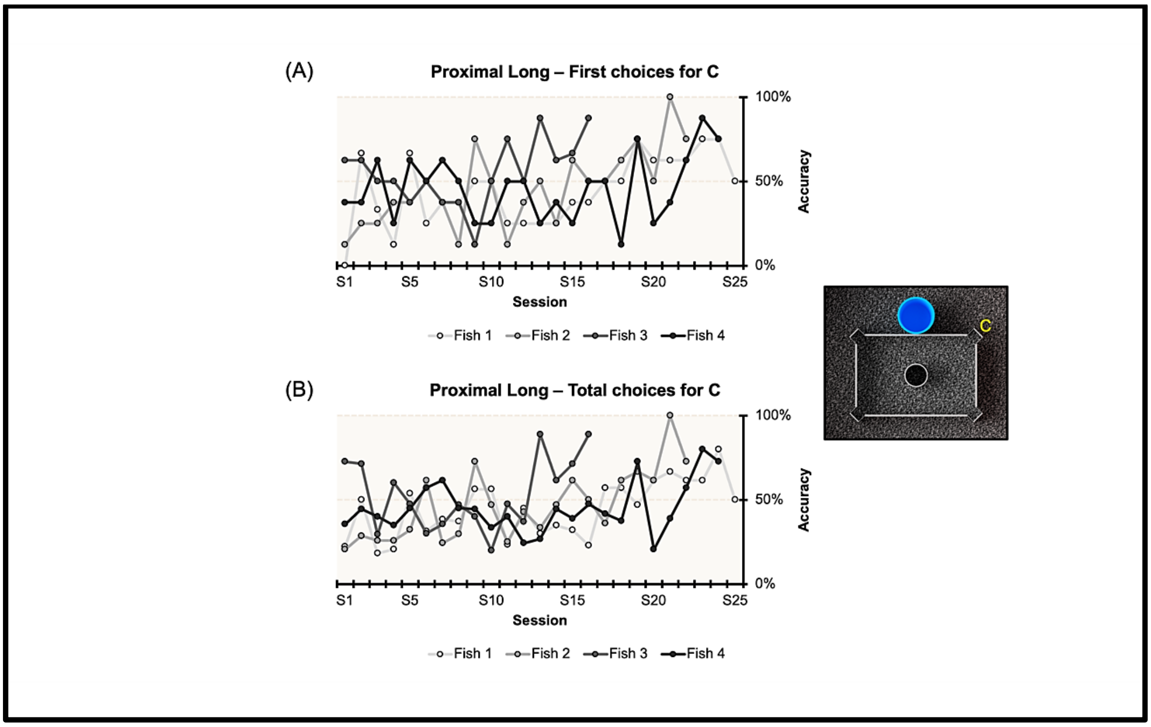


Figure 29. Results. First (A) and total (B) choices towards the Correct corner for PL (Proximal Long): individual curves. 3/4 fish met the learning criterion ≥ 70%.

Condition 3: Distal Short (DS)

Results are shown in Figure 30, considering the first and total choices [%] towards the Correct corner. 1/4 fish met the learning criterion ≥ 70%.

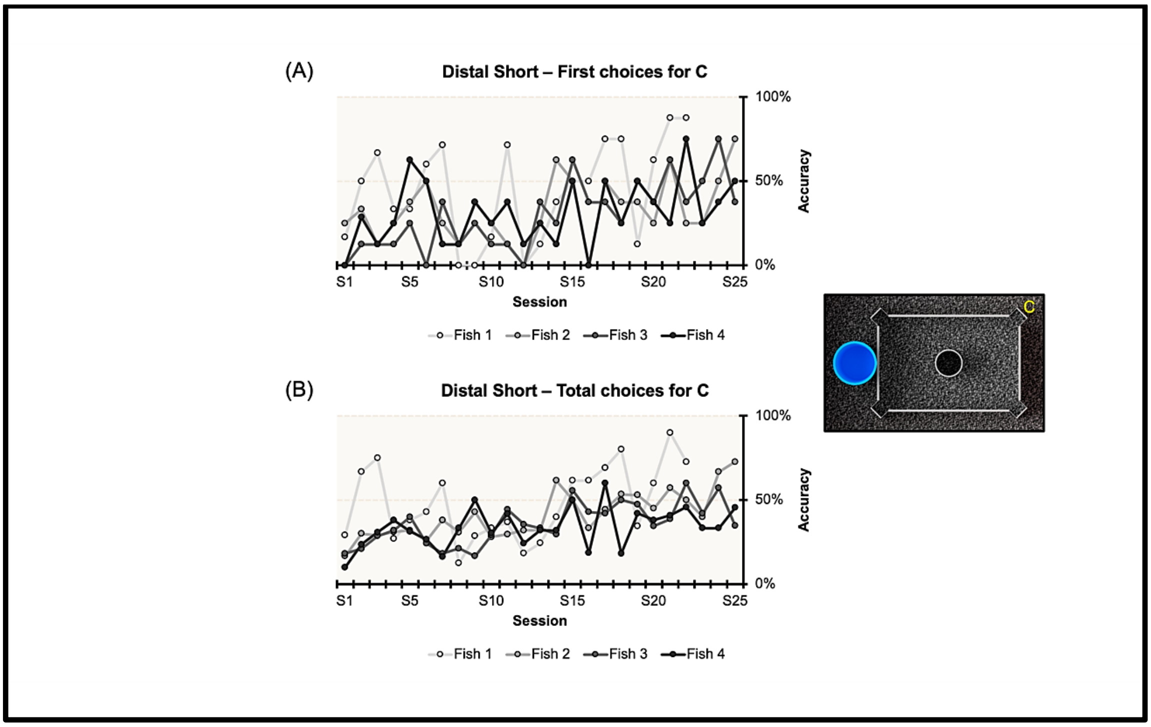


Figure 30. Results. First (A) and total (B) choices towards the Correct corner for DS (Distal Short): individual curves. 1/4 fish met the learning criterion ≥ 70%.

Condition 4: Distal Long (DL)

Results are shown in Figure 31, considering the first and total choices [%] towards the Correct corner. 0/4 fish met the learning criterion ≥ 70%.

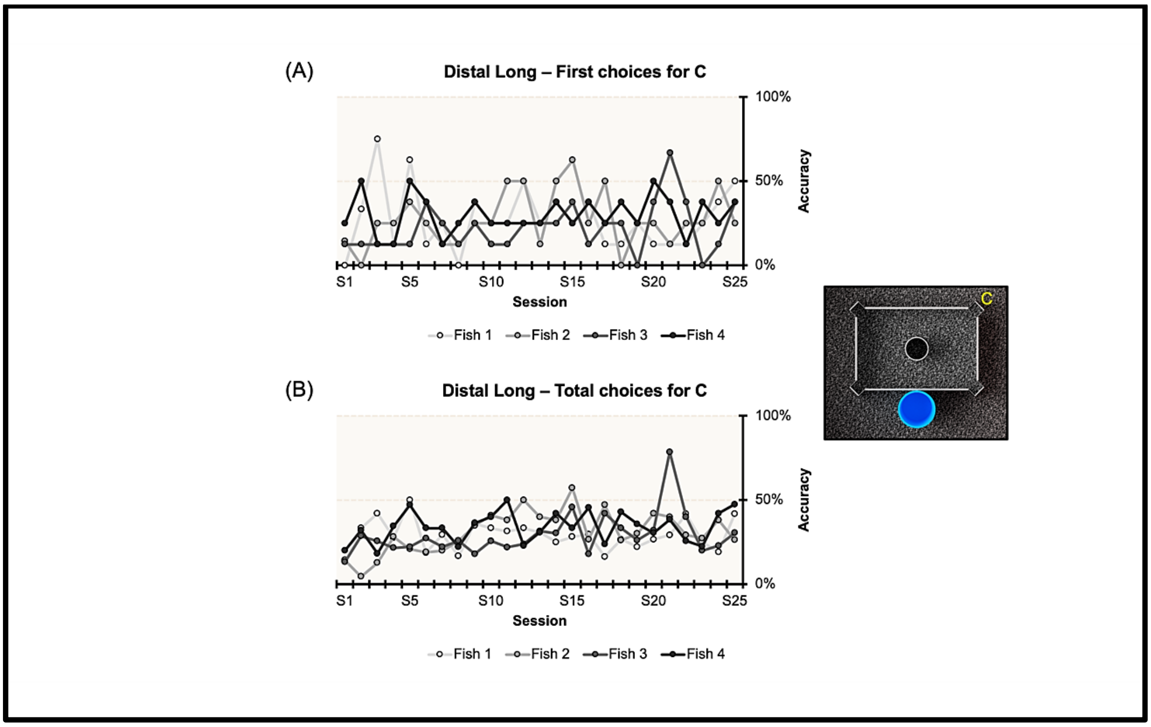


Figure 31. Results. First (A) and total (B) choices towards the Correct corner for DL (Distal Long): individual curves. 0/4 fish met the learning criterion ≥ 70%.

Corners by conditions (PS, PL, DS, DL) in the last four sessions of training

We analysed the choices made by fish towards the four corners of the arena, depending on the experimental condition, to evaluate the integrated use of geometry (transparent rectangular arena) and landmark (blue cylinder) in the sessions of training that all fish had in common (the last four).

A repeated measures ANOVA was performed by considering the total choices in frequencies [f]. The ANOVA with Session (the last four sessions of training) and Corner (C, N, R, F) as within-subject factors, and Condition (PS, PL, DS, DL) as between-subject factor, showed the following results: there was a significant effect of Session ($F_{(3,36)} = 3.07$, $p = .04$, $\eta_p^2 = .2$), Corner ($F_{(2,24)} = 69.88$, $p < .001$, $\eta_p^2 = .85$), Corner $*$ Condition ($F_{(6,24)} = 4.5$, $p = .003$, $\eta_p^2 = .53$), and Condition ($F_{(3,12)} = 28.38$, $p < .001$, $\eta_p^2 = .88$), while there were no significant effects of Session $*$ Condition ($F_{(9,36)} =$

1.28, p = .28), Session ∗ Corner ($F_{(4,51)}$ = 1.7, p = .16), and Session ∗ Corner ∗ Condition ($F_{(13,51)}$ = 1.43, p = .18).

A repeated measures ANOVA and a paired samples t-test were performed on the total choices [f], in the four experimental conditions separately.

In the Proximal Short condition, the ANOVA with Session (the last four sessions of training) and Corner (C, N, R, F) as within-subject factors, showed the following results: there was a significant effect of Corner ($F_{(3,9)}$ = 27.28, p = .01, η_p^2 = .9), while there were no significant effects of Session ($F_{(1,9)}$ = 5.41, p = .1) and Session ∗ Corner ($F_{(9,27)}$ = 1.64, p = .16). In the fourth last session of training, the t-test revealed significant differences among corners: C vs. N ($t_{(3)}$ = 6.97, p = .01, 95% CI [2.45, 6.55]); C vs. R ($t_{(3)}$ = 9.92, p = .002, 95% CI [3.23, 6.27]). In the third last session of training, the t-test revealed significant differences among corners: C vs. N ($t_{(3)}$ = 5.28, p = .01, 95% CI [2.19, 8.81]); C vs. R ($t_{(3)}$ = 25, p < .001, 95% CI [5.45, 7.05]); C vs. F ($t_{(3)}$ = 4.62, p = .02, 95% CI [1.71, 9.29]). In the second last session of training, the t-test revealed significant differences among corners: C vs. N ($t_{(3)}$ = 19.6, p < .001, 95% CI [6.7, 9.3]); C vs. R ($t_{(3)}$ = 11.52, p = .001, 95% CI [5.25, 9.25]); C vs. F ($t_{(3)}$ = 11.62, p = .001, 95% CI [5.45, 9.55]. In the last session of training, the t-test revealed significant differences among corners: C vs. N ($t_{(3)}$ = 29, p < .001, 95% CI [6.45, 8.05]; C vs. R ($t_{(3)}$ = 17.23, p < .001, 95% CI [6.73, 9.77]; C vs. F ($t_{(3)}$ = 13.86, p = .001, 95% CI [6.16, 9.84].

In the Proximal Long condition, the ANOVA with Session (the last four sessions of training) and Corner (C, N, R, F) as within-subject factors, showed the following results: there was a significant effect of Corner ($F_{(3,9)}$ = 150.20, p < .001, η_p^2 = .98), while there were no significant effects of Session ($F_{(3,9)}$ = .78, p = .53) and Session ∗ Corner ($F_{(9,27)}$ = 1.18, p = .35). In the fourth last session of training, the t-test revealed significant differences among corners: C vs. N ($t_{(3)}$ = 5.4, p = .01, 95% CI [2.77, 10.73]); C vs. R ($t_{(3)}$ = 5.56, p = .01, 95% CI [2.56, 9.44]; C vs. F ($t_{(3)}$ = 5.46, p = .01, 95% CI [2.71, 10.29]. In the third last session of training, the *t*-test revealed significant differences among corners: C vs. N ($t_{(3)}$ = 14.1, p = .001, 95% CI [5.23, 8.27]); C vs. R ($t_{(3)}$ = 8.52, p = .003, 95% CI [3.45, 7.55]); C vs. F ($t_{(3)}$ = 13, p = .001, 95% CI [4.91, 8.09]). In the second last session of training, the *t*-test revealed significant differences among corners: C vs. N ($t_{(3)}$ = 15.15, p = .001, 95% CI [5.73, 8.77]); C vs. R ($t_{(3)}$ = 33, p < .001, 95% CI [7.45, 9.05]); C vs. F ($t_{(3)}$ = 19.6, p < .001, 95% CI [6.7, 9.3]. In the last session of training, the *t*-test revealed significant differences among corners: C vs. N ($t_{(3)}$ = 7, p = .01, 95% CI [3.82, 10.18]); C vs. R ($t_{(3)}$ = 29, p < .001, 95% CI [6.45, 8.05]); C vs. F ($t_{(3)}$ = 13, p = .001, 95% CI [4.91, 8.09]).

In the Distal Short condition, the ANOVA with Session (the last four sessions of training) and Corner (C, N, R, F) as within-subject factors, showed the following results: there was a significant effect of Corner ($F_{(3,9)}$ = 21.34, p < .001, η_p^2 = .88), while there were no significant effects of Session ($F_{(3,9)}$ = 1.72, p = .23) and Session $*$ Corner ($F_{(9,27)}$ = 1.23, p = .32). In the fourth last session of training, the *t*-test revealed significant differences among corners: C vs. N ($t_{(3)}$ = 3.39, p = .04, 95% CI [.32, 10.18]); C vs. R ($t_{(3)}$ = 5.05, p = .02, 95% CI [2.68, 11.82]); C vs. F ($\mathrm{t}_{(3)}$ = 6.73, p = .01, 95% CI [3.03, 8.47]. In the third last session of training, the *t*-test revealed significant differences among corners: C vs. N ($t_{(3)}$ = 6.35, p = .01, 95% CI [2.74, 8.26]); C vs. R ($t_{(3)}$ = 5.02, p = .02, 95% CI [1.74, 7.76]). In the second last session of training, the *t*-test revealed significant differences among corners: C vs. N ($t_{(3)}$ = 5.17, p = .01, 95% CI [2.5, 10.5]); C vs. R ($t_{(3)}$ = 6.54, p = .01, 95% CI [3.72, 10.78]); N vs. F ($t_{(3)}$ = −3.58, p = .04, 95% CI [−4.25, −.25]; R vs. F ($t_{(3)}$ = −3.67, p = .04, 95% CI [−5.6, −.4]). In the last session of training, the *t*-test revealed significant differences among corners: C vs.

N ($t_{(3)}$ = 3.8, p = .03, 95% CI [.77, 8.73]); C vs. R ($t_{(3)}$ = 15.15, p = .001, 95% CI [5.73, 8.77]); C vs. F ($t_{(3)}$ = 4.44, p = .02, 95% CI [1.49, 9]).

In the Distal Long condition, the ANOVA with Session (the last four sessions of training) and Corner (C, N, R, F) as within-subject factors, showed the following results: there were no significant effects of Session ($F_{(3,9)}$ = 1.11, p = .4), Corner ($F_{(3,9)}$ = .91, p = .47), and Session $*$ Corner ($F_{(9,27)}$ = 1.59, p = .17).

In three out of four experimental conditions (Proximal Short, Proximal Long, Distal Short), fish chose the Correct corner more than the incorrect ones (Near, Rotational, Far), over the last four sessions of training. However, not all fish met the learning criterion ≥ 70%: 4/4 in the Proximal Short, 3/4 in the Proximal Long, and 1/4 in the Distal Short learned to resolve the task.

Corners by conditions (PS, PL, DS, DL) in the last two sessions of training

We analysed the choices made by fish towards the four corners of the arena, depending on the experimental condition, to evaluate the integrated use of geometry (transparent rectangular arena) and landmark (blue cylinder) in the last two sessions of training, that is, where a stronger effect of corners was expected.

Results are shown Figure 32.

A repeated measures ANOVA was performed by considering the total choices in frequencies [f]. The ANOVA with Session (the last two sessions of training) and Corner (C, N, R, F) as within-subject factors, and Condition (PS, PL, DS, DL) as between-subject factor, showed the following results: there was a significant effect of Corner ($F_{(3,36)}$ = 111.73, p < .001, η_p^2 = .9), Corner $*$ Condition ($F_{(9,36)}$ = 6.6, p < .001, η_p^2 = .62), and Condition ($F_{(3,12)}$ = 13.22, p < .001, η_p^2 = .77), while there were no significant effects of Session ($F_{(1,12)}$ = .003, p = .95), Session $*$ Condition ($F_{(3,12)}$ = .3,

$p = .82$), Session $\ast$ Corner ($F_{(2,26)} = 1.75$, $p = .19$), Session $\ast$ Corner $\ast$ Condition ($F_{(6,26)} = 1.71$, $p = .16$).

A repeated measures ANOVA and a paired samples *t*-test were performed on the total choices [f], in the four experimental conditions separately.

In the Proximal Short condition, the ANOVA with Session (the last two sessions of training) and Corner (C, N, R, F) as within-subject factors, showed the following results: there was a significant effect of Corner ($F_{(3,9)} = 223.74$, $p < .001$, $\eta_p^2 = .99$), while there were no significant effects of Session ($F_{(1,3)} = 1$, $p = .39$) and Session $\ast$ Corner ($F_{(3,9)} = 2.49$, $p = .13$). In the second last session of training, the *t*-test revealed significant differences among corners: C vs. N ($t_{(3)} = 19.6$, $p < .001$, 95% CI [6.7, 9.3]); C vs. R ($t_{(3)} = 11.52$, $p = .001$, 95% CI [5.25, 9.25]); C vs. F ($t_{(3)} = 11.62$, $p = .001$, 95% CI [5.45, 9.55]. In the last session of training, the *t*-test revealed significant differences among corners: C vs. N ($t_{(3)} = 29$, $p < .001$, 95% CI [6.45, 8.05]; C vs. R ($t_{(3)} = 17.23$, $p < .001$, 95% CI [6.73, 9.77]; C vs. F ($t_{(3)} = 13.86$, $p = .001$, 95% CI [6.16, 9.84].

In the Proximal Long condition, the ANOVA with Session (the last two sessions of training) and Corner (C, N, R, F) as within-subject factors, showed the following results: there was a significant effect of Corner ($F_{(3,9)} = 81.81$, $p < .001$, $\eta_p^2 = .97$), while there were no significant effects of Session ($F_{(1,3)} = .42$, $p = .56$) and Session $\ast$ Corner ($F_{(3,9)} = 2.6$, $p = .12$). In the second last session of training, the *t*-test revealed significant differences among corners: C vs. N ($t_{(3)} = 15.15$, $p = .001$, 95% CI [5.73, 8.77]); C vs. R ($t_{(3)} = 33$, $p < .001$, 95% CI [7.45, 9.05]); C vs. F ($t_{(3)} = 19.6$, $p < .001$, 95% CI [6.7, 9.3]. In the last session of training, the *t*-test revealed significant differences among corners: C vs. N ($t_{(3)} = 7$, $p = .01$, 95% CI [3.82, 10.18]); C vs. R ($t_{(3)} = 29$, $p < .001$, 95% CI [6.45, 8.05]); C vs. F ($t_{(3)} = 13$, $p = .001$, 95% CI [4.91, 8.09]).

In the Distal Short condition, the ANOVA with Session (the last two sessions of training) and Corner (C, N, R, F) as within-subject factors, showed the following results: there was a significant effect of Corner ($F_{(1,4)}$ = 33.61, p = .01, η_p^2 = .92), while there were no significant effects of Session ($F_{(1,3)}$ = .49, p = .53) and Session $*$ Corner ($F_{(3,9)}$ = 1.1, p = .19). In the second last session of training, the *t*-test revealed significant differences among corners: C vs. N ($t_{(3)}$ = 5.17, p = .01, 95% CI [2.5, 10.5]); C vs. R ($t_{(3)}$ = 6.54, p = .01, 95% CI [3.72, 10.78]); N vs. F ($t_{(3)}$ = −3.58, p = .04, 95% CI [−4.25, −.25]; R vs. F ($t_{(3)}$ = −3.67, p = .04, 95% CI [−5.6, −.4]). In the last session of training, the *t*-test revealed significant differences among corners: C vs. N ($t_{(3)}$ = 3.8, p = .03, 95% CI [.77, 8.73]); C vs. R ($t_{(3)}$ = 15.15, p = .001, 95% CI [5.73, 8.77]); C vs. F ($t_{(3)}$ = 4.44, p = .02, 95% CI [1.49, 9]).

In the Distal Long condition, the ANOVA with Session (the last two sessions of training) and Corner (C, N, R, F) as within-subject factors, showed the following results: there were no significant effects of Session ($F_{(1,3)}$ = .18, p = .7), Corner ($F_{(3,9)}$ = 1.95, p = .19), and Session $*$ Corner ($F_{(3,9)}$ = 1.54, p = .27).

In three out of four experimental conditions (Proximal Short, Proximal Long, Distal Short), fish chose the Correct corner more than the incorrect ones (Near, Rotational, Far), in both the second last and last session of training.

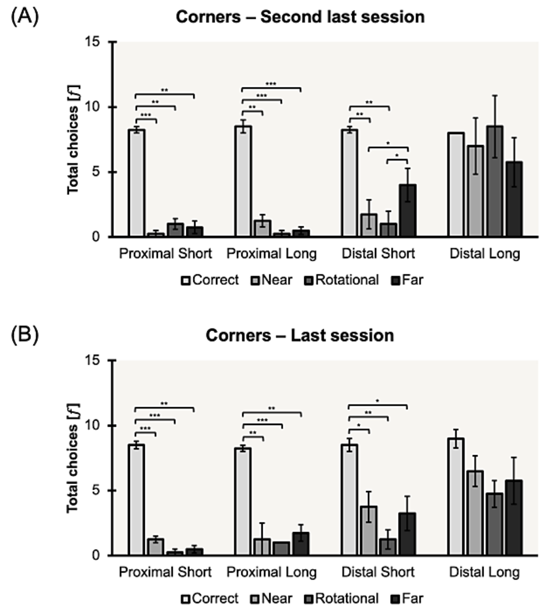


Figure 32. Corners by conditions (PS, PL, DS, DL) in the last two sessions of training: second last session (A), last session (B). Mean ± SEM are shown. Significant *p* values are indicated with asterisks (* $p = .05$; ** $p = .001$; *** $p < .001$).

Diagonals by conditions (PL, DS, DL) over the twenty-five sessions of training

We analysed the choices made by fish towards the two diagonals of the arena, depending on the experimental condition, to evaluate the use of geometry (transparent rectangular arena) regardless of the presence of landmark (blue cylinder) over the twenty-five sessions of training for unsuccessful fish at learning.

Results are shown in Figure 33.

A repeated measures ANOVA was performed by considering the total choices in frequencies [f]. The ANOVA with Sessions (all the twenty-five sessions of training) and Diagonal (CR, NF) as within-subject factors, and Condition (PL, DS, DL) as between-subject factor, showed the following results: there was a significant effect of Session ($F_{(24,120)} = 2.67$, $p < .001$, $\eta_p^2 = .35$) and Diagonal ($F_{(1,5)} = 45.8$, $p = .001$, $\eta_p^2 =$

.9), while there were no significant effects of Session ∗ Condition ($F_{(48,120)}$ = .87, p = .71), Diagonal ∗ Condition ($F_{(2,5)}$ = .54, p = .62), Session ∗ Diagonal ($F_{(24,120)}$ = .63, p = .9), Session ∗ Diagonal ∗ Condition ($F_{(48,120)}$ = .86, p = .72), and Condition ($F_{(2,5)}$ = 3.76, p = .1). A paired samples *t*-test showed a significant difference between CR and NF in session 5 ($t_{(7)}$ = 3.42, p = .01, 95% CI [.26, 2.12]), session 7 ($t_{(7)}$ = 4.11, p = .01, 95% CI [.41, 2.45]), session 10 ($t_{(7)}$ = 2.71, p = .03, 95% CI [.09, 1.79]), session 12 ($t_{(7)}$ = 2.95, p = .02, 95% CI [.15, 1.9]), session 13 ($t_{(7)}$ = 3.15, p = .02, 95% CI [.19, 1.99]), session 14 ($t_{(7)}$ = 2.53, p = .04, 95% CI [.04, 1.7]), session 15 ($t_{(7)}$ = 7.43, p < .001, 95% CI [1.1, 4.13]), session 21 ($t_{(7)}$ = 4.32, p = .003, 95% CI [.46, 2.55]), session 22 ($t_{(7)}$ = 2.92, p = .02, 95% CI [.14, 1.88]), and session 24 ($t_{(7)}$ = 2.53, p = .04, 95% CI [.04, 1.7]).

As regards the experimental conditions in which not all the fish met the learning criterion ≥ 70% towards the Correct corner, that is, Proximal Long (1/4 fish), Distal Short (3/4 fish), and Distal Long (4/4 fish), the use of the geometrically correct diagonal often emerged over the twenty-five sessions of training and reached a steady peak from session 12 to 15.

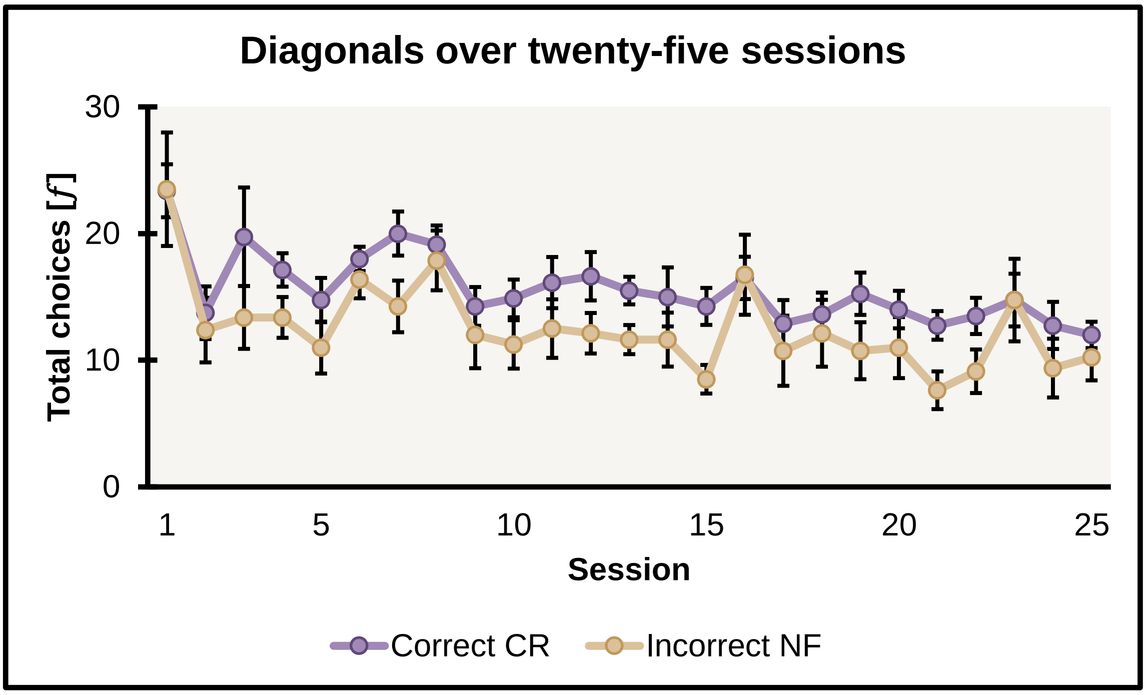


Figure 33. Diagonals by conditions (PL, DS, DL) over the twenty-five sessions of training. The chart considers only the three experimental conditions in which not all the fish met the learning criterion ≥ 70%. Mean ± SEM are shown.

Diagonals by conditions (PS, PL, DS, DL) separately

We analysed the choices made by fish towards the two diagonals of the arena, depending on the experimental condition, to evaluate the use of geometry (transparent rectangular arena) regardless of the presence of landmark (blue cylinder) in the last two session before learning for successful fish and the last two sessions of training for unsuccessful fish.

Results are shown in Figure 34.

A repeated measures ANOVA was performed by considering the total choices in frequencies [f]. The ANOVA with Session (the last two sessions before learning for PS and PL fish, the last two sessions of training for DS and DL fish) and Diagonal (CR, NF) as within-subject factors, and Condition (PS, PL, DS, DL) as between-subject factor, showed the following results: there was a significant effect of Diagonal ($F_{(1,12)}$ = 19.57, $p < .001$, $\eta_p^2 = .62$) and Condition ($F_{(3,12)} = 5.08$, $p = .02$, $\eta_p^2 = .56$), while there were no significant effects of Session ($F_{(1,12)} = 1.18$, $p = .3$), Session $*$ Condition ($F_{(3,12)} = .63$, $p = .61$), Diagonal $*$ Condition ($F_{(3,12)} = .96$, $p = .45$), Session $*$ Diagonal ($F_{(1,12)} = .06$, $p = .81$), and Session $*$ Diagonal $*$ Condition ($F_{(3,12)} = .64$, $p = .6$).

A paired samples *t*-test was performed to analyse the difference between CR and NF, in the four experimental conditions separately.

In the Proximal Short condition, the *t*-test showed a significant difference between CR and NF in the last session before learning ($t_{(3)} = 3.31$, $p = .05$, 95% CI [.18, 9.32]), but not in the second last ($t_{(3)} = .44$, $p = .69$). In the Proximal Long condition, the *t*-test showed a significant difference between CR and NF both in the second last session before learning ($t_{(3)} = 5.72$, $p = .01$, 95% CI [3.1, 10.9]) and in the last ($t_{(3)} = 3.07$, $p = .05$, 95% CI [−.23, 12.23]). In the Distal Short condition, the *t*-test did not show a significant difference between CR and NF, nor in the second last session of training, the twenty-fourth ($t_{(3)} = 2.61$, $p = .08$), neither in the last, the twenty-fifth ($t_{(3)} = 1.48$, $p = .24$). In the Distal Long condition, the *t*-test did not show a significant difference between CR and NF, nor in the second last session of training, the twenty-fourth ($t_{(3)} = 1.46$, $p = .24$), neither in the last, the twenty-fifth ($t_{(3)} = .71$, $p = .53$).

As regards the experimental conditions in which fish met the learning criterion ≥ 70% towards the Correct corner, that is, Proximal Short (4/4 fish) and Proximal Long (3/4 fish), the use of the geometrically correct diagonal for at least two consecutive sessions of training emerged only in PL fish.

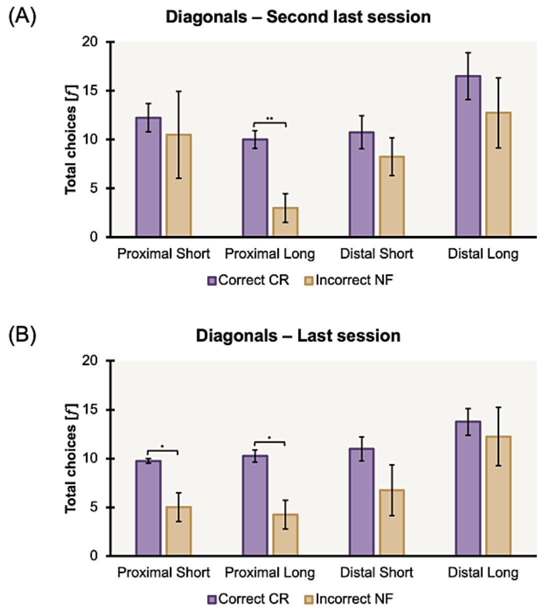


Figure 34. Diagonals by conditions (PS, PL, DS, DL) separately, in the last two sessions before learning for PS and PL, and in the last two sessions of training for DS and DL. Second last session (A), last session (B). Mean ± SEM are shown. Significant *p* values are indicated with asterisks (* $p = .05$; ** $p = .001$; *** $p < .001$).

Diagonals by conditions (PS, PL, DS) collapsed

We analysed the choices made by fish towards the two diagonals of the arena, by collapsing the experimental conditions in which fish learned to integrate geometry (transparent rectangular arena) with landmark (blue cylinder).

Results are shown in Figure 35.

A repeated measures ANOVA was performed by considering the total choices in frequencies $[f]$. The ANOVA with Session (the last two sessions before learning) and Diagonal (CR, NF) as within-subject factors, and Condition (PS, PL, DS) as between-subject factor, showed the following results: there was a significant effect of Diagonal ($F_{(1,5)} = 13.25$, $p = .02$, $\eta_p^2 = .73$), while there were no significant effects of Session

($F_{(1,5)}$ = 4.69, p = .08), Session ∗ Condition ($F_{(2,5)}$ = 1.20, p = .38), Diagonal ∗ Condition ($F_{(2,5)}$ = 1.09, p = .4), Session ∗ Diagonal ($F_{(1,5)}$ = 1.53, p = .27), Session ∗ Diagonal ∗ Condition ($F_{(2,5)}$ = .48, p = .64), and Condition ($F_{(2,5)}$ = 1.06, p = .41).

A paired samples *t*-test was performed to analyse the difference between CR and NF. The *t*-test showed a significant difference between CR and NF in the last session before learning ($t_{(7)}$ = 5.31, p = .001, 95% CI [.67, 3.04]), but not in the second last ($t_{(7)}$ = 1.9, p = .1).

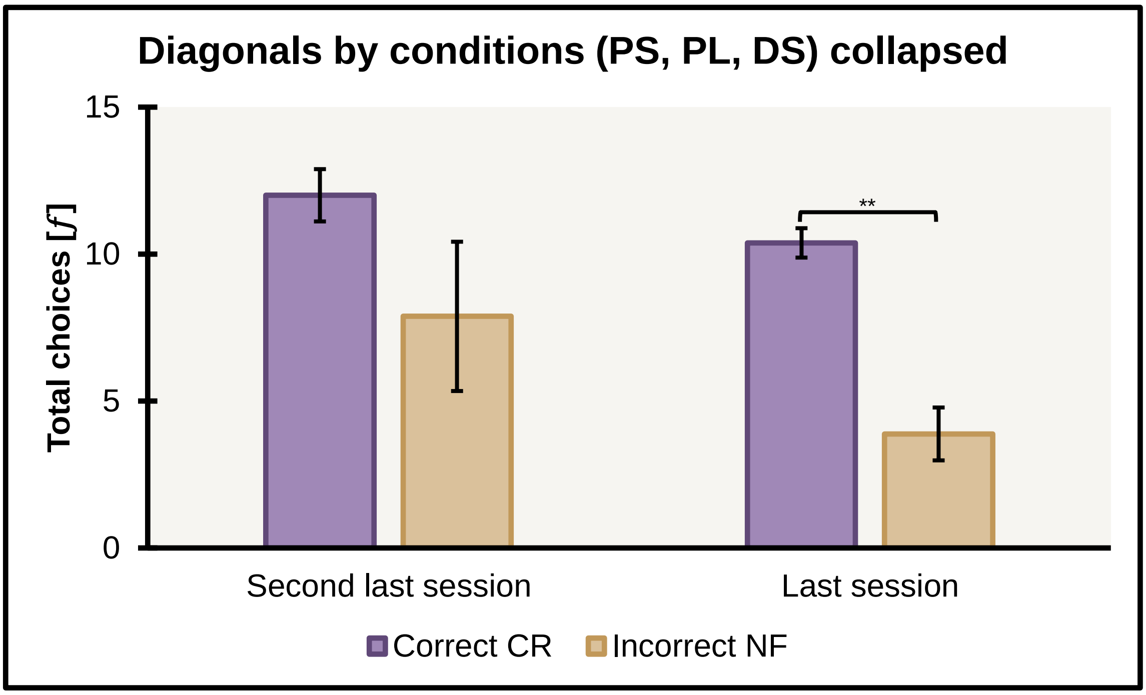


Figure 35. Diagonals by conditions (PS, PL, DS) collapsed, in the last two sessions before learning. Mean ± SEM are shown. Significant p values are indicated with asterisks (* p = .05; ** p = .001; *** p < .001).

Corners by conditions (PS, PL, DS) collapsed

We analysed the choices made by fish towards the four corners of the arena, by collapsing the experimental conditions in which fish learned to integrate geometry (transparent rectangular arena) with landmark (blue cylinder).

Results are shown in Figure 36.

Fish learned to resolve the reorientation tasks in 119.13 ± 20.65 trials (≈ 17 sessions of training). However, the mean number of trials between PS and PL significantly differed (PS: 78.5 ± 25.97; PL: 165.33 ± 19.23; $t_{(5)} = -2.5$, $p = .05$, 95% CI [−3.75, .03]).

A repeated measures ANOVA was performed by considering the total choices in frequencies [f]. The ANOVA with Session (the sessions of learning and validation) and Corner (C, N, R, F) as within-subject factors, and Condition (PS, PL, DS) as between-subject factor, showed the following results: there was a significant effect of Corner ($F_{(3,15)} = 341.73$, $p < .001$, $\eta_p^2 = .99$) and Session ∗ Corner ∗ Condition ($F_{(6,15)} = 4.39$, $p = .01$, $\eta_p^2 = .64$), while there were no significant effects of Session ($F_{(1,5)} = .08$, $p = .8$), Session ∗ Condition ($F_{(2,5)} = .1$, $p = .9$), Corner ∗ Condition ($F_{(6,15)} = .59$, $p = .73$), Session ∗ Corner ($F_{(3,15)} = 2.01$, $p = .16$), and Condition ($F_{(2,5)} = .02$, $p = .98$).

A paired samples *t*-test was performed to analyse the difference among corners (C, N, R, F) in the sessions of learning and validation separately, since they were both needed to support a successful performance. The *t*-test showed a significant difference between C vs. N (learning: $t_{(7)} = 29.93$, p < .001, 95% CI [5.15, 15.79]; validation: 28.04, $p < .001$, 95% CI [4.82, 14.8]), C vs. R (learning: $t_{(7)} = 19.78$, $p < .001$, 95% CI [3.36, 10.64]; validation: $t_{(7)} = 24.73$, $p < .001$, 95% CI [4.23, 13.27]), C vs. F (learning: $t_{(7)} = 21.18$, $p < .001$, 95% CI [3.61, 11.38]; validation: $t_{(7)} = 17.56$, $p < .001$, 95% CI [2.96, 9.46]).

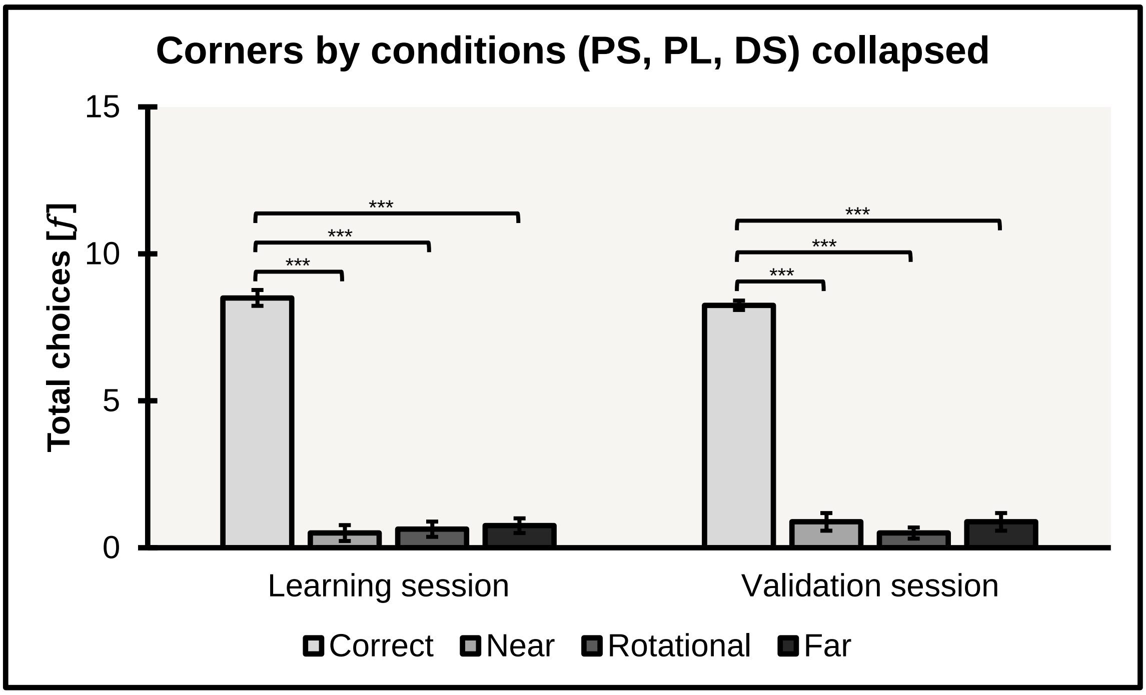


Figure 36. Corners by conditions (PS, PL, DS) collapsed, in the sessions of learning and validation. Mean ± SEM are shown. Significant *p* values are indicated with asterisks (* *p* = .05; ** *p* = .001; *** *p* < .001).

To estimate the learning performance, we considered also the first choices made by fish towards the four corners of the experimental arena. For each fish, the percentage of first choices in both the session of learning and validation is reported in Table 2.

Fish	Condition	% Correct Learning Session	% Correct Validation Session
1	PS	75	75
2	PS	100	87.5
3	PS	75	75
4	PS	75	75
2	PL	100	75
3	PL	66.67	87.5
4	PL	87.5	75
1	DS	87.5	87.5

Table 2. For each fish meeting the learning criterion ≥ 70% towards the Correct corner, the percentage of first choices in both the session of learning and validation is reported. In the learning session, PL fish #3 achieved 66.67% (approaching 70%) but highly improved its performance in the subsequent validation session.

Latency times by conditions (PS, PL, DS, DL) in the first and last session of training

We analysed the time spent by fish within the arena before going out (through a correct choice), in the first and last sessions of training, to evaluate potential improvement in latency times due to the proximity of the landmark to the Correct corner.

Results are shown in Figure 37.

A repeated measures ANOVA was performed by considering the total amount of time [s] that fish spent within the experimental arena before going out. The ANOVA with Session (the first and last session of training) as within-subject factor, and Condition (PS, PL, DS, DL) as between-subject factor, showed the following results: there was a significant effect of Session ($F_{(1,12)} = 109.41$, $p < .001$, $\eta_p^2 = .9$), Session $*$ Condition ($F_{(3,12)} = 11.74$, $p < .001$, $\eta_p^2 = .75$), and Condition ($F_{(3,12)} = 11.07$, $p < .001$, $\eta_p^2 = .74$).

A paired samples *t*-test was performed to analyse the difference between the total amount of time [s] in the first and last session of training, in the four experimental conditions separately.

In the Proximal Short condition, the *t*-test showed a significant difference between sessions ($t_{(3)} = 3.98$, $p = .03$, 95% CI [190.04, 1699.96]). In the Proximal Long condition, the *t*-test did not show a significant difference between sessions ($t_{(3)} = 2.83$, $p = .07$). In the Distal Short condition, the *t*-test showed a significant difference between sessions ($t_{(3)}$ = 12.79, p = .001, 95% CI [2112.52, 3511.48]). In the Distal Long condition, the *t*-test showed a significant difference between sessions ($t_{(3)}$ = 3.97, p = .03, 95% CI [344.87, 3124.13]).

In three out of four experimental conditions (Proximal Short, Distal Short, Distal Long), fish significantly decreased the time spent within the experimental arena before going out. Proximal Long fish did not show this strong tendency.

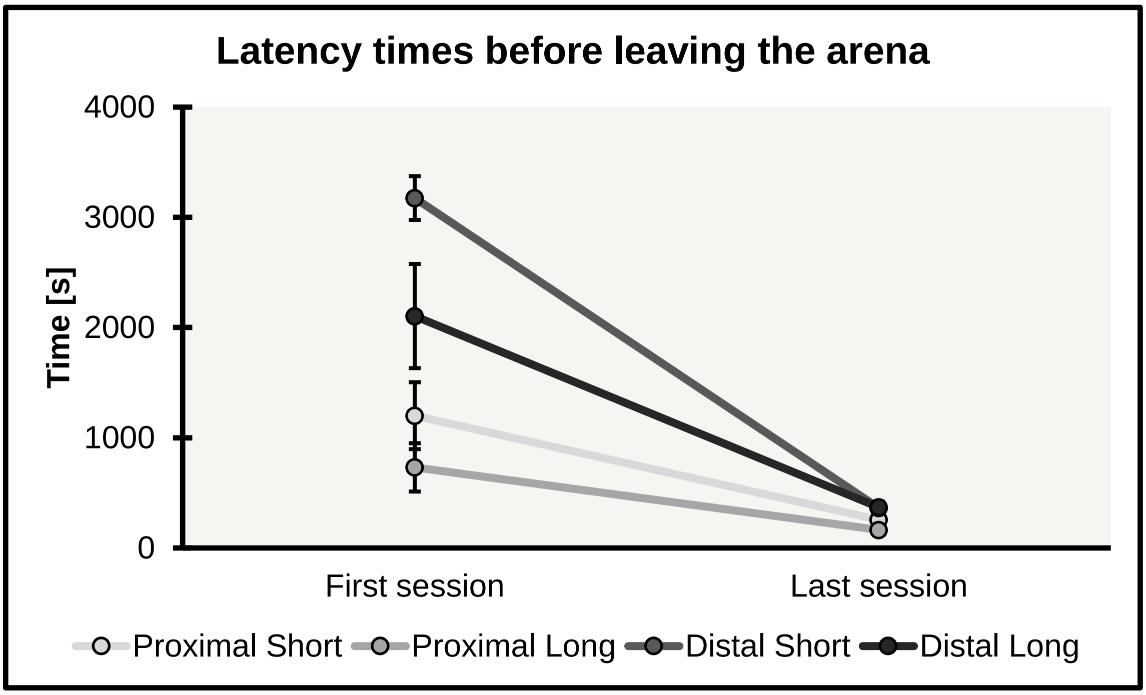


Figure 37. Latency times by conditions (PS, PL, DS, DL) in the first and last session of training. Mean ± SEM are shown.

Motion patterns by conditions (PS, PL, DS, DL) collapsed in the last two sessions of training: motion strategy

We analysed the choices made by fish towards the Correct corner of the arena as regards the strategy of approach (Wall-following vs. Centre-to-corner), to evaluate if consistent motion strategy could come out.

Results are shown in Figure 38A.

A repeated measures ANOVA was performed by considering the total choices towards the Correct corner in frequencies [f]. The ANOVA with Session (the last two sessions of training) and Strategy (Wall-following, Centre-to-corner) as within-subject factors, and Condition (PS, PL, DS, DL) as between-subject factor, showed the following results: there was a significant effect of Strategy ($F_{(1,12)} = 4364.63$, $p < .001$, $\eta_p^2 = 1$), while there were no significant effects of Session ($F_{(1,12)} = 1.15$, $p = .3$), Session $*$ Condition ($F_{(3,12)} = .79$, $p = .53$), Strategy $*$ Condition ($F_{(3,12)} = .39$, $p = .76$), Session $*$ Strategy ($F_{(1,12)} = 2.01$, $p = .18$), Session $*$ Strategy $*$ Condition ($\mathrm{F}_{(3,12)} = .81$, $p = .51$), and Condition ($F_{(3,12)} = .05$, $p = .98$).

Regardless of the experimental condition, all the fish used a wall-following (perimetrical) motion strategy to approach the Correct corner both in the second last and last session of training.

Motion patterns by conditions (PS, PL, DS, DL) collapsed in the last two sessions of training: motion direction

We analysed the choices made by fish towards the Correct corner of the arena as regards the direction of approach (Left vs. Right), to evaluate if consistent motion direction could come out.

Results are shown in Figure 38B.

A repeated measures ANOVA was performed by considering the total choices towards the Correct corner in frequencies [f]. The ANOVA with Session (the last two sessions of training) and Direction (Left, Right) as within-subject factors, and Condition (PS, PL, DS, DL) as between-subject factor, showed the following results: there were no significant effects of Session ($F_{(1,12)} = 1.64$, $p = .23$), Session $*$ Condition ($F_{(3,12)} =$

.79, $p = .52$), Direction ($F_{(1,12)} = 1.93$, $p = .19$), Direction ∗ Condition ($F_{(3,12)} = 1.15$, $p = .37$), Session ∗ Direction ($F_{(1,12)} = .3$, $p = .59$), Session ∗ Direction ∗ Condition ($F_{(3,12)} = .28$, $p = .84$), and Condition ($F_{(3,12)} = .16$, $p = .92$).

Regardless of the experimental condition, all the fish did not exhibit a preferential motion direction to approach the Correct corner both in the second last and last session of training.

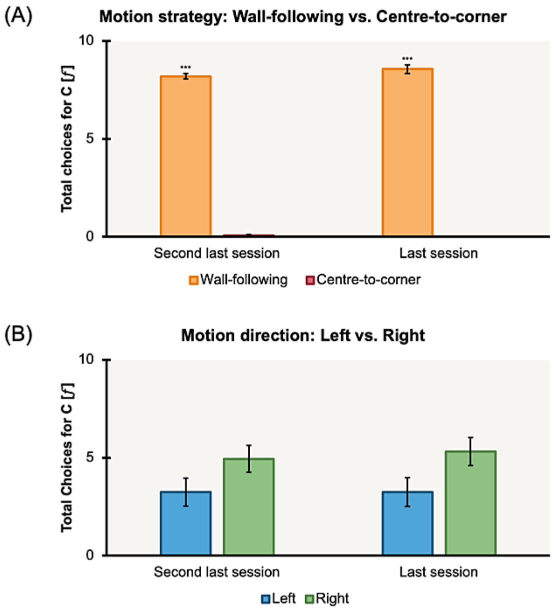


Figure 38. Motion patterns by conditions (PS, PL, DS, DL) in the last two sessions of training. Total choices [*ƒ*] for the Correct corner in relation to the use of motion strategy (A) and motion direction (B). Mean ± SEM are shown. Significant *p* values are indicated with asterisks (* $p = .05$; ** $p = .001$; *** $p < .001$).

3.3.4 Discussion

Study 7 explored reorientation behaviour of zebrafish within a rectangular transparent arena where a conspicuous three-dimensional landmark (a blue cylinder) was located outside. The position of this landmark varied among four experimental conditions: in the Proximal Short (PS), the landmark was placed near the Correct corner on the short side of the arena; in the Proximal Long (PL), the landmark was placed near the Correct corner on the long side of the arena; in the Distal Short (DS), the landmark was placed far from the Correct corner on the short side of the arena; in the Distal Long (DL), the landmark was placed far from the Correct corner on the long side of the arena.

We investigated whether zebrafish could learn to integrate, by means of two sensory pathways, the nonvisual geometry of the arena (two short/long surfaces on the left/right) with the visual landmark (a blue cylinder) to resolve the spatial symmetry and distinguish the Correct corner from the Rotational corner, which shared an equivalent metric-sense relationship. Besides that, we evaluated if the proximity of the landmark to the Correct corner could have affected the integration, also in relation to the length of surfaces (short vs. long). A previous study by Lee and colleagues (2015*a*) showed that untrained zebrafish failed at combining the nonvisual geometry of a rectangular arena with a cylindrical landmark, if observing the spontaneous reorientation behaviour of fish in a social-cued (working) memory task. The present study reproduced two experimental conditions (Exp. 4: Proximal landmark; Exp. 5: Distal landmark) and adapted their methods to a reference memory task in which fish were trained through an operant conditioning procedure (rewarded exit task: Baratti et al., 2020; 2021; Sovrano et al., 2002; 2003; 2005*a,b*; 2007; 2018; 2020*a,b*). The aim was to understand the impact of behavioural tasks – spontaneous choices in working memory vs. training over time in reference memory – on the integrated use of geometry and landmark in zebrafish.

Results showed an increasing range of difficulty at learning, depending on the proximity of the cylindrical landmark to the Correct corner. Half of fish (8/16 distributed as it follows: 4/4 PS; 3/4 PL; 1/4 DS) learned to reorient by combining the transparent arena’s shape with the blue cylinder, while the other half did not meet the learning criterion ≥ 70% towards the Correct corner. However, in three out of four experimental conditions (PS, PL, DS), fish chose the Correct corner more than the incorrect ones (Near, Rotational, Far) over the last four and last two sessions of training, while DL fish did not exhibit this preference. Fish used the cylindrical landmark when proximal to the target as well as when distal but placed on the short side of the rectangular arena.

Since zebrafish, by means of extra-visual sensory channels, learned to reorient within a rectangular transparent arena and systematically confused the two symmetric corners diagonally placed (Sovrano et al., 2020*b*), we evaluated the use of diagonals over the twenty-five sessions of training for the experimental conditions in which fish did not learn (PL, DS, DL) and in the last two sessions before learning for the experimental conditions in which fish learned (PS, PL, DS). Results showed that unsuccessful fish used the geometrically correct diagonal at least for two consecutive sessions, while successful fish did not. It is possible that somehow the “coding” of the arena’s shape has hindered the use of the landmark, anchoring reorientation behaviour at the geometric level. To better understand if overlapped codes may affect the integration or occur at different time points over an extensive training, further studies could design a pre-training phase in geometry-only conditions followed by a subsequent training phase in the presence of a landmark, also to explore blocking and/or overshadowing effects (Good, 2001; Hayward et al., 2003; Pearce et al., 2001; 2006; Wilson & Alexander, 2008).

An interesting difference arose between PS and PL conditions, if considering the mean number of trials to learn and the latency times before going out from the experimental arena: PL fish needed a higher number of trials but were faster, not showing a significant decrease in the time spent within the arena. Additionally, PL fish used the geometrically correct diagonal at least for two consecutive sessions of training (the second last and last before learning). An interpretation relates to the absolute distance, from the centre to the landmark, which was minor for PL fish rather than PS fish (Tommasi & Vallortigara, 2000). Even though the cylindrical landmark was proximal to the target corner in both the experimental conditions, when placed on the short side of the arena it was far from the centre (i.e., the starting position of the fish), while when placed on the long side it was near the centre. If reorientation behaviour of PL fish was partially driven by ego-centred mechanisms, we can hazard the hypothesis that the recruitment of different processes could have delayed long-term learning (i.e., more training trials needed) but made more effective the single response within a single trial (i.e., less time to do a choice).

Relating to a possible involvement of motion strategies (Wall-following vs. Centre-to-corner) and motion directions (Left vs. Right) to approach the Correct corner, results showed that at the population level fish exhibited the tendency to swim close to the physical perimeter of the arena and that such a behaviour was not lateralized, regardless of the experimental condition. This evidence adds theoretical complexity to previous findings in other species (insects: Wystrach, 2009; Wystrach & Beugnon, 2009; chickens: Pecchia & Vallortigara, 2010*a,b*; 2012; evolved agents: Dawson et al., 2010; Miglino & Lund, 2001; Ponticorvo & Miglino, 2010), but does not disclose the nature of processing beneath (e.g., sight-dependent mechanisms, geocentric representations grounded on other sensory systems). As such, the amount of evidence from spatial reorientation studies converges on what animals can do but not on how. Currently, it is unclear whether disoriented navigators mould representations of geometrical and featural cues through view-matching processes (global: Wystrach, 2009; local: Pecchia & Vallortigara, 2012), general associative rules (Dawson et al., 2010), motor action sequences (Miglino et al., 2009), or modular codes (Lee, 2017). Beside this, reorientation behaviour also occurs without visual coding (Gianni et al., 2018; Sovrano et al., 2018*a*; 2020*b*), starting from which the role of other sensory channels, such as hearing and haptics, should be critically addressed.

In general, these results highlight the impact of behavioural tasks – spontaneous choices in working memory vs. training over time in reference memory – on reorientation abilities of zebrafish. In contrast to Lee and colleagues (2015*a*), trained fish were able to combine the nonvisual geometry of the rectangular arena with the cylindrical blue landmark, most of all when proximal to the Correct corner, and to overcome the attractiveness-bias towards the Near corner. Evidence about the role of behavioural procedures on reorientation have suggested that training protocols aid fish in better representing spatial relationships within geometric layouts (visual: Baratti et al., 2021; nonvisual: Sovrano et al., 2020*b*), as well as in efficiently using conspicuous and local landmarks to identify particular locations (Sovrano et al., 2020*a*; Study 1, pp. 29-40; Study 2, pp. 40-60). In appetitive contexts where motivational states acquire an increment over time, food and companions may trigger cognitive adaptations to face high level demands in zebrafish, which is sensitive to this kind of rewards (Daggett et al., 2019; Kalueff et al., 2014; Sison & Gerlai, 2010).

Zebrafish has proven to possess notable spatial precision and memory in geometric reorientation tasks (Baratti et al., 2020; 2021; Lee et al., 2015*a*; 2013; 2012; Sovrano et al., 2020*b*), if compared to other vertebrates (Lee, 2017; Tommasi et al., 2012), together with a natural disposition for spatial learning in visual and nonvisual geometric frameworks. The increasingly spread of this species in behavioural neuroscience (Grunwald & Eisen, 2002; Levin & Cerutti, 2009; Luchiari et al., 2021), despite a lack of protocols to test its cognitive phenotypes (Bailey et al., 2015; Gerlai, 2011; Meshalkina et al., 2017), led us to choose zebrafish as a challenge to further explore what computations underlie spatial reorientation behaviour and boundary mapping.

Chapter 4: Extra-visual Sensory Systems and Motion Patterns

4.1 Study 8: Lateral line pharmacological ablation in zebrafish

4.1.1 Introduction

The capacity to reorient with environmental geometries has been widely explored within rectangular enclosures composed of visible surfaces, white or black, thus suggesting view-based processing of spatial information during reorientation behaviour, also supported by evidence in insects (Wystrach, 2009; Wystrach & Beugnon, 2009; Wystrach et al., 2011*a,b*) and birds (Pecchia & Vallortigara, 2010*a,b*; 2012). But recently, it has been shown that geometric spatial reorientation can occur even in situations of non-visibility. Children reorient within a rectangular transparent room (Gianni et al., 2018); eyed fishes do the same (Study 5, pp. 77-90; Sovrano et al., 2020*b*), and even blind fish (the cavefish *Astyanax mexicanus* and *Phreatichthys andruzzii*) resolve the geometric reorientation task in the absence of sight (Sovrano et al., 2018*a*).

Starting from the study by Sovrano and colleagues (2020*b*), zebrafish is one of the three eyed species that learned to reorient in the absence of visible surfaces, that is, within a rectangular transparent arena. It has been advanced the idea that the lateral line (LL) could have played a crucial part in reorientation behaviour, as an alternative sensory system than sight.

The lateral line is a unique system of aquatic organisms that, through hair cells called “neuromasts”, detects hydrodynamic stimuli when the fish is moving around and contribute to spatial knowledge (Bisazza et al., 2014; Bleckmann, 1994; Bleckmann & Zelick, 2009; Burt de Perera, 2004; Burt de Perera et al., 2005; Holdbrook & Burt de

Perera, 2009; Sguanci et al., 2010; Sutherland et al., 2009; Teyke, 1989; Teyke & Schaerer, 1994; Windsor et al., 2010; 2008). This system is placed on the body of aquatic organisms (fish, amphibians, cyclostomes) and consists of an anterior head canal system (ALLs) and a posterior trunk canal system (PLLs), in which the projections of the anterior (NLLa) and the posterior (NLLp) nerves are topographically organised upon the posterior lateral line lobe (Maler et al., 1973; Mirjany & Faber, 2011). The lateral line is considered the precursor of the vertebrates' inner ear (van Bergeijk, 1967).

In the past years, researchers from several domains started going into the LL neurobiology, also by analysing the ototoxic effect of pharmacological drugs on neuromasts. Antibiotics, antineoplastics, diuretics, and industrial solvents are somehow associated with vestibulo-cochlear disfunctions with potential hearing loss and/or vestibular damages after treatments in humans (Forge & Schacht, 2000). Similar effects have been found both in blind and eyed fishes, showing that the LL could be reversibly damaged through the administration of aminoglycoside antibiotics, which led to hair cells loss and behavioural alterations (Blaxter & Fuiman, 1989; Harris et al., 2003; Kaus, 1987; Montgomery et al., 1997; 2003; Murakami et al., 2003; Owens et al., 2009; Santos et al., 2006; Song & Popper, 1995; Van Trump et al., 2010).

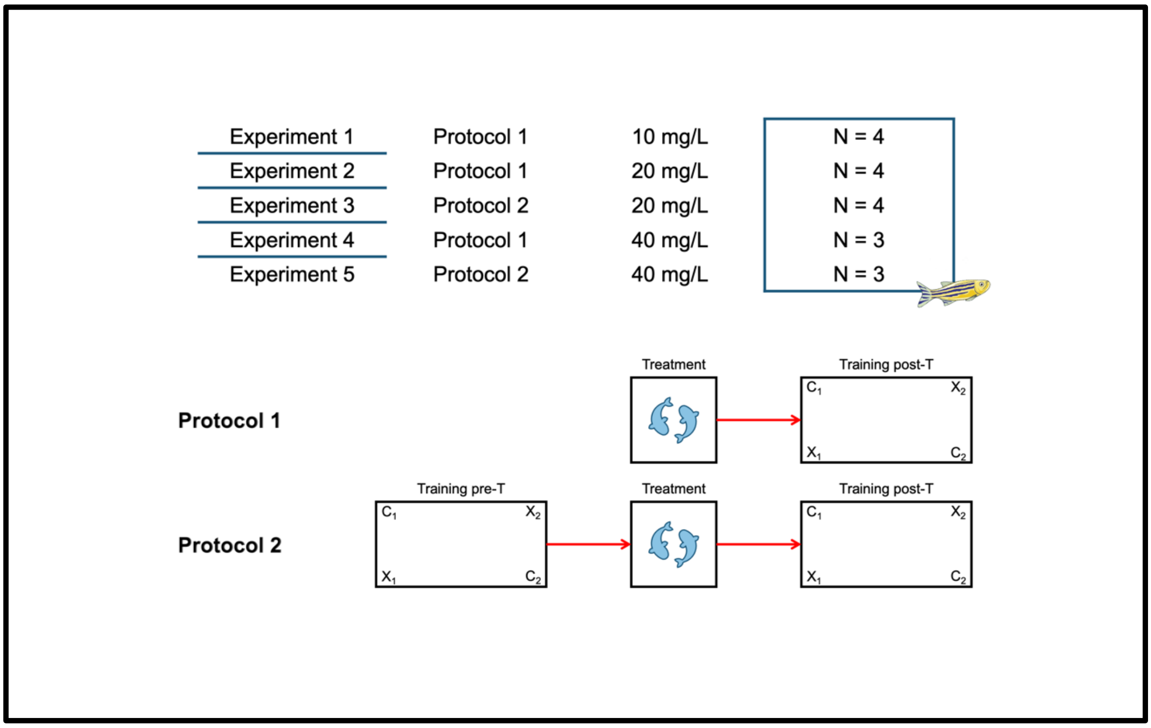
Among aminoglycosides, streptomycin and gentamicin have been used for long (Blaxter & Fuiman, 1989; Kaus, 1987; Montgomery et al., 1997; 2003; Owens et al., 2009; Song & Popper, 1995), although there is debate about their range of action (i.e., if they induce targeted effects on superficial and/or canal neuromasts: Van Trump et al., 2010) and their applicability for behavioural investigations (Brown et al., 2011). Compared to other aminoglycosides, gentamicin sulfate has been reported to induce a lower degree of ototoxicity (Song et al., 1995; Van Trump et al., 2010), together with the possibility to use protective substances to prevent irreversible damages (Ou et al., 2010). Gentamicin is a common broad-spectrum antibiotic for human purposes for the treatment of gram-negative bacteria *Pseudomonas aeruginosa*, gram-positive *Staphylococcus aureus*, and some mycoplasmas (Mingeot-Leclercq et al., 1999; Mingeot-Leclercq & Tulkens, 1999).

Study 8 aimed at exploring whether a LL pharmacological ablation through gentamicin sulfate affected reorientation behaviour of zebrafish, trying to verify if the LL is critically recruited in geometric mapping over time, as suggested by Sovrano and colleagues (2018*a*; 2020*b*; Study 5, pp. 77-90). As done with tortoises (Study 3, pp. 60-70; Study 4, pp. 70-76), zebrafish motion patters at reorienting were carefully explored, also through control experiments.

4.1.2 Materials and Methods

Subjects were 34 adult male zebrafish (wild type), ranging from 3 to 5 cm in body-length. 18 fish took part in Experiments 1-5, while 16 fish took part in Control Experiments 1-4. Housing conditions were the same as described above (p. 31).

Two protocols were designed: Protocol 1 consisted of two phases (treatment, behavioural observation after treatment); Protocol 2 consisted of three phases (behavioural observation before treatment, treatment, behavioural observations after treatment). Five experiments were scheduled, by handling the type of protocol (1 or 2) and the dosage of gentamicin (10, 20, or 40 mg/L) (Figure 39). Fish were subjected to a chronic exposure to gentamicin sulfate (NB-42-01363-10g; Neo Biotech Co., Ltd.), which begins 48 hrs before the training, on Friday morning. During the training, from Monday to Friday, fish were daily exposed to the drug that was refreshed every 24 hrs, after the training session. As regards the behavioural observation, fish were trained to reorient by performing the rewarded exit task over 10 sessions as usual but running two sessions per day with an intersession interval (ISI) of 3 hrs, to reduce the period of pharmacological exposure. As for all the previous experiments (Study 1-7), fish were required to meet an accuracy threshold ≥ 70% per two subsequent sessions of training (learning and validation). Fish that did not achieve the criterion post-treatment were left in their home tanks for 25 days with no drug (washout) and then trained again for 10 sessions, after the recovery of LL neuromasts (Harris et al., 2003; Song et al., 1995).



Experiment 1	Protocol 1	10 mg/L	N = 4
Experiment 2	Protocol 1	20 mg/L	N = 4
Experiment 3	Protocol 2	20 mg/L	N = 4
Experiment 4	Protocol 1	40 mg/L	N = 3
Experiment 5	Protocol 2	40 mg/L	N = 3

Figure 39. Schematic representation of the experimental design.

The following variables were measured: the mean number of trials $[f]$ to meet the learning criterion ≥ 70%; the first and total choices [proportion] towards each diagonal (geometrically Correct $C_{1,2}$ vs. Incorrect $X_{1,2}$) in the sessions of learning and validation; the first and total choices $[f]$ for the correct $C_{1,2}$ in relation to motion strategy (Wall-following vs. Centre-to-corner) and motion direction (Left vs. Right), as proportion indexes: [(Wall-following) / (Wall-following + Centre-to-corner)]; [(Left) / (Left + Right)]. Values higher than .50 indicated a preference for the Wall-following motion strategy and Left motion direction; conversely, values lower than .50 indicated a preference for the Centre-to-corner motion strategy and Right motion direction.

For each experiment, a repeated measures ANOVA was performed to evaluate, in the last two sessions of training (Learning, Validation), the use of: 1. spatial geometry (Correct $C_{1,2}$ vs. Incorrect $X_{1,2}$); motion strategy (Wall-following vs. Centre-to-corner); motion direction (Left vs. Right). As regards Protocol 2, all the variables were analysed before and after treatment. A repeated measures ANOVA was then performed after collapsing the five experiments and considering only the fish that met the learning criterion (16/18), to evaluate the same variables as above, but in relation to the protocol (1 or 2) and dosage (10, 20, or 40 mg/L). For clarity, all the statistic tests applied are reported as a supplementary material in a summarising table ("Supplementary Material" section, pp. 214-222, Suppl_1).

Although previous studies have already shown LL hair cells loss after exposing zebrafish to 0.001% and 0.002% concentration of gentamicin sulfate (Song et al., 1995; Van Trump et al., 2010), a staining procedure of mitochondria with DASPEI (2-[4-(Dimethylamino)styryl]-1-ethylpyridinium iodide; Sigma) was performed to verify that the treatment developed for the present study had ablated the LL of zebrafish properly. DASPEI is a nonspecific fluorescent vital dye to label hair cell mitochondria, thus, which can be used to visualise the loss of neuromasts due to a pharmacological ablation. 8 naïve fish (CTRL, N = 4; 10 mg/L, N = 2; 40 mg/L, N = 2) were stained in 0.005% DASPEI solution for 30 min following the 48 hrs gentamicin or sham exposure. After the incubation in DASPEI medium, fish were rinsed three times (4 min each) in fresh water, anesthetized with 0.001% tricaine methanesulfonate (MS-222; Sigma) for 5 min, and observed under a stereo microscope (SteREO Discovery.V8; Filter Set 38 HE (489038-9901-000); Carl Zeiss, GmbH). The images were acquired through a mobile stand-alone colour microscope camera (ZEISS Axiocam ERc 5s). To reduce at minimum the number of fish subjected to the staining procedure, and according to ethical statements, the intermediate dosage of 20 mg/L was not evaluated.

At last, four control experiments were run to specially explore the use of motion patterns within visible geometric layouts, drawing a comparison between goal-oriented behaviour in situations of transparency and visibility. The rewarded exit task was performed within a rectangular white arena (Ctrl Exp. 1; N = 4) as in Baratti and colleagues (2020), and by reproducing three experiments of Study 6 (pp. 90-100; see also Baratti et al., 2021), that is, use of boundary distance (Ctrl Exp. 2; N = 4), use of freestanding corners (Ctrl Exp. 3; N = 4), and use of boundary length (Ctrl Exp. 4; N = 4).

The proportion of total choices for the geometrically correct corners $C_{1,2}$ was analysed in relation to motion patterns as above, after collapsing the sessions of learning and validation.

4.1.3 Results

Experiment 1: protocol 1, 10 mg/L gentamicin

A repeated measures ANOVA was performed by considering the proportion of total choices. Results are shown in Figure 40. The ANOVA with Geometry (Correct $C_{1,2}$, Incorrect $X_{1,2}$) and Session (Learning, Validation) as within-subject factors showed the following results: there was a significant effect of Geometry ($F_{(1,3)} = 2234.18$, $p < .001$,

η_p^2 = .99), while there were no significant effects of Session ($F_{(1,3)}$ = 2.665e-13, p = 1) and Geometry × Session ($F_{(1,3)}$ = .07, p = .81).

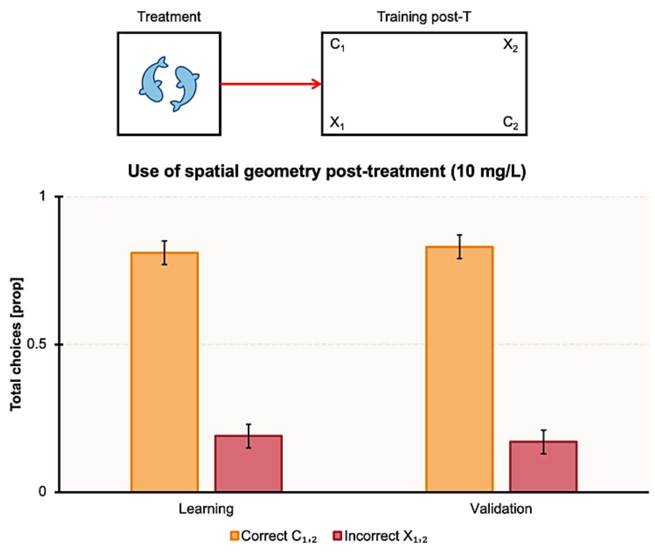


Figure 40. Results of Experiment 1. Total choices [proportion] for the geometrically Correct $C_{1,2}$ vs. Incorrect $X_{1,2}$ corners in the sessions of learning and validation, after 10 mg/L gentamicin administration. Mean ± SEM are shown.

After the treatment, 4/4 fish exposed to 10 mg/L gentamicin learned to reorient within the rectangular transparent arena, choosing more the geometrically correct corners. Fish met the accuracy threshold ≥ 70% in 63.75 ± 5.57 trials (≈ 9 training sessions).

Experiment 2: protocol 1, 20 mg/L gentamicin

A repeated measures ANOVA was performed by considering the proportion of total choices. Results are shown in Figure 41. The ANOVA with Geometry (Correct $C_{1,2}$, Incorrect $X_{1,2}$) and Session (Learning, Validation) as within-subject factors showed the following results: there was a significant effect of Geometry ($F_{(1,2)}$ = 33.05, p = .03, η_p^2 = .94), while there were no significant effects of Session ($F_{(1,2)}$ = −1.705e-13, p = 1) and Geometry × Session ($F_{(1,2)}$ = .2, p = .70).

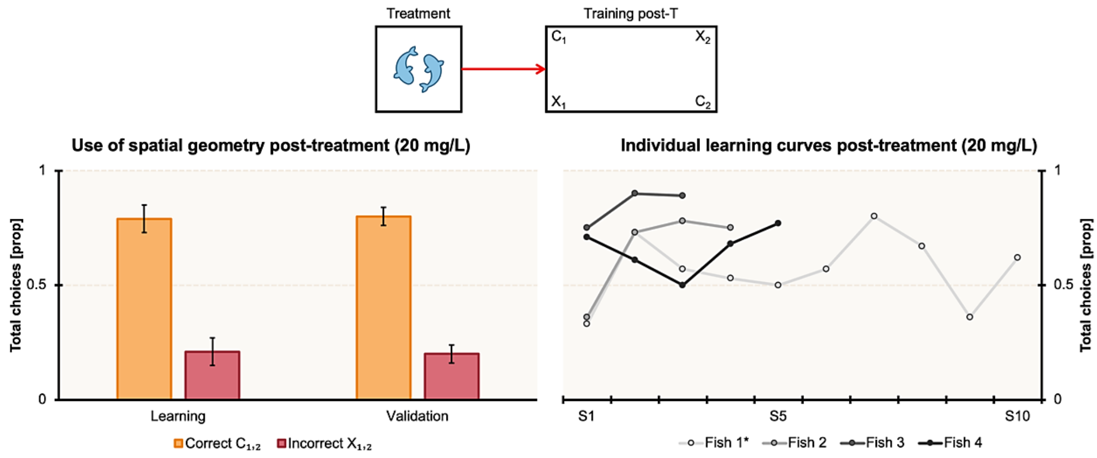


Figure 41. Results of Experiment 2. On the left, mean performance of fish that learned: total choices [proportion] for the geometrically Correct $C_{1,2}$ vs. Incorrect $X_{1,2}$ corners in the sessions of learning and validation, after 20 mg/L gentamicin administration. Mean ± SEM are shown. On the right, learning curves for each fish: total choices [proportion] for the geometrically Correct $C_{1,2}$ corners over 10 training sessions. After the treatment, 1/4 fish did not meet the accuracy threshold ≥ 70%.

After the treatment, 3/4 fish exposed to 20 mg/L gentamicin learned to reorient within the rectangular transparent arena, choosing more the geometrically correct corners. Fish met the accuracy threshold ≥ 70% in 24 ± 5.29 trials (≈ 4 training sessions). 25 days post-treatment (washout), 1/4 fish learned in 40 trials (5 training sessions).

Experiment 3: protocol 2, 20 mg/L gentamicin

A repeated measures ANOVA was performed by considering the proportion of total choices. Results are shown in Figure 42. The ANOVA with Treatment (Before, After), Geometry (Correct $C_{1,2}$, Incorrect $X_{1,2}$), and Session (Learning, Validation) as within-subject factors showed the following results: there was a significant effect of Geometry ($F_{(1,2)}$ = 747.9, p = .001, η_p^2 = .99), while there were no significant effects of Treatment ($F_{(1,2)}$ = −1.199e-14, p = 1), Session ($F_{(1,2)}$ = −1.776e-14, p = 1), Treatment × Geometry ($F_{(1,2)}$ = 13.17, p = .07), Treatment × Session ($F_{(1,2)}$ = 2.665e-13, p = 1), Geometry × Session ($F_{(1,2)}$ = 12, p = .07), and Treatment × Geometry × Session ($F_{(1,2)}$ = 1.23, p = .38).

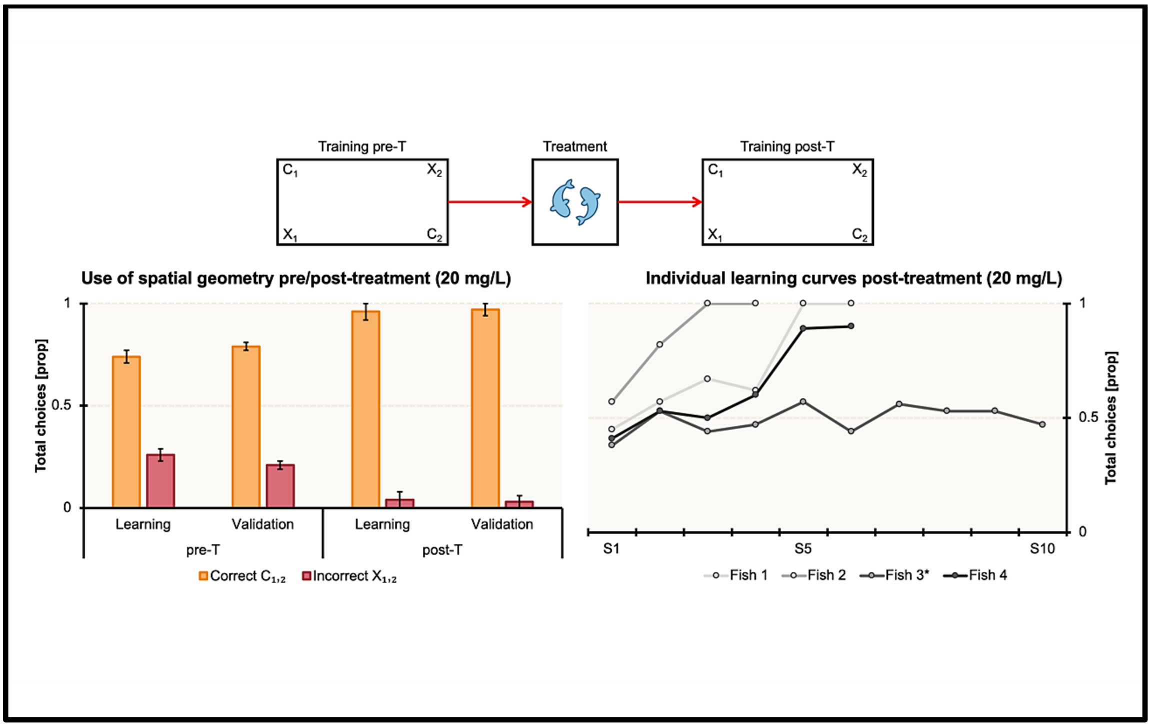


Figure 42. Results of Experiment 3. On the left, mean performance of fish that learned: total choices [proportion] for the geometrically Correct $C_{1,2}$ vs. Incorrect $X_{1,2}$ corners in the sessions of learning and validation, before and after 20 mg/L gentamicin administration. Mean ± SEM are shown. On the right, learning curves for each fish: total choices [proportion] for the geometrically Correct $C_{1,2}$ corners over 10 training sessions. After the treatment, 1/4 fish did not meet the accuracy threshold ≥ 70%.

Before the treatment, 4/4 fish exposed to 20 mg/L gentamicin learned to reorient within the rectangular transparent arena, choosing more the geometrically correct corners. Fish met the accuracy threshold ≥ 70% in 59.5 ± 12.66 trials (≈ 9 training sessions). After the treatment, 3/4 fish learned again, achieving the criterion in 32.67 ± 8.35 trials (≈ 5 training sessions). 25 days post-treatment (washout) 1/4 fish learned in 32 trials (4 training sessions).

Experiment 4: protocol 1, 40 mg/L gentamicin

A repeated measures ANOVA was performed by considering the proportion of total choices. Results are shown in Figure 43. The ANOVA with Geometry (Correct $C_{1,2}$, Incorrect $X_{1,2}$) and Session (Learning, Validation) as within-subject factors showed the following results: there was a significant effect of Geometry ($F_{(1,2)} = 91$, $p = .01$, $\eta_p^2 = .98$), while there were no significant effects of Session ($F_{(1,2)} = -1.705e\text{-}13$, $p = 1$) and Geometry × Session ($F_{(1,2)} = 1$, $p = .42$).

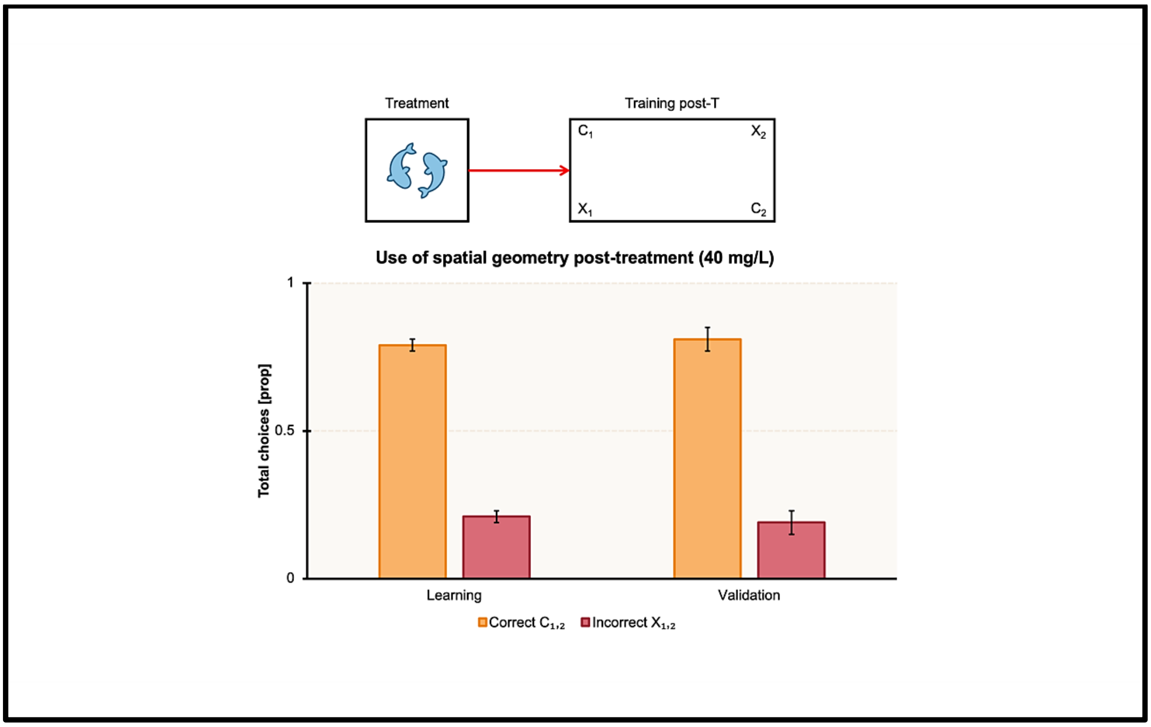


Figure 43. Results of Experiment 4. Total choices [proportion] for the geometrically Correct $C_{1,2}$ vs. Incorrect $X_{1,2}$ corners in the sessions of learning and validation, after 40 mg/L gentamicin administration. Mean ± SEM are shown.

After the treatment, 3/3 fish exposed to 40 mg/L gentamicin learned to reorient within the rectangular transparent arena, choosing more the geometrically correct corners. Fish met the accuracy threshold ≥ 70% in 39.67 ± .33 trials (≈ 5 training sessions).

Experiment 5: protocol 2, 40 mg/L gentamicin

A repeated measures ANOVA was performed by considering the proportion of total choices. Results are shown in Figure 44. The ANOVA with Treatment (Before, After), Geometry (Correct $C_{1,2}$, Incorrect $X_{1,2}$), and Session (Learning, Validation) as within-subject factors showed the following results: there was a significant effect of Geometry ($F_{(1,2)} = 157.76$, $p = .01$, $\eta_p^2 = .99$), while there were no significant effects of Treatment

($F_{(1,2)}$ = −4.737e-15, p = 1), Session ($F_{(1,2)}$ = 2.132e-13, p = 1), Treatment × Geometry ($F_{(1,2)}$ = .18, p = .71), Treatment × Session ($F_{(1,2)}$ = −1.279e-13, p = 1), Geometry × Session ($F_{(1,2)}$ = 9.44, p = .09), and Treatment × Geometry × Session ($F_{(1,2)}$ = .73, p = .48).

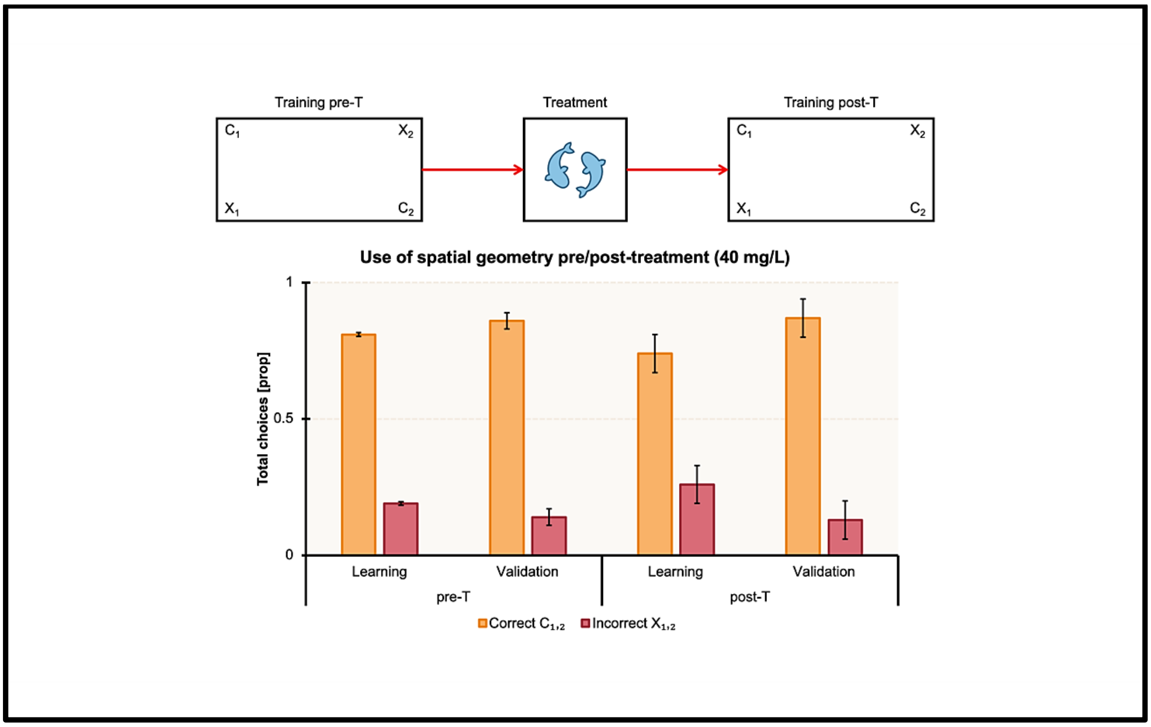


Figure 44. Results of Experiment 5. Total choices [proportion] for the geometrically Correct $C_{1,2}$ vs. Incorrect $X_{1,2}$ corners in the sessions of learning and validation, before and after 40 mg/L gentamicin administration. Mean ± SEM are shown.

Before and after the treatment, 3/3 fish exposed to 40 mg/L gentamicin learned to reorient within the rectangular transparent arena, choosing more the geometrically correct corners. Fish met the accuracy threshold ≥ 70% in 76 ± 16.17 trials (≈ 12 training sessions) before, and 64 ± 24.44 trials (≈ 11 training sessions) after[10].

---

[10] The number of training sessions exceeds the maximum of 10 because one fish took 108 and 96 trials, pre- and post-treatment, respectively. Its performance was around .65 during the tenth session, so it was provided another couple of sessions to meet the criterion entirely.

DASPEI Imaging

Neuromasts typically exhibit a stereotypical arrangement: they can be placed on the skin (“superficial” neuromasts, laid out in groups on the head, trunk, and caudal fin) or enclosed within fluid-filled canals beneath the scales (“canal” neuromasts, laid out in networks on the head and individually on the trunk: Bleckmann, 1994; Bleckmann & Zelick, 2009).

The evaluation of the LL neuromasts left after the pharmacological treatment was done through a qualitative assessment of the photographs acquired under the stereo microscope. Figure 45 provides a summarizing output panel where different sections, from the head to the trunk of zebrafish, were labelled, in ctrl, 10, and 40 mg/L treated fish. The qualitative analysis showed that neuromasts were clearly visible and vital in the LL of ctrl fish (around the eye, on the operculum, and along the trunk where they were arranged in a stereotypical pattern of vertical stripes); at 10 mg/L, a few cells were still present (e.g., on the operculum); at 40 mg/L no cells survived. The imaging revealed that the LL of ctrl fish was intact, at 10 mg/L gentamicin the LL was heavily impaired, whereas at 40 mg/L gentamicin the LL was entirely ablated.

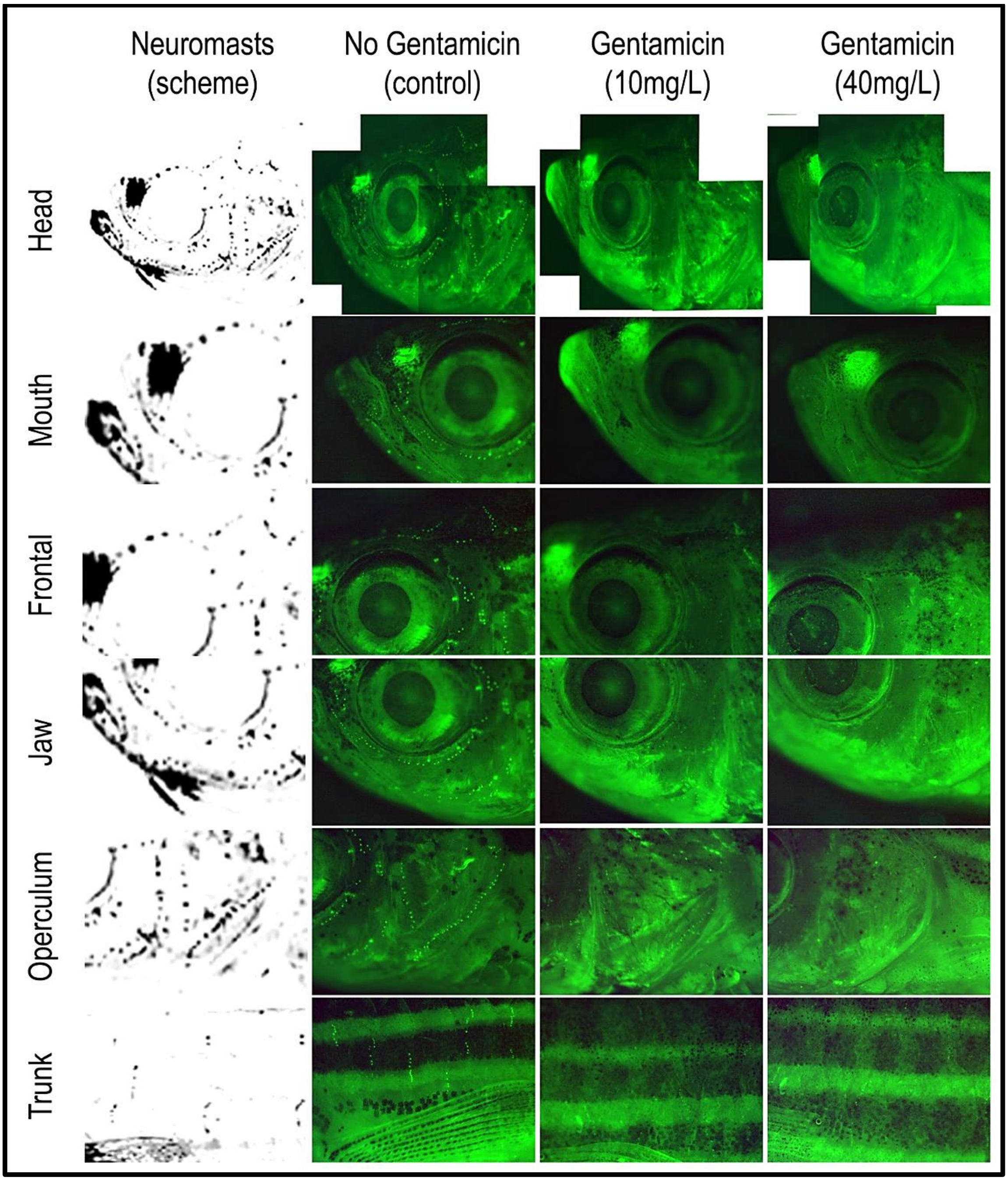


Figure 45. DASPEI labelling of the zebrafish LL in untreated (ctrl, no gentamicin) and treated (10, 40 mg/L gentamicin) groups. The photographs are lateral views of the head, mouth, frontal region, jaw, operculum, and trunk. A scheme of neuromasts depicting their averaged organization is also provided (left).

The staining procedure with DASPEI supported the effectiveness of the treatment and suggested that the LL was not crucially involved for reorientation behaviour to perceive the nonvisual spatial geometry.

Use of spatial geometry and motion patterns after treatment in the five experiments collapsed

A repeated measures ANOVA was performed to evaluate the use of spatial geometry, by considering the proportion of total choices. The ANOVA with Geometry (Correct $C_{1,2}$, Incorrect $X_{1,2}$) and Session (Learning, Validation) as within-subject factors, and Protocol (1, 2) as between-subject factor, showed the following results: there was a significant effect of Geometry ($F_{(1,14)} = 272.46$, $p < .001$, $\eta_p^2 = .95$), while there were no significant effects of Geometry × Protocol ($F_{(1,14)} = 3.35$, $p = .09$), Session ($F_{(1,14)} = 3.808\text{e-}13$, $p = 1$), Session × Protocol ($F_{(1,14)} = 0$, $p = 1$), Geometry × Session ($F_{(1,14)} = 2.98$, $p = .11$), Geometry × Session × Protocol ($F_{(1,14)} = .89$, $p = .36$), and Protocol ($F_{(1,14)} = 9.364\text{e-}14$, $p = 1$). The ANOVA with Geometry (Correct $C_{1,2}$, Incorrect $X_{1,2}$) and Session (Learning, Validation) as within-subject factors, and Dosage (10, 20, 40 mg/L) as between-subject factor, showed the following results: there was a significant effect of Geometry ($F_{(1,13)} = 235.7$, $p < .001$, $\eta_p^2 = .95$), while there were no significant effects of Geometry × Dosage ($F_{(2,13)} = 1.3$, $p = .31$), Session ($F_{(1,13)} = -2.375\text{e-}13$, $p = 1$), Session × Dosage ($F_{(2,13)} = -7.996\text{e-}15$, $p = 1$), Geometry × Session ($F_{(1,13)} = 2$, $p = .18$), Geometry × Session × Dosage ($F_{(2,13)} = .735$, $p = .50$), and Dosage ($F_{(2,13)} = -2.380\text{e-}14$, $p = 1$).

To estimate the learning performance, both the first and total choices made by fish towards the two diagonals of the experimental arena were considered. However, the total choices only were analysed, to be consistent with Study 7 (pp. 100-130). For each fish, the proportion of first choices in both the sessions of learning and validation after the treatment (i.e., those that all the fish have in common) is reported in Table 3, as a supplementary confirmation data.

Fish	Experiment	Proportion Correct Learning Session	Proportion Correct Validation Session
1	1	.75	.88
2	1	.70	.88
3	1	.75	.75
4	1	.88	.75
6	2	.71	.75
7	2	.88	.88
8	2	.1	.75
9	3	.75	1
10	3	.88	1
12	3	.75	.88
13	4	.75	.75
14	4	.75	.80
15	4	.75	.88
16	5	.75	.75
17	5	.75	1
18	5	.75	.75

Table 3. For each fish that met the criterion ≥ 70% towards the geometrically correct diagonal, the proportion of first choices in both the sessions of learning and validation is reported. Fish #5 (Experiment 2) and fish #11 (Experiment 3) did not learn to resolve the geometric reorientation task after the treatment, but they learned after 25 days post-treatment (fish #5: learning = .79; validation = .80; fish #11: learning = .80; validation = .77).

A repeated measures ANOVA was performed to evaluate motion strategies, by considering the proportion of total choices for the Correct $C_{1,2}$. Results are shown in Figure 46A. The ANOVA with Strategy (Wall-following, Centre-to-corner) and Session

(Learning, Validation) as within-subject factors, and Protocol (1, 2) as between-subject factor, showed the following results: there was a significant effect of Strategy ($F_{(1,14)}$ = 185.11, p < .001, η_p^2 = .93), while there were no significant effects of Strategy × Protocol ($F_{(1,14)}$ = 3.71, p = .08), Session ($F_{(1,14)}$ = −2.623e-14, p = 1), Session × Protocol ($F_{(1,14)}$ = −2.914e-15, p = 1), Strategy × Session ($F_{(1,14)}$ = .75, p = .4), Strategy × Session × Protocol ($F_{(1,14)}$ = 1.58, p = .23), and Protocol ($F_{(1,14)}$ = −1.457e-15, p = 1). The ANOVA with Strategy (Wall-following, Centre-to-corner) and Session (Learning, Validation) as within-subject factors, and Dosage (10, 20, 40 mg/L) as between-subject factor, showed the following results: there was a significant effect of Strategy ($F_{(1,13)}$ = 154.06, p < .001, η_p^2 = .92), while there were no significant effects of Strategy × Dosage ($F_{(2,13)}$ = .24, p = .79), Session ($F_{(1,13)}$ = −3.340e-14, p = 1), Session × Dosage ($F_{(2,13)}$ = −1.367e-16, p = 1), Strategy × Session ($F_{(1,13)}$ = .04, p = .85), Strategy × Session × Dosage ($F_{(2,13)}$ = 2.17, p = .15), and Dosage ($F_{(2,13)}$ = 1.911e-15, p = 1).

A repeated measures ANOVA was performed to evaluate motion directions, by considering the proportion of total choices for the Correct $C_{1,2}$. Results are shown in Figure 46B. The ANOVA with Direction (Left, Right) and Session (Learning, Validation) as within-subject factors, and Protocol (1, 2) as between-subject factor, showed the following results: there were no significant effects of Direction ($F_{(1,14)}$ = .89, p = .36), Direction × Protocol ($F_{(1,14)}$ = .58, p = .46), Session ($F_{(1,14)}$ = 3.886e-15, p = 1), Session × Protocol ($F_{(1,14)}$ = 2.186e-15, p = 1), Direction × Session ($F_{(1,14)}$ = .08, p = .79), Direction × Session × Protocol ($F_{(1,14)}$ = .98, p = .34), and Protocol ($F_{(1,14)}$ = −2.231e-15, p = 1). The ANOVA with Direction (Left, Right) and Session (Learning, Validation) as within-subject factors, and Dosage (10, 20, 40 mg/L) as between-subject factor, showed the following results: there were no significant effects of Direction ($F_{(1,13)}$ = 2.22, p = .16), Direction × Dosage ($F_{(2,13)}$ = 1.7, p = .22), Session ($F_{(1,13)}$ = −3.181e-15,

$p = 1$), Session × Dosage ($F_{(2,13)} = -7.732\text{e-}17$, $p = 1$), Direction × Session ($F_{(1,13)} = .19$, $p = .67$), Direction × Session × Dosage ($F_{(2,13)} = .14$, $p = .87$), and Dosage ($F_{(2,13)} = -4.398\text{e-}16$, $p = 1$).

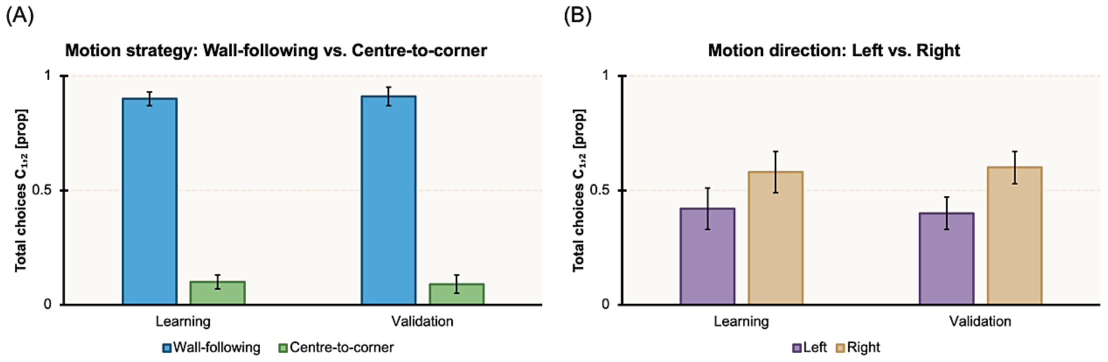


Figure 46. Motion patterns in the five experiments collapsed. Total choices [proportion] for the geometrically Correct $C_{1,2}$ corners in relation to the use of motion strategy (A) and motion direction (B). Mean ± SEM are shown.

All the fish that learned to reorient (16/18) used a consistent motion strategy to approach the geometrically correct corners, displaying a swim behaviour close to the physical perimeter of the transparent arena (i.e., wall-following). Moreover, such a strategy was not lateralized, at least, at the population level.

About individual motion strategy: at learning, 16/16 fish showed a preference for Wall-following (one-sample t-test: $t_{(15)} = 29.56$, $p < .001$, 95% CI [5.10, ∞]); at validation, 15/16 showed a preference for Wall-following (one-sample t-test: $t_{(14)}$ =

32.17, $p < .001$, 95% CI [.5.65, ∞]), while 1/6 no preference. About individual motion direction, which was calculated on wall-following fish: at learning, 6/16 fish showed a preference for Left (one-sample *t*-test: $t_{(5)} = 4.27$, $p < .004$, 95% CI [.59, ∞]), 7/16 fish showed a preference for Right (one-sample *t*-test: $t_{(6)} = 8.47$, $p < .001$, 95% CI [1.53, ∞]), 3/16 neither; at validation, 4/15 showed a preference for Left (one-sample *t*-test: $t_{(3)} = 3.94$, $p < .02$, 95% CI [.39, ∞]), 9/15 fish showed a preference for Right (one-sample *t*-test: $t_{(8)} = 5.72$, $p < .001$, 95% CI [.93, ∞]), 1/15 neither.

Control Experiments: motion patterns in a rectangular white arena, and by teasing apart the geometric cues of distance, corners, and length

Motion patterns of zebrafish during reorientation within a rectangular white arena, where the metric attributes are merged, and within fragmented layouts, where these attributes are present, but singularly, were analysed. The aim was to verify whether the motion patterns remained similar or diverged under the influence of continuous rather than fragmented geometric layouts. In such a case, the sessions of learning and validation were collapsed. Results are shown in Figure 47.

In all the four experiments, fish learned to reorient by geometry (Rectangular white: $C_{1,2}$ vs. $X_{1,2}$: $t_{(3)} = 8.02$, $p = .004$, 95% CI [41.48, 96.09]; Distance: $C_{1,2}$ vs. $X_{1,2}$: $t_{(3)} = 9.79$, $p = .002$, 95% CI [35.66, 70.04]; Corners: $C_{1,2}$ vs. $X_{1,2}$: $t_{(3)} = 11.91$, $p = .001$, 95% CI [44.68, 77.27]; Length: $C_{1,2}$ vs. $X_{1,2}$: $t_{(3)} = 29.56$, $p < .001$, 95% CI [51.59, 64.04]).

Concerning motion strategies, a paired samples *t*-test was performed to analyse the difference between Wall-following and Centre-to-corner to approach the geometrically Correct $C_{1,2}$ corners. Zebrafish showed a preference towards the Wall-following motion strategy within the rectangular white arena ($t_{(3)} = 5.06$, $p = .002$, 95% CI [.37, 4.67]), but not within fragmented layouts (distance: $t_{(3)} = 1.02$, $p = .38$; corners:

$t_{(3)} = -.15$, $p = .89$; length: $t_{(3)} = 1.6$, $p = .21$). Since motion directions relate to the Wall-following strategy, they were analysed for Control Experiment 1 only, revealing any effect of Direction ($t_{(3)} = -.03$, $p = .98$).

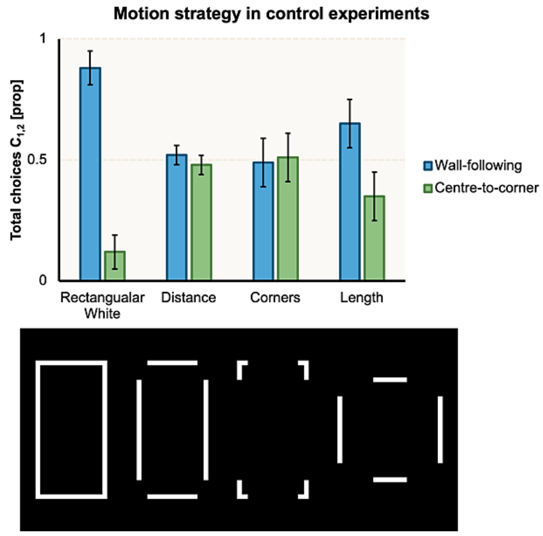


Figure 47. Motion strategy in the four control experiments. Total choices [proportion] for the geometrically Correct $C_{1,2}$ corners in relation to the use of Wall-following vs. Centre-to-corner strategy of motion. Mean ± SEM are shown. A schematic representation of the geometric layouts is provided below the chart.

Results showed that in the presence of white surfaces, zebrafish used the wall-following strategy of motion (as well as in the transparent arena), while in the presence of isolated metric cues, zebrafish did not show this preference and relied on the visual cues to reorient.

4.1.4 Discussion

Study 8 explored whether a pharmacological ablation of the zebrafish lateral line (LL) could affect their capacity to reorient, over training, by the nonvisual spatial geometry provided by a rectangular transparent arena. For the purpose, five experiments were run, by handling the type of protocol (1: training after treatment; 2: training before and after treatment) and the gentamicin sulfate dosage (10, 20, 40 mg/L).

Results showed that almost all the zebrafish (16/18) that were exposed to increasing dosages of gentamicin learned to reorient using the geometric layout of transparent surfaces. These findings are consistent with previous results (Study 5, pp. 77-90; Sovrano et al., 2020*b*), but further reveals that the acquired capacity of nonvisual boundary mapping does not crucially depend on the LL. In support of this, the DASPEI staining have picked out the effect of gentamicin at inducing LL hair cells loss, leading to exclude this system as a crucial extra-visual sensory channel for reorientation.

However, since fish behavioural patterns are the result of heteromodal integration (Rodríguez et al., 2021; Kasumyan, 2003), and functional overlap among sensory systems has been documented (Braun & Coombs, 2000; Higgs & Radford, 2016; Kalmijn, 1989), it is possible that the damaged LL has been counterbalanced by other channels (first, the sense of touch) to ensure reorientation. Secondly, performing a pharmacological ablation instead of other surgical techniques, such as microdissections of the brain structures associated with the LL pathway (i.e., the medial octavolateralis nucleus, the ventrolateral nucleus of torus semicircularis, and the tectum opticum: Bleckmann & Zelick, 2009), may have been enough to inhibit – but not to destroy – the LL functionalities.

Once excluding the LL as the priority channel for boundary mapping of nonvisual surfaces, we wanted to widen the analysis on motion patterns, proper to understand if fish learned to reorient through consistent swim behaviour (e.g., close to the physical perimeter of the arena). Thus, motion strategy (Wall-following vs. Centre-to-corner) and motion direction (Left vs. Right) were analysed in fish that learned to resolve the geometric task, showing a strong preference for wall-fallowing motion, irrespective of left-right direction. However, these findings are consistent with previous results in zebrafish (Study 7, pp. 100-130), where all the fish exhibited the same behavioural tendency, but irrespective of learning.

At that point, we presented fish with the rectangular white arena (where the metric attributes were blended: Baratti et al., 2020) and with fragmented layouts (where one single geometric attribute at a time was present: Study 6, pp. 90-100; Baratti et al., 2021), to control zebrafish motion patterns in the presence of visible surfaces. Interestingly, we observed wall-following motion within the opaque arena only, but not within the three fragmented layouts (boundary distance, freestanding corners, boundary length). One intriguing explanation would be that zebrafish can experience the physical continuity of spatial geometry (visible or not) through haptic/tactile stimulation, while they use sight as a primary system when the spatial geometry is demerged in its components. This view may acquire importance in the light of orientation abilities in blind people, who could develop *ad hoc* strategies to build self- and world-based spatial representations. Two recent studies by Nardi and colleagues (2022; 2020) have shown that blindfolded participants successfully relied on auditory landmark cues to reorient within a circular search space. In blind mice as well, interesting findings have demonstrated that sight was not needed to extract global-shape relationships to reorient. In fact, blind mice behaved as eyed ones within rectangular settings while varying several parameters, showing both higher accuracy when increasing the salience of boundaries, and peculiar trajectories (i.e., patrolling of 3D edges: Normandin et al., 2022).

In blind cavefish, geometric reorientation capacities have been found (Sovrano et al., 2018*a*). Due to their physiology and ecology, the LL of hypogean fish have more neuromasts than eyed fish (Schemmel, 1967; Teyke, 1990; Yoshizawa et al., 2010; 2012), although the number and density of these cells vary also among eyed fish, probably as an adaptation to different environments (Bleckmann & Zelick, 2009; Dijkgraaf, 1963; 1964; 1989; Webb, 1989*a,b*). Behavioural differences associated to LL morphological specificities may be expected and they would be addressed in terms of pharmacological ablation and motion strategies, to widen the knowledge about the (supposed) deterministic role of sight during geometry-based reorientation and its newly observed multisensory interactions.

Chapter 5: General Discussion

The present Thesis aimed at investigating reorientation behaviour of three species of fish (the zebrafish *D. rerio*, the redtail splitfin fish *X. eiseni*, the goldfish *C. auratus*) and one species of reptiles (the Hermann tortoise *T. hermanni*), to extensively dissect three issues: (1) the use of environmental geometry with and without landmarks; (2) the role of two behavioural tasks, one driven by spontaneous behaviour and the other by learning; (3) the involvement of extra-visual sensory systems in the case of visual transparency and/or the implementation of motion patterns. The project has been designed to apply a comparative approach, across species and methodologies, as a chief purpose.

Studies 1-4 examined geometry-based reorientation alone and in conjunction with distinctive landmarks in two animal models of great interest: the zebrafish, since its popularity in the field of behavioural neuroscience (Levin & Cerutti, 2009; Lin et al., 2016; Orger & Polavieja, 2017), and the Hermann tortoise, that is, currently an undiscovered animal whose behavioural and cognitive phenotype is poorly understood. Studies 5-7 examined the potential impact of behavioural tasks on reorientation capacities, where those procedures based on spontaneous behaviour (short term, social-cued memory task) have been compared with those based on learning processes over time (long term, rewarded exit task). Study 5 was run by observing three teleost fishes, while studies 6 and 7 focused on zebrafish. Study 8 examined the (supposed) recruitment of fish lateral line (LL) during reorientation within a geometrically informative layout of transparent surfaces, to test nonvisual “coding” of global-shape parameters. Additionally, motion patterns were evaluated to attempt explaining how animals can reorient in situations of no visual stimulation, such as transparency or blindness. This study started evaluating zebrafish but shows the way forward to the use of cavefish species. An overview of the studies is presented in Table 4.

Study	Species	Scientific question	Major findings
No. 1	*D. rerio*	Integrated use of environmental geometry and blue wall landmark over training (learning behaviour)	• Fish learned to integrate. • Blue wall landmark removal: fish chose in accord with geometry. • Affine transformation: fish chose in accord with geometry and left-right arrangement of landmark.
No. 2	*D. rerio*	Landmark-use with or without the influence of informative environmental geometry over training (learning behaviour)	• Fish learned to use corner panel landmarks under the influence of informative opaque geometry (rectangular arena). • Affine transformation: fish chose in accord with landmark. • Diagonal transposition: fish chose in accord with landmark. • Partial removal of panels: fish chose at random. • Total removal of panels: fish chose in accord with geometry in total choices only. • Fish did not learn to use corner panel landmarks under the influence of uninformative opaque geometry (square opaque arena). • Fish learned to use corner panel landmarks under the influence of uninformative transparent geometry (transparent square arena).

No. 3	*T. hermanni*	Geometry-based reorientation over training (learning behaviour)	• Tortoises learned to use geometry to reorient. • Motion patterns (strategy and direction) found at the individual level. • More time spent in the perimetrical zone of the arena.
No. 4	*T. hermanni*	Integrated use of environmental geometry and blue wall landmark over training (learning behaviour)	• Tortoises learned to combine geometry and landmark (blue wall) to reorient. • Wall-following motion strategy found at the population level; left-right motion direction found at the individual level.
No. 5	*D. rerio*, *X. eiseni*, *C. auratus*	Geometry-based reorientation through nonvisual, i.e., transparent, surfaces (spontaneous vs. learning behaviour)	• Untrained fish did not spontaneously use transparent surfaces to reorient, both in the case of no and short exploratory experience. • Trained fish used transparent surfaces over time to reorient. • Differences among species found in total choices [*f*]. *D. rerio* did more choice attempts over 5 days (Exp. 1-2); *C. auratus* did more choice attempts at geometric test (Exp. 3).
No. 6	*D. rerio*	Use of isolated geometric cues (distance, corners, length) over training (learning behaviour)	• Fish used boundary distance, freestanding corners, and boundary length when provided singularly (i.e., not interconnected into a polygon).

No. 7	*D. rerio*	Integrated use of environmental geometry and 3D cylindrical blue landmark over training (learning behaviour)	• Integration of transparent geometry and 3D landmark depended on proximity and length (increasing range of difficulty: proximal short > proximal long > distal short > distal long). • Residual use of transparent geometry in unsuccessful fish. • Irrespective of learning, wall-following motion strategy found at the population level.
No. 8	*D. rerio*	Geometry-based reorientation through nonvisual, i.e., transparent, surfaces after LL pharmacological ablation (learning behaviour)	• Fish learned to used transparent surfaces (before and) after LL pharmacological ablation through gentamicin antibiotic. • DASPEI mitochondria fluorescent staining showed LL hair cell loss in treated fish. • Wall-following motion strategy found at the population level for both opaque and transparent global shape, but not for isolated attributes (control experiments).

Table 4. Overview of the eight studies and major findings.

Study 1 (*Conspicuous landmark (blue wall) in zebrafish*) showed that zebrafish learned to use the spatial geometry of the rectangular opaque arena in conjunction with a conspicuous blue wall landmark to identify one target location (i.e., the correct corner position) that provided a reward, irrespective of landmark proximity and length. Fish also showed a preference for spatial geometry after removing the blue wall landmark (Test 1), and for spatial geometry and left-right arrangement of landmark, as acquired over training, after affine transformation (Test 2). Results are consistent with previous literature in several other vertebrate species (Tommasi et al., 2012; Vallortigara, 2009), including fish (Lee et al., 2012*c*). Secondarily, the effect of learning processes emerged in overcoming the attractiveness-bias of the landmark when near the correct corner position. This suggests that long-term memory mechanisms can improve the strength of associative rules at integrating different sources of spatial information to allow goal-oriented behaviours.

Study 2 (*Local landmarks (corner panels) in zebrafish*) revealed landmark-use by zebrafish under the influence of (1) informative opaque geometry, i.e., the rectangular white arena; (2) uninformative transparent geometry, i.e., the square transparent arena. In both cases, fish learned to use one local landmark characterised by a distinctive pattern to locate the target location, irrespective of spatial geometry. The preference exhibited for the panel after the affine transformation test puts emphasis on featural cues as a sufficient information for reorienting, even within environments that are defined by their metric. However, fish failed at learning how to reorient under the influence of uninformative opaque geometry (the square white arena), while varying the size of the experimental space (large vs. small). Although this finding seems not consistent (if the landmark is enough, the fish will learn), we could appeal to salience effects (Fernandes et al., 2021; Gatto et al., 2020; Santacà et al., 2021) as a deterministic aspect for zebrafish reorientation. Is this the case of visual landmarks arranged at the corners of a transparent, i.e., nonvisual, arena, where it is likely that they strongly caught the fish attention, ensuring them to reorient properly. As well as in Study 1, also in Study 2 learning processes aided the fish in bypassing the tendency to approach the corner position near the correct (Sovrano et al., 2020*a*), no longer using the landmark as a beacon. Since spontaneous use of corner panel landmarks has not been investigated in zebrafish yet, detailed comparison between these two species cannot be drawn at the moment on this issue.

Study 3 (*Environmental geometry in tortoises*) and Study 4 (*Conspicuous landmark (blue wall) in tortoises*) indicated that Hermann tortoises learned to use the distinctive geometry of the arena alone (i.e., boundary mapping) and in conjunction with the conspicuous cue provided by the blue wall, while varying its proximity in relation to the correct corner. Tortoises not only chose the two symmetric corners with a short/long wall on the left/right, but also one exact target location, that is, the corner with a short/long blue wall on the left/right. In doing so, they displayed a wall-following strategy of motion at the population level and left/right preferential directions at the individual level.

While lateralised behaviours of Hermann tortoises have started being explored a few years ago (social recognition: Sovrano et al., 2018*b*; righting reflex: Stancher et al., 2006), the focus on motion patterns, in terms of exploratory activity within enclosed spaces, has been introduced here for the first time. The rationale beneath that was to understand how (or rather, through what mechanisms) navigators can represent in mind global-shape parameters and featural cues for reorienting. View-matching processes (global: Wystrach, 2009; local: Pecchia & Vallortigara, 2012), general associative rules (Dawson et al., 2010), motor action sequences (Miglino et al., 2009), modular codes (Lee, 2017), or tactile-like stimulation (Gianni et al., 2018; Sovrano et al., 2018*a*; 2020*a*; Study 5 and Study 8) are not mutually exclusive options since each of them can explain one piece of the puzzle.

A special mention should be done to the so-called “wall-following behaviour”. Sometimes called “wall-touching” or “centrophobism” behaviour (Besson & Martin, 2004; Creed & Miller, 1990; Götz & Biesinger, 1985), it consists in exploring a bounded environment staying close to its perimeter. This behaviour has been observed in several animal groups, such as mammals (rodents: Barnett, 1963; Treit & Fundytus, 1988; Simon et al., 1994; blind humans: Hill et al., 1993; Kallai et al., 2007; 2005), blind fish (Abdel-Latif et al., 1990; Breder & Gresser, 1941; Gertychowa, 1970; Sharma et al., 2009; Teyke, 1985; 1989), and even invertebrates (Basil & Sandemann, 2000; Creed & Miller, 1990; Jeanson et al., 2003; Besson & Martin, 2004; Martin, 2004). In other words, navigators could ground exploratory activities on thigmotaxis, that is, all those automatic behaviours relative to tactile/haptic stimulation (Fraenkel & Gunn, 1961). As a result of the association of spatial cues with locomotor activity in time and space (Kallai et al., 2005; 2007), the wall-following behaviour can be considered an adaptive goal-oriented strategy (O'Keefe & Nadel, 1978; Oman et al., 2000), which involves action-based mental representations. However, it has also been suggested that such a tendency to move in perimetrical, i.e., peripheral, areas is a stress-related measure of anxiety and fear when exploring novel environments (Choleris et al., 2001; Jeanson et al., 2003; Kallai et al., 2007; Ohl et al.,, 2001; Pellow & File, 1986; Simon et al., 1994; Treit & Fundytus, 1988). Previous evidence has further reported that thigmotaxis over time prevented animals to use mapping-like strategies to resolve different kind of spatial tasks within Morris-type mazes (Graziano et al., 2003; Kallai et al., 2005). Beyond any definitions, it could be assumed that wall-following behaviours allow navigators to establish 3D extended surfaces of bounded environments, to get protection (e.g., "staying in periphery to avoid exposed, open space") or acquire spatial knowledge (e.g., "staying in periphery to determine a reference frame from which starting to build new spatial relationships). It is also possible that rearing conditions within enclosed spaces, such as tanks or fences, affect motion patterns. Starting from this speculation, it would be interesting to study wall-following strategy after having reared animals within environments with comfortable, familiar, central areas (e.g., enriched with natural objects) or where no peripheral areas are present (e.g., within X-shaped mazes). However, it has been reported that fish raised within rectangular vs. circular home tanks reoriented similarly by using spatial geometry (Brown et al., 2007; Sovrano & Chiandetti, 2017), even if in these studies motion patterns have not been investigated.

In Study 3 and Study 4, wall-following behaviours came out by analysing the discrete choices and the time spent within the three zones of the arena (perimetrical vs. diagonal vs. neutral), in reference to the two geometrically correct corner positions. We were interested in observing if this strategy could be exhibited by tortoises once they had learned to reorient in accord with spatial geometry alone (Study 3) or in conjunction with landmark (Study 4). It seems that the tactile exploration along the perimeter has been useful to tortoises for determining the metric component of boundary-length, while the conspicuous landmark has been associated to the frame on visual basis, regardless of its positional sense (left or right) and proximity (near or far).

Study 5 (*Nonvisual environmental geometry in zebrafish, redtail splitfin fish, and goldfish*) showed that all the three species learned to reorient within an enclosed space composed of transparent surfaces, which prevented the fish to use sight for determining its spatial geometry. By contrast, untrained fish did not spontaneously reorient in that environment, also showing interspecific differences in the number of choices for the corner positions. In this study, we have shown to what extent the behavioural task (spontaneous vs. rewarded), thus, the memory system involved (working vs. reference), can affect the use of spatial geometry under visual transparency. To explain the good performance of fish at learning despite the surface's transparency, the recruitment of extra-visual sensory channels has been proposed, focusing on the lateral line (LL) system. Also known as «touch-at-a-distance» (Dijkgraaf, 1963; 1964; 1989), the LL allows perceiving short-range stationary or moving objects to familiarise with the environment, especially in bind cavefish (Bisazza et al., 2014; Bleckmann, 1994; Bleckmann & Zelick, 2009; Burt de Perera, 2004; Burt de Perera et al., 2005; Gertychowa, 1970; Hassan, 1989; 1992; Hassan et al., 1992; Holdbrook & Burt de Perera, 2009; John, 1957; Sguanci et al., 2010; Sutherland et al., 2009; Teyke, 1985; 1989; Teyke & Schaerer, 1994; Von Campenhausen et al., 1981; Weissert & Von Campenhausen, 1981; Windsor et al., 2010; 2008). From a previous work by Sovrano and colleagues (2018*a*) with hypogean cavefish, the LL had already been considered as a key system for reorienting in the case of blindness. However, observing geometric reorientation in situations of prevented visibility in eyed fish would have stressed the role of this system also in species that mainly use sight in everyday activities. At that point, the role of the LL could only be inferred from behavioural observations and not directly established.

Study 6 (*Isolated environmental geometric cues in zebrafish*) revealed that zebrafish learned to use boundary distance, freestanding corners, and boundary length when presented one by one, for reorienting. Here we showed that a repeated and consistent experience over time aided the fish in using these three parameters, unlike what it was found in the previous study by Lee and colleagues (2013) with untrained zebrafish. In the case of spontaneous behaviour, fish underperformed with freestanding corners and boundary length, using only boundary distance to reorient. As reported in Study 1 and Study 5, the type of task strongly affected reorientation, where learning processes acquired relevance in helping the fish to experience the space in a kind of appetitive conditioning over time (Bouton & Peck, 1989; Daggett et al., 2019; Kalueff et al., 2014; Silveira et al., 2019; Sison & Gerlai, 2010).

Study 7 (*3D outside landmark (blue cylinder) in zebrafish*) led to interesting results. First, we found an increasing range of difficulty at combining the nonvisual spatial geometry with the conspicuous 3D landmark, which depends on the landmark proximity to the correct corner position. Zebrafish performed better when the blue cylinder was proximal (proximal short > proximal long) and underperformed when the blue cylinder was distal (distal short > distal long). Second, we found that unsuccessful fish used spatial geometry halfway along the training (i.e., choosing more the two geometrically correct corners), while successful fish did not. This finding may suggest that fish reorientation behaviour has been anchored at the geometric level, thus blocking the process of associating the distal landmark (probably, not so salient) with the spatial frame. Third, a difference between proximal short and proximal long conditions came out in the mean number of trials to learn (proximal long > proximal short), in the latency times before going out from the experimental arena (proximal long < proximal short), and in the use of spatial geometry (proximal long > proximal short). One argument relates to absolute distance from the arena's centre to the landmark position, which was minor in the proximal long condition (Newcombe & Ratliff, 2007; Pecchia & Vallortigara, 2010*b*; Tommasi & Vallortigara, 2000). The cylindrical landmark proximal to the target location but placed on the long side was nearer the centre than the cylindrical landmark proximal to the target location but placed on the short side. An overlap of different processes, world- and self-based, could have delayed long-term learning (i.e., more training trials needed) but made more effective the single response within repeated single trials (i.e., less time to do a choice). Fourth, the analysis on motion patterns (strategy and direction) indicated non-lateralised wall-following behaviour irrespective of the learning task. In other words, the tendency to swim in the peripheral areas before approaching the arena's corridors at corners was shared among the fish, despite the experimental conditions. This means that wall-following behaviours occurred in any case, regardless of the geometric task, and that such a strategy was not the way through which zebrafish integrated geometry and landmark for reorienting. Although that contrasts with what we have previously reported, different task's demands (geometry only vs. geometry + landmark) may partly explain the inconsistency of results. Fifth, training over time allowed fish to combine the nonvisual geometry of the rectangular arena with the cylindrical blue landmark, most of all in proximal conditions, and to overcome the attractiveness-bias found by Lee and colleagues (2015*a*).

Study 8 (*Lateral line pharmacological ablation in zebrafish*) replicated Study 5 with the aim to directly establish if the LL was crucially recruited during geometric reorientation in situations of visual transparency. This investigation was run in zebrafish only, for which some literature is available as regards the LL system and the effect of aminoglycoside antibiotics (Ghysen & Dambly-Chaudiere, 2004; Gompel et al., 2001; Ma & Raible, 2009; Ou et al., 2010; Owens et al., 2009; Raible & Kruse, 2000; Santos et al., 2006). After administering gentamicin sulfate at different concentrations (10, 20, 40 mg/L), with a subsequent ablation of the LL as supported by the DASPEI mitochondria fluorescent staining, almost all the fish reoriented properly not showing any behavioural impairment. As such, the LL must be excluded within a range of possible systems used by fish to detect the nonvisual global shape for reorienting or, rather, it could be reconsidered as working in synch with other sensory channels (e.g., touch, chemoreception). Once put aside the LL, the analysis has been focused on motion patterns, not only for transparent but also for opaque geometry. As regards opaque geometry, motion patterns have been evaluated in four cases: global shape, boundary distance, freestanding corners, and boundary length (as in Baratti et al., 2020; 2021; Study 6, pp. 90-100). Interestingly, zebrafish used the wall-following motion strategy under the influence of a distinctive global shape, both transparent and opaque, but they did not the same when presented with isolated cues (where no motion preference was found). It is possible that explorations of physical perimeters occur as a robust tendency of fish for detecting continuous environmental geometries as collapsed within an all-in-one polygon, while discrete metric units would require view-based processes applied to single parameters. This view is not totally consistent with Normandin and colleagues (2022), where they observed that blind mice used 3D edges to reorient by «patrolling the borders», thus showing wall-following behaviour. However, since the analyses on strategies are not comparable between the studies, as well as the comparison between reorientation under blindness vs. visual transparency conditions, no clear conclusions can be drawn. It has been reported that blind cavefish displayed wall-following behaviour with exploratory functions in situations of darkness (Sharma et al., 2009); starting from that, more targeted investigations could be designed to widely explore whether orientation in space by touch is consistent even during disoriented navigation.

Apart from exploring several aspects of geometric spatial reorientation, the present Thesis pursued a line of comparison, across species, methodologies, and sensory channels. This approach has led to the following considerations.

Three species of fish reoriented under visual transparency conditions thanks to learning processes, but not in the case of spontaneous behaviour. Minor interspecific differences were found in choice frequency, where zebrafish did more attempts (probably due to natural traits), and goldfish took longer to learn. However, these three species behaved similarly as regards the use of transparent surfaces defining a distinctive global shape, also supporting spatial reorientation under adverse circumstances (i.e., must find a food source within a visually deprived, unenriched, environment) as a shared skill among teleosts, despite ecological specificities (description on pp. 22-27). Besides fish, one reptile species has been carefully observed, leading to interesting comparative insights. The use of environmental geometry (alone and in conjunction with a conspicuous landmark) was found, together with wall-following explorations of boundaries to determine global shape parameters for reorienting. This tendency to move within peripheral areas, close to the perimeter, is akin to that displayed by zebrafish and could represent a goal-oriented behavioural pattern that remote vertebrates have in common to explore enclosed environments. Even If tortoise cognition is less understood and described than fish cognition (Bridgeman & Tattersall, 2019; Gutnick et al., 2020; Mueller-Paul et al., 2014; 2012; Santacà et al., 2019; 2020; Wilkinson et al., 2010; Wilkinson & Glass, 2018), our findings indicate that also reptile models can reorient within a geometric space with precision to meet a survival need. Even species that hibernate (and often aestivate) for long can benefit from orientation by extended terrain structures such as riverbeds or hill-like surfaces.

The comparison between spontaneous and acquired behaviour has added evidence to what previously reported in teleosts (Baratti et al., 2022) and mice (Lee et al., 2015*b*) as regards the role of different memory tests (short-term, “working” vs. long-term, “reference”) on reorientation. We can suppose that the rewarded exit task designed to train fish and tortoise to reorient in several experimental situations required learning processes allowing them to overcome the constraints of predispositions to improve other related abilities, such as landmark-use (as directional cues). The dissociation between working vs. reference memory in spatial domain, as the expression of hippocampal vs. striatal neural networks (Broglio et al., 2010; Durán et al., 2010; 2008; López et al., 2000*a,b*; 2001; 2003; Portavella & Vargas, 2005; Rodríguez et al., 2002; Salas et al., 1996*a,b*), must be considered highly dependent on specific task's demands where, probably, attentional factors determine short-term memories and motivational states (that increase over time) long-term ones.

In respect of sensory systems, the use of LL has been opposed to sight to establish if reorientation behaviour within transparent environments could be fulfilled by this special channel. Although we had to exclude the LL, the investigation on motion patterns led to consider the recruitment of exploratory modalities driven by touch for determining spatial geometric characteristics during reorientation. As such, a promising link between other vertebrates and humans takes place, in consideration of the orientation mechanisms used to face situations of visual deprivation or impairments (Giudice, 2018; Schinazi et al., 2016)

In more than thirty years research, geometric spatial reorientation has been meticulously investigated from several perspectives, thanks to philosophical, behavioural, and brain-centred domains. This four-year Ph.D. research project has provided to me the opportunity to extensively look into really specific issues, mainly approaching them through behavioural assessments and analyses in fish species and tortoises. The future perspectives of this topic probably rely in investigating all the aspects of spatial reorientation (e.g., role of memory, behavioural impairments due to drugs, focal lesions or even pollutants) through other kind of analyses, more centred on biochemical and neurobiological processes underlying geometry- and landmark-based representations, with a special focus on neural networks involved in multisensory exploratory strategies. The purpose is to contribute to a better understanding of reorientation capacities as a widespread, primitive, and sophisticated cognitive spatial skill in everyday activities of living organisms.

References

1. Abdel-Latif, H., Hassan, E. S., & Von Campenhausen, C. (1990). Sensory performance of blind Mexican cave fish after destruction of the canal neuromasts. *Naturwissenschaften*, *77*(5), 237–239. https://doi.org/10.1007/BF01138492
2. Abdollahpour, H., Falahatkar, B., & Lawrence, C. (2020). The effect of photoperiod on growth and spawning performance of zebrafish, *Danio rerio*. *Aquaculture Reports*, *17*, 100295. https://doi.org/10.1016/j.aqrep.2020.100295
3. Abramenko, M. I., Kravchenko, O. V., & Velikoivanenko, A. E. (1997). Population genetic structure of the goldfish *Carassius auratus* gibelio diploid-triploid complex from the Don River Basin. *Journal of Ichthyology*, 37, 56–65.
4. Agrillo, C., Miletto Petrazzini, M. E., Tagliapietra, C., & Bisazza, A. (2012). Inter-specific differences in numerical abilities among teleost fish. *Frontiers in Psychology*, *3*, 483. https://doi.org/10.3389/fpsyg.2012.00483
5. Aiello, B. R., Hardy, A. R., Westneat, M. W., & Hale, M. E. (2018). Fins as mechanosensors for movement and touch-related behaviors. *Integrative and Comparative Biology*, *58*(5), 844–859. https://doi.org/10.1093/icb/icy065
6. Albertazzi, L., Rosa-Salva, O., Da Pos, O., & Sovrano, V. A. (2017). Fish are sensitive to expansion-contraction color effects. *Animal Behavior and Cognition*, *4*(3), 349–364. https://doi.org/10.26451/abc.04.03.12.2017
7. Arthur, D., & Levin, E. D. (2001). Spatial and non-spatial visual discrimination learning in zebrafish (*Danio rerio*). *Animal Cognition*, *4*(2), 125–131. https://doi.org/10.1007/s100710100111
8. Avdesh, A., Martin-Iverson, M. T., Mondal, A., Chen, M., Askraba, S., Morgan, N., ... & Martins, R. N. (2012). Evaluation of color preference in

zebrafish for learning and memory. *Journal of Alzheimer's Disease*, *28*(2), 459–469.

9. Avdesh, A., Martin-Iverson, M. T., Mondal, A., Chen, M., Verdile, G., & Martins, R. N. (2010). Natural colour preference in the zebrafish (*Danio rerio*). Proceedings of Measuring Behavior, 155–157.

10. Bailey, J. M., Oliveri, A. N., & Levin, E. D. (2015). Pharmacological analyses of learning and memory in zebrafish (*Danio rerio*). *Pharmacology Biochemistry and Behavior*, *139*, 103–111. https://doi.org/10.1016/j.pbb.2015.03.006

11. Baratti, G., Potrich, D., & Sovrano, V. A. (2020). The environmental geometry in spatial learning by zebrafish (*Danio rerio*). *Zebrafish*, *17*(2), 131–138. https://doi.org/10.1089/zeb.2019.1845

12. Baratti, G., Potrich, D., Lee, S. A., Morandi-Raikova, A., & Sovrano, V. A. (2022). The geometric world of fishes: A synthesis on spatial reorientation of teleosts. *Animals*, *12*(7), 881. https://doi.org/10.3390/ani12070881

13. Baratti, G., Rizzo, A., Miletto Petrazzini, M. E., & Sovrano, V. A. (2021). Learning by Doing: The Use of Distance, Corners and Length in Rewarded Geometric Tasks by Zebrafish (*Danio rerio*). *Animals*, *11*(7), 2001. https://www.mdpi.com/2076-2615/11/7/2001

14. Barnett S. A. (1963). *The rat: a study in behavior*. Metheun, London.

15. Basil, J., & Sandeman, D. (2000). Crayfish (*Cherax destructor*) use tactile cues to detect and learn topographical changes in their environment. *Ethology*, *106*(3), 247–259. https://doi.org/10.1046/j.1439-0310.2000.00524.x

16. Besson, M., & Martin, J. R. (2004). Centrophobism/thigmotaxis, a new role for the mushroom bodies in *Drosophila*. *Journal of Neurobiology*, *62*(3), 386–396. https://doi.org/10.1002/neu.20111

17. Bicanski, A., & Burgess, N. (2018). A neural-level model of spatial memory and imagery. *Elife*, *7*, e33752. https://doi.org/10.7554/eLife.33752

18. Bingman, V. P., Riters, L. V., Strasser, R., & Gagliardo, A. (1998) Neuroethology of avian navigation. In R. Balda, I. Pepper-Berg, & A. Kamil (Eds.), *Animal Cognition in Nature* (pp. 201–226). Cambridge: Academic Press.

19. Bisazza, A., Tagliapietra, C., Bertolucci, C., Foà, A., & Agrillo, C. (2014). Non-visual numerical discrimination in a blind cavefish (*Phreatichthys andruzzii*). *Journal of Experimental Biology*, *217*(11), 1902–1909. https://doi.org/10.1242/jeb.101683

20. Bitterman, M. E. & Schoel, W. M. (1970) Instrumental learning in animals: Parameters of reinforcement. *Annual Review of Psychology*, *21*, 367–436. https://doi.org/10.1146/annurev.ps.21.020170.002055

21. Blaxter, J. H., & Fuiman, L. A. (1989). Function of the free neuromasts of marine teleost larvae. In S. Coombs, P. Görner, & H. Münz (Eds.), *The mechanosensory lateral line* (pp. 481–499). New York: Springer. https://doi.org/10.1007/978-1-4612-3560-6_24

22. Bleckmann, H. (1994). Reception of hydrodynamic stimuli in aquatic and semiaquatic animals. In W. Rathmayer (Ed.), *Progress in Zoology*, vol. 41 (pp. 1—115). Frankfurt: Fisher Verlag.

23. Bleckmann, H., & Zelick, R. (2009). Lateral line system of fish. *Integrative Zoology*, *4*(1), 13–25. https://doi.org/10.1111/j.1749-4877.2008.00131.x

24. Bouton, M. E., & Peck, C. A. (1989). Context effects on conditioning, extinction, and reinstatement in an appetitive conditioning preparation. *Animal Learning & Behavior*, *17*(2), 188–198. https://doi.org/10.3758/BF03207634

25. Braun, C. B., & Coombs, S. (2000). The overlapping roles of the inner ear and lateral line: the active space of dipole source detection. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *355*(1401), 1115–1119. https://doi.org/10.1098/rstb.2000.0650

26. Breder C. M., & Gresser, E. B. (1941) Correlation between structural eye defects and behavior in the Mexican blind characin. *Zoologica*, *26*, 123–131.

27. Bridgeman, J. M., & Tattersall, G. J. (2019). Tortoises develop and overcome position biases in a reversal learning task. *Animal Cognition*, *22*(2), 265–275. https://doi.org/10.1007/s10071-019-01243-8

28. Broglio, C., Rodríguez, F., Gómez, A., Arias, J. L., & Salas, C. (2010). Selective involvement of the goldfish lateral pallium in spatial memory. *Behavioural Brain Research*, *210*(2), 191–201. https://doi.org/10.1016/j.bbr.2010.02.031

29. Broglio, C., Rodríguez, F., & Salas, C. (2003). Spatial cognition and its neural basis in teleost fishes. *Fish and Fisheries*, *4*(3), 247–255. https://doi.org/10.1046/j.1467-2979.2003.00128.x

30. Brown, C., Laland, K., & Krause, J. (2011). *Fish cognition and behavior.* New Jersey: John Wiley & Sons.

31. Brown, A. D., Mussen, T. D., Sisneros, J. A., & Coffin, A. B. (2011). Reevaluating the use of aminoglycoside antibiotics in behavioral studies of the lateral line. *Hearing research*, *272*(1–2), 1–4. https://doi.org/10.1016/j.heares.2010.10.014

32. Brown, A. A., Spetch, M. L., & Hurd, P. L. (2007). Growing in circles: Rearing environment alters spatial navigation in fish. *Psychological Science*, *18*(7), 569–573. https://doi.org/10.1111/j.1467-9280.2007.01941.x

33. Burgess, N. (2008). Spatial cognition and the brain. *Annals of the New York Academy of Sciences*, *1124*(1), 77–97. https://doi.org/10.1196/annals.1440.002

34. Burt de Perera, T. (2004). Spatial parameters encoded in the spatial map of the blind Mexican cave fish, *Astyanax fasciatus*. *Animal Behaviour*, *68*(2), 291–295. https://doi.org/10.1016/j.anbehav.2003.11.009

35. Burt de Perera, T., de Vos, A., & Guilford, T. (2005). The vertical component of a fish's spatial map. *Animal Behaviour*, *70*(2), 405–409. https://doi.org/10.1016/j.anbehav.2004.11.007

36. Caro, T. M., Roper, R., Young, M., & Dank, G. R. (1979). Inter-observer reliability. *Behaviour*, *69*(3–4), 303–315. https://doi.org/10.1163/156853979X00520

37. Chan, E., Baumann, O., Bellgrove, M. A., & Mattingley, J. B. (2012). From objects to landmarks: the function of visual location information in spatial navigation. *Frontiers in Psychology*, *3*, 304. https://doi.org/10.3389/fpsyg.2012.00304

38. Chelazzi, G., & Delfino, G. (1986). A field test on the use of olfaction in homing by *Testudo hermanni* (Reptilia: Testudinidae). *Journal of Herpetology*, 20(3), 451–455. https://doi.org/10.2307/1564513

39. Chelazzi, G., & Francisci, F. (1979). Movement patterns and homing behaviour of Testudo hermanni Gmelin (Reptilia Testudinidae). *Italian Journal*

of Zoology, *13*(2–3), 105–127. https://doi.org/10.1080/00269786.1979.10736332

40. Cheng, K. (1986). A purely geometric module in the rat's spatial representation. *Cognition*, *23*(2), 149–178. https://doi.org/10.1016/0010-0277(86)90041-7

41. Cheng, K. (2008). Whither geometry? Troubles of the geometric module. *Trends in Cognitive Sciences*, *12*(9), 355–361. https://doi.org/10.1016/j.tics.2008.06.004

42. Cheng, K., & Gallistel, C. R. (1984). Testing the geometric power of an animal's spatial representation. In H. Roitblat, T. G. Bever, & H. Terrace (Eds.), *Animal Cognition* (pp. 409–423). Lawrence Erlbaum Associates.

43. Cheng, K., Huttenlocher, J., & Newcombe, N. S. (2013). 25 years of research on the use of geometry in spatial reorientation: a current theoretical perspective. *Psychonomic Bulletin & Review*, *20*(6), 1033–1054. https://doi.org/10.3758/s13423-013-0416-1

44. Cheng, K., & Newcombe, N. S. (2005). Is there a geometric module for spatial orientation? Squaring theory and evidence. *Psychonomic Bulletin & Review*, *12*(1), 1–23. https://doi.org/10.3758/BF03196346

45. Cheung, A., Stürzl, W., Zeil, J., & Cheng, K. (2008). Information content of panoramic images: II. View-based navigation in nonrectangular experimental arenas. *Journal of Experimental Psychology: Animal Behavior Processes*, *34*(1), 15–30.

46. Choi, T. Y., Choi, T. I., Lee, Y. R., Choe, S. K., & Kim, C. H. (2021). Zebrafish as an animal model for biomedical research. *Experimental &*

Molecular Medicine, *53*(3), 310–317. https://doi.org/10.1038/s12276-021-00571-5

47. Choleris, E., Thomas, A. W., Kavaliers, M., & Prato, F. S. (2001). A detailed ethological analysis of the mouse open field test: effects of diazepam, chlordiazepoxide and an extremely low frequency pulsed magnetic field. *Neuroscience & Biobehavioral Reviews*, *25*(3), 235–260. https://doi.org/10.1016/S0149-7634(01)00011-2

48. Collett, T. S., Cartwright, B. A., Smith, B. A. (1986). Landmark learning and visuo-spatial memory in gerbils. *Journal of Comparative Physiology (A)*, *158*(6), 835−851. https://doi.org/10.1007/BF01324825

49. Collett, T. S., & Graham, P. (2004). Animal navigation: path integration, visual landmarks and cognitive maps. *Current Biology*, *14*(12), R475–R477. https://doi.org/10.1016/j.cub.2004.06.013

50. Collett, T. S., & Collett, M. (2002). Memory use in insect visual navigation. *Nature Reviews Neuroscience*, *3*(7), 542–552. https://doi.org/10.1038/nrn872

51. Colwill, R. M., Raymond, M. P., Ferreira, L., & Escudero, H. (2005). Visual discrimination learning in zebrafish (*Danio rerio*). *Behavioural Processes*, *70*(1), 19–31. https://doi.org/10.1016/j.beproc.2005.03.001

52. Creed, R. P., & Miller, J. R. (1990). Interpreting animal wall-following behavior. *Experientia*, *46*(7), 758–761. https://doi.org/10.1007/BF01939959

53. Currier, W. R. (2013). How does sexual selection and breeding biology play a role in the conservation of the threatened *Xenotoca eiseni*? *Undergraduate Honors Theses.* Paper 337. University of Colorado at Boulder.

54. Daggett, J. M., Brown, V. J., & Brennan, C. H. (2019). Food or friends? What motivates zebrafish (*Danio rerio*) performing a visual discrimination. *Behavioural Brain Research*, *359*, 190–196. https://doi.org/10.1016/j.bbr.2018.11.002

55. Darland, T., & Dowling, J. E. (2001). Behavioral screening for cocaine sensitivity in mutagenized zebrafish. *Proceedings of the National Academy of Sciences*, *98*(20), 11691–11696. https://doi.org/10.1073/pnas.191380698

56. Dawson, M. R. W., Kelly, D. M., Spetch, M. L., & Dupuis, B. (2010). Using perceptrons to explore the reorientation task. *Cognition*, *114*(2), 207–226. https://doi.org/10.1016/j.cognition.2009.09.006

57. Dehaene, S., Izard, V., Pica, P., & Spelke, E. (2006). Core knowledge of geometry in an Amazonian indigene group. *Science, 311*(5759), 381–384. https://doi.org/10.1126/science.1121739

58. Dijkgraaf, S. (1963). The functioning and significance of the lateral-line organs. *Biological Reviews*, *38*(1), 51–105. https://doi.org/10.1111/j.1469-185X.1963.tb00654.x

59. Dijkgraaf, S. (1964). The supposed use of the lateral line as an organ of hearing in fish. *Experientia*, *20*(10), 586–587. https://doi.org/10.1007/BF02150313

60. Dijkgraaf, S. (1989). A Short Personal Review of the History of Lateral Line Research. In S. Coombs, P. Görner, & H. Münz (Eds.), *The Mechanosensory Lateral Line* (pp. 7–14). Springer, New York, NY. https://doi.org/10.1007/978-1-4612-3560-6_2

61. Dilks, D. D., Julian, J. B., Paunov, A. M., & Kanwisher, N. (2013). The occipital place area is causally and selectively involved in scene perception.

Journal of Neuroscience, *33*(4), 1331–1336. https://doi.org/10.1523/JNEUROSCI.4081-12.2013

62. Doeller, C. F., & Burgess, N. (2008). Distinct error-correcting and incidental learning of location relative to landmarks and boundaries. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(15), 5909–5914. https://doi.org/10.1073/pnas.0711433105

63. Doeller, C. F., King, J. A., & Burgess, N. (2008). Parallel striatal and hippocampal systems for landmarks and boundaries in spatial memory. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(15), 5915–5920. https://doi.org/10.1073/pnas.0801489105

64. Durán, E., Ocaña, F. M., Broglio, C., Rodríguez, F., & Salas, C. (2010). Lateral but not medial telencephalic pallium ablation impairs the use of goldfish spatial allocentric strategies in a “hole-board” task. *Behavioural Brain Research*, *214*(2), 480–487. https://doi.org/10.1016/j.bbr.2010.06.010

65. Durán, E., Ocaña, F. M., Gómez, A., Jiménez-Moya, F., Broglio, C., Rodríguez, F., & Salas, C. (2008). Telencephalon ablation impairs goldfish allocentric spatial learning in a “hole-board” task. *Acta Neurobiologiae Experimentalis*, 68, 519–525.

66. Echevarria, D. J., Jouandot, D. J., & Toms, C. N. (2011). Assessing attention in the zebrafish: Are we there yet? *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *35*(6), 1416–1420. https://doi.org/10.1016/j.pnpbp.2011.01.020

67. Epstein, R. A. (2005). The cortical basis of visual scene processing. *Visual Cognition*, *12*(6), 954–978. https://doi.org/10.1080/13506280444000607

68. Epstein, R. A. (2008). Parahippocampal and retrosplenial contributions to human spatial navigation. *Trends in Cognitive Sciences*, *12*(10), 388–396. https://doi.org/10.1016/j.tics.2008.07.004

69. Epstein, R. A., Patai, E. Z., Julian, J. B., & Spiers, H. J. (2017). The cognitive map in humans: spatial navigation and beyond. *Nature Neuroscience*, *20*(11), 1504–1513. https://doi.org/10.1038/nn.4656

70. Epstein, R., & Kanwisher, N. (1998). A cortical representation of the local visual environment. *Nature*, *392*(6676), 598–601. https://doi.org/10.1038/33402

71. Etienne, A. S. (1992). Navigation of a small mammal by dead reckoning and local cues. *Current Directions in Psychological Science*, *1*(2), 48–52. https://doi.org/10.1111/1467-8721.ep11509737

72. Fernandes, A. M., Mearns, D. S., Donovan, J. C., Larsch, J., Helmbrecht, T. O., Kölsch, Y., ... & Baier, H. (2021). Neural circuitry for stimulus selection in the zebrafish visual system. *Neuron*, *109*(5), 805–822. https://doi.org/10.1016/j.neuron.2020.12.002

73. Fodor, J. A. (1983). *The modularity of mind: an essay on faculty psychology.* Cambridge: MIT press.

74. Forge, A., & Schacht, J. (2000). Aminoglycoside antibiotics. *Audiology and Neurotology*, *5*(1), 3-22. https://doi.org/10.1159/000013861

75. Fraenkel, G. S., & Gunn, D. L. (1961). *The orientation of animals: kineses, taxes and compass reactions.* New York: Dover.

76. Galeotti, P., Sacchi, R., Pellitteri-Rosa, D., & Fasola, M. (2007). Olfactory discrimination of species, sex, and sexual maturity by the Hermann's tortoise

Testudo hermanni. *Copeia*, 2007(4), 980–985. https://doi.org/10.1643/0045-8511(2007)7[980:ODOSSA]2.0.CO;2

77. Gallistel, C. R. (1990). *The organization of learning*. MIT Press.

78. Gatto, E., Lucon-Xiccato, T., Bisazza, A., Manabe, K., & Dadda, M. (2020). The devil is in the detail: Zebrafish learn to discriminate visual stimuli only if salient. *Behavioural Processes*, *179*, 104215. https://doi.org/10.1016/j.beproc.2020.104215

79. Gatto, E., Santacà, M., Verza, I., Dadda, M., & Bisazza, A. (2021). Automated Operant Conditioning Devices for Fish. Do They Work? *Animals*, *11*(5), 1397. https://doi.org/10.3390/ani11051397

80. Gerlai, R. (2011). Associative learning in zebrafish (*Danio rerio*). *Methods in Cell Biology*, *101*, 249–270. https://doi.org/10.1016/B978-0-12-387036-0.00012-8

81. Gertychowa, R. (1970). Studies on the ethology and space orientation of the blind cave fish *Anoptichthys jordani Hubbs* et Innes 1936 (Characidae). *Folia Biologica*, *18*(1), 9–69.

82. Ghysen, A., & Dambly-Chaudiere, C. (2004). Development of the zebrafish lateral line. *Current Opinion in Neurobiology*, *14*(1), 67–73. https://doi.org/10.1016/j.conb.2004.01.012

83. Gianni, E., De Zorzi, L., & Lee, S. A. (2018). The developing role of transparent surfaces in children's spatial representation. *Cognitive Psychology*, *105*, 39–52. https://doi.org/10.1016/j.cogpsych.2018.05.003

84. Giudice, N. A. (2018). Navigating without Vision: Principles of Blind Spatial Cognition. In *Handbook of Behavioral and Cognitive Geography* (pp. 260–288). Edward Elgar Publishing: Cheltenham, UK; Northampton, MA, USA.

85. Gompel, N., Cubedo, N., Thisse, C., Thisse, B., Dambly-Chaudière, C., & Ghysen, A. (2001). Pattern formation in the lateral line of zebrafish. *Mechanisms of Development*, *105*(1–2), 69–77. https://doi.org/10.1016/S0925-4773(01)00382-3

86. Götz, K. G., & Biesinger, R. (1985). Centrophobism in *Drosophila melanogaster*. *Journal of Comparative Physiology A*, *156*(3), 329–337. https://doi.org/10.1007/BF00610726

87. Gouteux, S., & Spelke, E. S. (2001). Children's use of geometry and landmarks to reorient in an open space. *Cognition*, *81*(2), 119–148. https://doi.org/10.1016/S0010-0277(01)00128-7

88. Gouteux, S., Thinus-Blanc, C., & Vauclair, J. (2001). Rhesus monkeys use geometric and nongeometric information during a reorientation task. *Journal of Experimental Psychology: General*, *130*(3), 505–519.

89. Graziano, A., Petrosini, L., & Bartoletti, A. (2003). Automatic recognition of explorative strategies in the Morris water maze. *Journal of Neuroscience Methods*, *130*(1), 33–44. https://doi.org/10.1016/S0165-0270(03)00187-0

90. Grill-Spector, K. (2003). The neural basis of object perception. *Current Opinion in Neurobiology*, *13*(2), 159–166. https://doi.org/10.1016/S0959-4388(03)00040-0

91. Grunwald, D. J., & Eisen, J. S. (2002). Headwaters of the zebrafish—emergence of a new model vertebrate. *Nature Reviews Genetics*, *3*(9), 717–724. https://doi.org/10.1038/nrg892

92. Gutnick, T., Weissenbacher, A., & Kuba, M. J. (2020). The underestimated giants: operant conditioning, visual discrimination and long-

term memory in giant tortoises. *Animal Cognition*, *23*(1), 159–167. https://doi.org/10.1007/s10071-019-01326-6

93. Haight, J. L., & Schroeder, J. A. (2011). Spatial cognition in Zebrafish. In Kalueff & J. Cachat (Eds.), *Zebrafish Models in Neurobehavioral Research* (pp. 235–248). Humana Press, Totowa, NJ. https://doi.org/10.1007/978-1-60761-922-2_11

94. Hailey, A. (1990). Adult survival and recruitment and the explanation of an uneven sex ratio in a tortoise population. *Canadian Journal of Zoology*, *68*(3), 547–555. https://doi.org/10.1139/z90-080

95. Harris, J. A., Cheng, A. G., Cunningham, L. L., MacDonald, G., Raible, D. W., & Rubel, E. W. (2003). Neomycin-induced hair cell death and rapid regeneration in the lateral line of zebrafish (*Danio rerio*). *Journal of the Association for Research in Otolaryngology*, *4*(2), 219–234. https://doi.org/10.1007/s10162-002-3022-x

96. Hassan, E. S. (1989). Hydrodynamic imaging of the surroundings by the lateral line of the blind cave fish *Anoptichthys jordani*. In S. Coombs, P. Görner, & H. Münz, H. (Eds.), *The mechanosensory lateral line* (pp. 217–227). Springer, New York, NY. https://doi.org/10.1007/978-1-4612-3560-6_10

97. Hassan, E. S. (1992). Mathematical description of the stimuli to the lateral line system of fish derived from a three-dimensional flow field analysis. *Biological Cybernetics*, *66*(5), 443–452. https://doi.org/10.1007/BF00197725

98. Hassan, E., Abdel-Latif, H., & Biebricher, R. (1992). Studies on the effects of Ca2++ and Co++ on the swimming behavior of the blind Mexican cave fish. *Journal of Comparative Physiology A*, *171*(3), 413–419. https://doi.org/10.1007/BF00223971

99. Hayward, A., McGregor, A., Good, M. A., & Pearce, J. M. (2003). Absence of overshadowing and blocking between landmarks and the geometric cues provided by the shape of a test arena. *The Quarterly Journal of Experimental Psychology Section B*, *56*(1b), 114–126. https://doi.org/10.1080/02724990244000214

100. Hermer, L., & Spelke, E. S. (1994). A geometric process for spatial reorientation in young children. *Nature*, *370*(6484), 57–59. https://doi.org/10.1038/370057a0

101. Hermer, L., & Spelke, E. S. (1996). Modularity and development: The case of spatial reorientation. *Cognition*, *61*(3), 195–232. https://doi.org/10.1016/S0010-0277(96)00714-7

102. Hermer-Vazquez, L., Moffet, A., & Munkholm, P. (2001). Language, space, and the development of cognitive flexibility in humans: The case of two spatial memory tasks. *Cognition*, *79*(3), 263–299. https://doi.org/10.1016/S0010-0277(00)00120-7

103. Hermer-Vazquez, L., Spelke, E. S., & Katsnelson, A. S. (1999). Sources of flexibility in human cognition: Dual-task studies of space and language. *Cognitive Psychology*, *39*(1), 3–36. https://doi.org/10.1006/cogp.1998.0713

104. Higgs, D. M., & Radford, C. A. (2016). The potential overlapping roles of the ear and lateral line in driving “acoustic” responses. In J. A. Sisneros (Ed.), *Fish hearing and bioacoustics: An anthology in honor of Arthur N. Popper and Richard R. Fay* (pp. 255–270). Cham, Switzerland: Springer. https://doi.org/10.1007/978-3-319-21059-9_12

105. Hill, E. W., Rieser, J. J., Hill, M. M., Hill, M., Halpin, J., & Halpin, R. (1993). How persons with visual impairments explore novel spaces: Strategies

of good and poor performers. *Journal of Visual Impairment & Blindness*, *87*(8), 295–301. https://doi.org/10.1177/0145482X9308700805

106. Holbrook, R. I., & Burt de Perera, T. (2009). Separate encoding of vertical and horizontal components of space during orientation in fish. *Animal Behaviour*, *78*(2), 241–245. https://doi.org/10.1016/j.anbehav.2009.03.021

107. Hupbach, A., & Nadel, L. (2005). Reorientation in a rhombic environment: No evidence for an encapsulated geometric module. *Cognitive Development*, *20*(2), 279–302. https://doi.org/10.1016/j.cogdev.2005.04.003

108. Huttenlocher, J., & Lourenco, S. F. (2007). Coding location in enclosed spaces: Is geometry the principle? *Developmental Science*, *10*(6), 741–746. https://doi.org/10.1111/j.1467-7687.2007.00609.x

109. Jeanson, R., Blanco, S., Fournier, R., Deneubourg, J. L., Fourcassié, V., & Theraulaz, G. (2003). A model of animal movements in a bounded space. *Journal of Theoretical Biology*, *225*(4), 443–451. https://doi.org/10.1016/S0022-5193(03)00277-7

110. John, K . R . (1957) . Observations on the behavior of blind and blinded fish. *Copeia*, 1957(2), 123–132. https://doi.org/10.2307/1439399

111. Julian, J. B., Ryan, J., Hamilton, R. H., & Epstein, R. A. (2016). The occipital place area is causally involved in representing environmental boundaries during navigation. *Current Biology*, *26*(8), 1104–1109. https://doi.org/10.1016/j.cub.2016.02.066

112. Kallai, J., Makany, T., Csatho, A., Karadi, K., Horvath, D., Kovacs-Labadi, B., ... & Jacobs, J. W. (2007). Cognitive and affective aspects of thigmotaxis strategy in humans. *Behavioral Neuroscience*, *121*(1), 21–30.

113. Kallai, J., Makany, T., Karadi, K., & Jacobs, W. J. (2005). Spatial orientation strategies in Morris-type virtual water task for humans. *Behavioural Brain Research*, *159*(2), 187–196. https://doi.org/10.1016/j.bbr.2004.10.015

114. Kalmijn, A. J. (1989). Functional evolution of lateral line and inner ear sensory systems. In S. Coombs, P. Görner, & H. Münz (Eds.), *The mechanosensory lateral line* (pp. 187–215). New York: Springer. https://doi.org/10.1007/978-1-4612-3560-6_9

115. Kalueff, A. V., Gebhardt, M., Stewart, A. M., Cachat, J. M., Brimmer, M., Chawla, J. S., ... & Schneider, and the Zebrafish Neuroscience Research Consortium, H. (2013). Towards a comprehensive catalog of zebrafish behavior 1.0 and beyond. *Zebrafish*, *10*(1), 70–86. https://doi.org/10.1089/zeb.2012.0861

116. Kalueff, A. V., Stewart, A. M., & Gerlai, R. (2014). Zebrafish as an emerging model for studying complex brain disorders. *Trends in Pharmacological Sciences*, *35*(2), 63–75. https://doi.org/10.1016/j.tips.2013.12.002

117. Kasumyan, A. O. (2003). The lateral line in fish: structure, function, and role in behavior. *Journal of Ichthyology*, *43*(2), S175–S213.

118. Kaus, S. (1987). The effect of aminoglycoside antibiotics on the lateral line organ of *Aplocheilus lineatus* (Cyprinodontidae). *Acta Oto-Laryngologica*, *103*(3–4), 291–298. https://doi.org/10.3109/00016488709107285

119. Keinath, A. T., Julian, J. B., Epstein, R. A., & Muzzio, I. A. (2017). Environmental geometry aligns the hippocampal map during spatial reorientation. *Current Biology*, *27*(3), 309–317. https://doi.org/10.1016/j.cub.2016.11.046

120. Kelly, D. M., Spetch, M. L., & Heth, C. D. (1998). Pigeons' (*Columba livia*) encoding of geometric and featural properties of a spatial environment. *Journal of Comparative Psychology*, *112*(3), 259–269. https://doi.org/10.1037/0735-7036.112.3.259

121. Learmonth, A. E., Nadel, L., & Newcombe, N. S. (2002). Children's use of landmarks: Implications for modularity theory. *Psychological Science*, *13*(4), 337–341. https://doi.org/10.1111/j.0956-7976.2002.00461.x

122. Learmonth, A. E., Newcombe, N. S., Sheridan, N., & Jones, M. (2008). Why size counts: Children’s spatial reorientation in large and small enclosures. *Developmental Science*, *11*(3), 414–426. https://doi.org/10.1111/j.1467-7687.2008.00686.x

123. Lee, S. A. (2017). The boundary-based view of spatial cognition: A synthesis. *Current Opinion in Behavioral Sciences, 16*, 58–65. https://doi.org/10.1016/j.cobeha.2017.03.006

124. Lee, S. A., Ferrari, A., Vallortigara, G., & Sovrano, V. A. (2015*a*). Boundary primacy in spatial mapping: Evidence from zebrafish (*Danio rerio*). *Behavioural Processes*, *119*, 116–122. https://doi.org/10.1016/j.beproc.2015.07.012

125. Lee, S. A., Miller, J. F., Watrous, A. J., Sperling, M. R., Sharan, A., Worrell, G. A., ... & Lega, B. (2018). Electrophysiological signatures of spatial boundaries in the human subiculum. *Journal of Neuroscience*, *38*(13), 3265–3272. https://doi.org/10.1523/JNEUROSCI.3216-17.2018

126. Lee, S. A., Sovrano, V. A., & Spelke, E. S. (2012*a*). Navigation as a source of geometric knowledge: Young children’s use of length, angle,

distance, and direction in a reorientation task. *Cognition*, *123*(1), 144–161. https://doi.org/10.1016/j.cognition.2011.12.015

127. Lee, S. A., & Spelke, E. S. (2008). Children's use of geometry for reorientation. *Developmental science*, *11*(5), 743–749. https://doi.org/10.1111/j.1467-7687.2008.00724.x

128. Lee, S. A., & Spelke, E. S. (2010*a*). A modular geometric mechanism for reorientation in children. *Cognitive Psychology*, *61*(2), 152–176. https://doi.org/10.1016/j.cogpsych.2010.04.002

129. Lee, S. A., & Spelke, E. S. (2010*b*). Two systems of spatial representation underlying navigation. *Experimental Brain Research*, *206*(2), 179–188. https://doi.org/10.1007/s00221-010-2349-5

130. Lee, S. A., & Spelke, E. S. (2011). Young children reorient by computing layout geometry, not by matching images of the environment. *Psychonomic bulletin & Review*, *18*(1), 192–198. https://doi.org/10.3758/s13423-010-0035-z

131. Lee, S. A., Spelke, E. S., & Vallortigara, G. (2012*b*). Chicks, like children, spontaneously reorient by three-dimensional environmental geometry, not by image matching. *Biology Letters*, *8*(4), 492–494. https://doi.org/10.1098/rsbl.2012.0067

132. Lee, S. A., Shusterman, A., & Spelke, E. S. (2006). Reorientation and landmark-guided search by young children: Evidence for two systems. *Psychological Science*, *17*(7), 577–582. https://doi.org/10.1111/j.1467-9280.2006.01747.x

133. Lee, S. A., Tucci, V., Sovrano, V. A., & Vallortigara, G. (2015*b*). Working memory and reference memory tests of spatial navigation in mice (*Mus*

musculus). *Journal of Comparative Psychology*, *129*(2), 189–197. https://doi.org/10.1037/a0039129

134. Lee, S. A., Vallortigara, G., Flore, M., Spelke, E. S., & Sovrano, V. A. (2013). Navigation by environmental geometry: the use of zebrafish as a model. *Journal of Experimental Biology*, *216*(19), 3693–3699. https://doi.org/10.1242/jeb.088625

135. Lee, S. A., Vallortigara, G., Ruga, V., & Sovrano, V. A. (2012*c*). Independent effects of geometry and landmark in a spontaneous reorientation task: a study of two species of fish. *Animal Cognition*, *15*(5), 861–870. https://doi.org/10.1007/s10071-012-0512-z

136. Lelek, A. (1987). *The freshwater fishes of Europe. Threatened fishes of Europe.* Aula-Verlag, Wiesbaden, Germany.

137. Lever, C., Burton, S., Jeewajee, A., O’Keefe, J., & Burgess, N. (2009). Boundary vector cells in the subiculum of the hippocampal formation. *Journal of Neuroscience*, *29*(31), 9771–9777. https://doi.org/10.1523/JNEUROSCI.1319-09.2009

138. Levin, E. D., Cerutti, D. T. (2009). Behavioral Neuroscience of Zebrafish. In J. J. Buccafusco (Ed.), *Methods of Behavior Analysis in Neuroscience.* 2nd edition. Boca Raton: CRC Press/Taylor & Francis.

139. Liew, W. C., & Orbán, L. (2013). Zebrafish sex: a complicated affair. *Briefings in Functional Genomics*, *13*(2), 172–187. https://doi.org/10.1093/bfgp/elt041

140. Lin, C. Y., Chiang, C. Y., & Tsai, H. J. (2016). Zebrafish and Medaka: new model organisms for modern biomedical research. *Journal of Biomedical Science*, *23*(1), 19. https://doi.org/10.1186/s12929-016-0236-5

141. Loomis, J. M., Klatzky, R. L., Golledge, R. G., Cicinelli, J. G., Pellegrino, J. W., & Fry, P. A. (1993). Nonvisual navigation by blind and sighted: assessment of path integration ability. *Journal of Experimental Psychology: General*, *122*(1), 73–91. https://doi.org/10.1037/0096-3445.122.1.73

142. López, J. C., Bingman, V. P., Rodríguez, F., Gómez, Y., & Salas, C. (2000*a*). Dissociation of place and cue learning by telencephalic ablation in goldfish. *Behavioral Neuroscience*, *114*(4), 687–699. https://doi.org/10.1037/0735-7044.114.4.687

143. López, J. C., Broglio, C., Rodríguez, F., Thinus-Blanc, C., & Salas, C. (1999). Multiple spatial learning strategies in goldfish (*Carassius auratus*). *Animal Cognition*, *2*(2), 109–120. https://doi.org/10.1007/s100710050031

144. López, J., Gómez, Y., Rodríguez, F., Broglio, C., Vargas, J., & Salas, C. (2001). Spatial learning in turtles. *Animal Cognition*, *4*(1), 49−59. https://doi.org/10.1007/s100710100091

145. López, J. C., Rodríguez, F., Gómez, Y., Vargas, J. P., Broglio, C., & Salas, C. (2000*b*). Place and cue learning in turtles. *Learning & Behavior*, *28*(4), 360–372. https://doi.org/10.3758/BF03200270

146. López, J. C., Vargas, J. P., Gómez, Y., & Salas, C. (2003). Spatial and non-spatial learning in turtles: the role of medial cortex. *Behavioural Brain Research*, *143*(2), 109–120. https://doi.org/10.1016/S0166-4328(03)00030-5

147. Lorenzoni, M., Corboli, M., Ghetti, L., Pedicillo, G., Carosi, A. (2007). Growth and reproduction of the goldfish *Carassius auratus*: a case study from Italy. In Gheradi F. (Ed.), *Biological Invaders in Inland Waters: Profiles, Distribution, and Threats* (pp. 259–273). Springer-Verlag: New York, NY, USA. https://doi.org/10.1007/978-1-4020-6029-8_13

148. Lourenco, S. F., Addy, D., & Huttenlocher, J. (2009). Location representation in enclosed spaces: What types of information afford young children an advantage? *Journal of Experimental Child Psychology*, *104*(3), 313–325. https://doi.org/10.1016/j.jecp.2009.05.007

149. Lubyk, D. M., Dupuis, B., Gutiérrez, L., & Spetch, M. L. (2012). Geometric orientation by humans: Angles weigh in. *Psychonomic Bulletin & Review*, *19*(3), 436−442. https://doi.org/10.3758/s13423-012-0232-z

150. Lubyk, D. M., Spetch, M. L., Zhou, R., Pisklak, J., & Mou, W. (2013). Reorientation in diamond-shaped environments: Encoding of features and angles in enclosures versus arrays by adult humans and pigeons (*Columbia livia*). *Animal Cognition*, *16*(4), 565−581. https://doi.org/10.1007/s10071-012-0594-7

151. Luchiari, A. C., Málaga-Trillo, E., Tran, S., & Gerlai, R. (2021). Zebrafish cognition and behavior. *Frontiers in Behavioral Neuroscience*, *15*, 659501. https://doi.org/10.3389/fnbeh.2021.659501

152. Ma, E. Y., & Raible, D. W. (2009). Signaling pathways regulating zebrafish lateral line development. *Current Biology*, *19*(9), R381–R386. https://doi.org/10.1016/j.cub.2009.03.057

153. Mackintosh, N. J. (1971). Reward and aftereffects of reward in the learning of goldfish. *Journal of Comparative and Physiological Psychology*, *76*(2), 225–232. https://doi.org/10.1037/h0031405

154. Maguire, E. (2001). The retrosplenial contribution to human navigation: a review of lesion and neuroimaging findings. *Scandinavian Journal of Psychology*, *42*(3), 225–238. https://doi.org/10.1111/1467-9450.00233

155. Maitland, P. S. (2004). *Keys to the freshwater fish of Britain and Ireland, with notes on their distribution and ecology.* Freshwater Biological Association, Amblesid, UK.

156. Maler, L., Karten, H. J., & Bennett, M. V. (1973). The central connections of the anterior lateral line nerve of *Gnathonemus petersii*. *Journal of Comparative Neurology*, *151*(1), 67–84. https://doi.org/10.1002/cne.901510106

157. Martin, J. R. (2004). A portrait of locomotor behaviour in *Drosophila* determined by a video-tracking paradigm. *Behavioural Processes*, *67*(2), 207–219. https://doi.org/10.1016/j.beproc.2004.04.003

158. Meshalkina, D. A., Kizlyk, M. N., Kysil, E. V., Collier, A. D., Echevarria, D. J., Abreu, M. S., ... & Kalueff, A. V. (2017). Understanding zebrafish cognition. *Behavioural Processes*, *141*, 229–241. https://doi.org/10.1016/j.beproc.2016.11.020

159. Michotte, A., Thinès, G., & Crabbé, G. (1991). Amodal completion of perceptual structures (E. Miles & T.R. Miles, Trans.). In G. Thinès, A. Costall, & G. Butterworth (Eds.), *Michotte's experimental phenomenology of perception* (pp. 140–167). Hillsdale, NJ: Erlbaum. (Original work published 1964).

160. Miglino, O., & Lund, H. H. (2001). Do rats need Euclidean cognitive maps of the environmental shape? *Cognitive Processes*, *3*, 22–33.

161. Miglino, O., Ponticorvo, M., & Bartolomeo, P. (2009). Place cognition and active perception: a study with evolved robots. *Connection Science*, *21*(1), 3–14. https://doi.org/10.1080/09540090802364769

162. Miletto Petrazzini, M. E., Pecunioso, A., Dadda, M., & Agrillo, C. (2019). The Impact of brain lateralization and anxiety-like behaviour in an extensive

operant conditioning task in zebrafish (*Danio rerio*). *Symmetry*, *11*(11), 1395. https://doi.org/10.3390/sym11111395

163. Miller, N., & Gerlai, R. (2011) Shoaling in zebrafish: what we don't know. Reviews in the Neurosciences 22(1), 17–25. https://doi.org/10.1515/rns.2011.004

164. Miller, N., & Gerlai, R. (2012). From schooling to shoaling: patterns of collective motion in zebrafish (*Danio rerio*). *PloS one*, *7*(11), e48865. https://doi.org/10.1371/journal.pone.0048865

165. Mingeot-Leclercq, M. P., Glupczynski, Y., & Tulkens, P. M. (1999). Aminoglycosides: activity and resistance. *Antimicrobial Agents and Chemotherapy*, *43*(4), 727–737. https://doi.org/10.1128/AAC.43.4.727

166. Mingeot-Leclercq, M. P., & Tulkens, P. M. (1999). Aminoglycosides: nephrotoxicity. *Antimicrobial Agents and Chemotherapy*, *43*(5), 1003–1012. https://doi.org/10.1128/AAC.43.5.1003

167. Mirjany, M., & Faber, D. S. (2011). Characteristics of the anterior lateral line nerve input to the Mauthner cell. *Journal of Experimental Biology*, *214*(20), 3368–3377. https://doi.org/10.1242/jeb.056226

168. Montgomery, J. C., Baker, C. F., & Carton, A. G. (1997). The lateral line can mediate rheotaxis in fish. *Nature*, *389*(6654), 960–963. https://doi.org/10.1038/40135

169. Montgomery, J. C., McDonald, F., Baker, C. F., Carton, A. G., & Ling, N. (2003). Sensory integration in the hydrodynamic world of rainbow trout. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *270*(Suppl_2), S195-S197. https://doi.org/10.1098/rsbl.2003.0052

170. Morris, R. G. (1981). Spatial localization does not require the presence of local cues. *Learning and Motivation*, *12*(2), 239–260. https://doi.org/10.1016/0023-9690(81)90020-5

171. Morris, R. G. M., Garrud, P., Rawlins, J. A., & O'Keefe, J. (1982). Place navigation impaired in rats with hippocampal lesions. *Nature*, *297*(5868), 681–683. https://doi.org/10.1038/297681a0

172. Mouritsen, H. (2018). Long-distance navigation and magnetoreception in migratory animals. *Nature*, *558*(7708), 50−59. https://doi.org/10.1038/s41586-018-0176-1

173. Mueller-Paul, J., Wilkinson, A., Aust, U., Steurer, M., Hall, G., & Huber, L. (2014). Touchscreen performance and knowledge transfer in the red-footed tortoise (*Chelonoidis carbonaria*). *Behavioural Processes*, *106*, 187–192. https://doi.org/10.1016/j.beproc.2014.06.003

174. Mueller-Paul, J., Wilkinson, A., Hall, G., & Huber, L. (2012). Radial-arm-maze behavior of the red-footed tortoise (*Geochelone carbonaria*). *Journal of Comparative Psychology*, *126*(3), 305–317. https://doi.org/10.1037/a0026881

175. Müller, M., & Wehner, R. (1988). Path integration in desert ants, *Cataglyphis fortis*. *Proceedings of the National Academy of Sciences*, *85*(14), 5287–5290. https://doi.org/10.1073/pnas.85.14.5287

176. Murakami, S. L., Cunningham, L. L., Werner, L. A., Bauer, E., Pujol, R., Raible, D. W., & Rubel, E. W. (2003). Developmental differences in susceptibility to neomycin-induced hair cell death in the lateral line neuromasts of zebrafish (*Danio rerio*). *Hearing Research*, *186*(1–2), 47–56. https://doi.org/10.1016/S0378-5955(03)00259-4

177. Nanay, B. (2018). The importance of amodal completion in everyday perception. *i-Perception*, *9*(4). https://doi.org/10.1177/2041669518788887

178. Nardi, D., Carpenter, S. E., Johnson, S. R., Gilliland, G. A., Melo, V. L., Pugliese, R., ... & Kelly, D. M. (2022). Spatial reorientation with a geometric array of auditory cues. *Quarterly Journal of Experimental Psychology*, *75*(2), 362–373. https://doi.org/10.1177/1747021820913295

179. Nardi, D., Twyman, A. D., Holden, M. P., & Clark, J. M. (2020). Tuning in: can humans use auditory cues for spatial reorientation? *Spatial Cognition & Computation*, *20*(2), 83–103. https://doi.org/10.1080/13875868.2019.1702665

180. Nardini, M., Thomas, R. L., Knowland, V. C., Braddick, O. J., & Atkinson, J. (2009). A viewpoint-independent process for spatial reorientation. *Cognition*, *112*(2), 241–248. https://doi.org/10.1016/j.cognition.2009.05.003

181. Newcombe, N. S., & Huttenlocher, J. (2006). Development of spatial cognition. In D. Kuhn, & R. S. Siegler (Eds.), *Handbook of child psychology: Cognition, perception, and language*, vol. 2 (6th ed., pp. 734–776). New York: Wiley.

182. Newcombe, N. S., & Ratliff, K. R. (2007). Explaining the development of spatial reorientation: Modularity-plus-language versus the emergence of adaptive combination. In J. M. Plumert & J. P. Spencer (Eds.), *The emerging spatial mind* (pp. 63–76). New York: Oxford University Press.

183. Nico, L. & Schofield, P. J. (2006). *Carassius auratus*. *USGS Non-indigenous Aquatic Species Database*, Gainesville, FL.

184. Normandin, M. E., Garza, M. C., Ramos-Alvarez, M. M., Julian, J. B., Eresanara, T., Punjaala, N., ... & Muzzio, I. A. (2022). Navigable Space and Traversable Edges Differentially Influence Reorientation in Sighted and Blind

Mice. *Psychological Science*, *33*(6), 925–947. https://doi.org/10.1177/09567976211055373

185. Ohl, F., Holsboer, F., & Landgraf, R. (2001). The modified hole board as a differential screen for behavior in rodents. *Behavior Research Methods, Instruments, & Computers*, *33*(3), 392–397. https://doi.org/10.3758/BF03195393

186. O'Keefe, J., & Nadel, L. (1978). *The hippocampus as a cognitive map.* Oxford: Clarendon Press.

187. Oliveira, J., Silveira, M., Chacon, D., & Luchiari, A. (2015). The zebrafish world of colors and shapes: preference and discrimination. *Zebrafish*, *12*(2), 166–173. https://doi.org/10.1089/zeb.2014.1019

188. Oman, C. M., Shebilske, W. L., Richards, J. T., Tubré, T. C., Beall, A. C., & Natapoff, A. (2000). Three dimensional spatial memory and learning in real and virtual environments. *Spatial Cognition and Computation*, *2*(4), 355–372. https://doi.org/10.1023/A:1015548105563

189. Orger, M. B., & de Polavieja, G. G. (2017). Zebrafish behavior: opportunities and challenges. *Annual Review of Neuroscience*, *40*, 125–147. https://doi.org/10.1146/annurev-neuro-071714-033857

190. Ou, H. C., Santos, F., Raible, D. W., Simon, J. A., & Rubel, E. W. (2010). Drug screening for hearing loss: using the zebrafish lateral line to screen for drugs that prevent and cause hearing loss. *Drug Discovery Today*, *15*(7–8), 265–271. https://doi.org/10.1016/j.drudis.2010.01.001

191. Owens, K. N., Coffin, A. B., Hong, L. S., Bennett, K. O. C., Rubel, E. W., & Raible, D. W. (2009). Response of mechanosensory hair cells of the zebrafish

lateral line to aminoglycosides reveals distinct cell death pathways. *Hearing Research*, *253*(1–2), 32–41. https://doi.org/10.1016/j.heares.2009.03.001

192. Park, S., Brady, T. F., Greene, M. R., & Oliva, A. (2011). Disentangling scene content from spatial boundary: complementary roles for the parahippocampal place area and lateral occipital complex in representing real-world scenes. *Journal of Neuroscience*, *31*(4), 1333–1340. https://doi.org/10.1523/JNEUROSCI.3885-10.2011
193. Park, S., & Chun, M. M. (2009). Different roles of the parahippocampal place area (PPA) and retrosplenial cortex (RSC) in panoramic scene perception. *Neuroimage*, *47*(4), 1747–1756. https://doi.org/10.1016/j.neuroimage.2009.04.058
194. Paszkowski, C. A., Tonn, W. M., Piironen, J. & Holopainen, I. J. (1990). Behavioural and population-level aspects of intraspecific competition in crucian carp. *Annales Zoologici Fennici*, 27, 77–85.
195. Pearce, J. M., Graham, M., Good, M. A., Jones, P. M., & McGregor, A. (2006). Potentiation, overshadowing, and blocking of spatial learning based on the shape of the environment. *Journal of Experimental Psychology: Animal Behavior Processes*, *32*(3), 201–214. https://doi.org/10.1037/0097-7403.32.3.201
196. Pearce, J. M., Ward-Robinson, J., Good, M., Fussell, C., & Aydin, A. (2001). Influence of a beacon on spatial learning based on the shape of the test environment. *Journal of Experimental Psychology: Animal Behavior Processes*, *27*(4), 329–344. https://doi.org/10.1037/0097-7403.27.4.329

197. Pecchia, T., & Vallortigara, G. (2010*a*). Reorienting strategies in a rectangular array of landmarks by domestic chicks (*Gallus gallus*). *Journal of Comparative Psychology*, *124*(2), 147–158. https://doi.org/10.1037/a0019145

198. Pecchia, T., & Vallortigara, G. (2010*b*). View-based strategy for reorientation by geometry. *Journal of Experimental Biology*, *213*(17), 2987–2996. https://doi.org/10.1242/jeb.043315

199. Pecchia, T., & Vallortigara, G. (2012). Spatial reorientation by geometry with freestanding objects and extended surfaces: a unifying view. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1736), 2228–2236. https://doi.org/10.1098/rspb.2011.2522

200. Pellitteri-Rosa, D., Sacchi, R., Galeotti, P., Marchesi, M., & Fasola, M. (2010). Do Hermann's tortoises (*Testudo hermanni*) discriminate colours? An experiment with natural and artificial stimuli. *Italian Journal of Zoology*, *77*(4), 481–491. https://doi.org/10.1080/11250000903464067

201. Pellow, S., & File, S. E. (1986). Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: a novel test of anxiety in the rat. *Pharmacology Biochemistry and Behavior*, *24*(3), 525–529. https://doi.org/10.1016/0091-3057(86)90552-6

202. Piironen, J. & Holopainen, I. J. (1988). Length structure and reproductive potential of crucian carp (*Carassius carassius L.*) populations in some small forests ponds. *Annales Zoologici Fennici*, 25, 203–208.

203. Piller, K. R., Kenway-Lynch, C. S., Camak, D. T., & Domínguez-Domínguez, O. (2015). Phylogeography and population structure of the imperiled Redtail Splitfin (Goodeidae: *Xenotoca eiseni*): implications for conservation. *Copeia*, *103*(2), 440–454. https://doi.org/10.1643/CI-14-067

204. Ponticorvo, M., & Miglino, O. (2010). Encoding geometric and non-geometric information: a study with evolved agents. *Animal Cognition*, *13*(1), 157–174. https://doi.org/10.1007/s10071-009-0255-7

205. Portavella, M., & Vargas, J. P. (2005). Emotional and spatial learning in goldfish is dependent on different telencephalic pallial systems. *European Journal of Neuroscience*, *21*(10), 2800–2806. https://doi.org/10.1111/j.1460-9568.2005.04114.x

206. Pradhan, A., & Olsson, P. E. (2015). Zebrafish sexual behavior: role of sex steroid hormones and prostaglandins. *Behavioral and Brain Functions*, *11*(1), 23. https://doi.org/10.1186/s12993-015-0068-6

207. Putland, R. L., Montgomery, J. C., & Radford, C. A. (2018). Ecology of fish hearing. *Journal of Fish Biology*, *95*(1), 39–52. https://doi.org/10.1111/jfb.13867

208. Raible, D. W., & Kruse, G. J. (2000). Organization of the lateral line system in embryonic zebrafish. *Journal of Comparative Neurology*, *421*(2), 189–198. https://doi.org/10.1002/(SICI)1096-9861(20000529)421:2<189::AID-CNE5>3.0.CO;2-K

209. Ratliff, K. R., & Newcombe, N. S. (2008). Reorienting when cues conflict: Evidence for an adaptive-combination view. *Psychological Science*, *19*(12), 1301–1307. https://doi.org/10.1111/j.1467-9280.2008.02239.x

210. Ritchie, M. G., Hamill, R. M., Graves, J. A., Magurran, A. E., Webb, S. A., & Macías García, C. (2007). Sex and differentiation: population genetic divergence and sexual dimorphism in Mexican goodeid fish. *Journal of Evolutionary Biology*, *20*(5), 2048–2055. https://doi.org/10.1111/j.1420-9101.2007.01357.x

211. Rodríguez, F., Duran, E., Vargas, J. P., Torres, B., & Salas, C. (1994). Performance of goldfish trained in allocentric and egocentric maze procedures suggests the presence of a cognitive mapping system in fishes. *Learning & Behavior*, *22*(4), 409–420. https://doi.org/10.3758/BF03209160

212. Rodrıguez, F., López, J. C., Vargas, J. P., Broglio, C., Gómez, Y., & Salas, C. (2002). Spatial memory and hippocampal pallium through vertebrate evolution: insights from reptiles and teleost fish. *Brain Research Bulletin*, *57*(3–4), 499–503. https://doi.org/10.1016/S0361-9230(01)00682-7

213. Rodríguez, F., Quintero, B., Amores, L., Madrid, D., Salas-Peña, C., & Salas, C. (2021). Spatial Cognition in Teleost Fish: Strategies and Mechanisms. *Animals*, *11*(8), 2271. https://doi.org/10.3390/ani11082271

214. Rogers L. J., Vallortigara G., Andrew R. J. (2013). *Divided Brains: The Biology and Behaviour of Brain Asymmetries.* Cambridge University Press. https://doi.org/10.1017/CBO9780511793899

215. Salas, C., Broglio, C., Rodríguez, F., López, J. C., Portavella, M., & Torres, B. (1996*a*). Telencephalic ablation in goldfish impairs performance in a 'spatial constancy' problem but not in a cued one. *Behavioural Brain Research*, *79*(1–2), 193–200. https://doi.org/10.1016/0166-4328(96)00014-9

216. Salas, C., Rodríguez, F., Vargas, J. P., Durán, E., & Torres, B. (1996*b*). Spatial learning and memory deficits after telencephalic ablation in goldfish trained in place and turn maze procedures. *Behavioral Neuroscience*, *110*(5), 965–980. https://doi.org/10.1037/0735-7044.110.5.965

217. Santacà, M., Dadda, M., Petrazzini, M. E. M., & Bisazza, A. (2021). Stimulus characteristics, learning bias and visual discrimination in zebrafish

(*Danio rerio*). *Behavioural Processes*, *192*, 104499. https://doi.org/10.1016/j.beproc.2021.104499

218. Santacà, M., Miletto Petrazzini, M. E., Agrillo, C., & Wilkinson, A. (2019). Can reptiles perceive visual illusions? Delboeuf illusion in red-footed tortoise (*Chelonoidis carbonaria*) and bearded dragon (*Pogona vitticeps*). *Journal of Comparative Psychology*, *133*(4), 419–427. https://doi.org/10.1037/com0000176

219. Santacà, M., Miletto Petrazzini, M. E., Wilkinson, A., & Agrillo, C. (2020). Red-footed tortoises (*Chelonoidis carbonaria*) do not perceive the Delboeuf illusion. *Canadian Journal of Experimental Psychology*, *74*(3), 201–206. https://doi.org/10.1037/cep0000203

220. Santos, F., MacDonald, G., Rubel, E. W., & Raible, D. W. (2006). Lateral line hair cell maturation is a determinant of aminoglycoside susceptibility in zebrafish (*Danio rerio*). *Hearing Research*, *213*(1–2), 25–33. https://doi.org/10.1016/j.heares.2005.12.009

221. Schemmel, C. (1967). Vergleichende Untersuchungen an den Hautsinnesorganen ober- und unterirdisch lebender *Astyanax-Formen*. *Zeitschrift für Morphologie der Tiere*, *61*(2), 255–316. https://doi.org/10.1007/BF00400988

222. Schenk, F., & Morris, R. G. M. (1985). Dissociation between components of spatial memory in rats after recovery from the effects of retrohippocampal lesions. *Experimental Brain Research*, *58*(1), 11–28. https://doi.org/10.1007/BF00238949

223. Schinazi, V. R., Thrash, T., & Chebat, D. R. (2016). Spatial navigation by congenitally blind individuals. *Wiley Interdisciplinary Reviews: Cognitive Science*, *7* (1), 37–58. https://doi.org/10.1002/wcs.1375

224. Séguinot, V., Maurer, R., & Etienne, A. S. (1993). Dead reckoning in a small mammal: the evaluation of distance. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology*, *173*(1), 103–113. https://doi.org/10.1007/BF00209622

225. Sguanci, S., Ceccolini, F., & Berti, R. (2010). Non visual discrimination of shapes in the blind cave cyprinid *Phreatichthys andruzzii* Vinciguerra 1924. *Ethology Ecology & Evolution*, *22*(4), 353–358. https://doi.org/10.1080/03949370.2010.510038

226. Sharma, S., Coombs, S., Patton, P., & De Perera, T. B. (2009). The function of wall-following behaviors in the Mexican blind cavefish and a sighted relative, the Mexican tetra (*Astyanax*). *Journal of Comparative Physiology A*, *195*(3), 225–240. https://doi.org/10.1007/s00359-008-0400-9

227. Shusterman, A., Lee, S. A., & Spelke, E. S. (2011). Cognitive effects of language on human navigation. *Cognition*, *120*(2), 186–201. https://doi.org/10.1016/j.cognition.2011.04.004

228. Silveira, M. M., Ferreira de Souza, J., Pires Moreira, A. L., Silva, P. F., & Luchiari, A. C. (2019). Memory retention of appetitive and aversive conditioning in the damselfish *Stegastes fuscus*. *Journal of Fish Biology*, *95*(3), 772–780. https://doi.org/10.1111/jfb.14063

229. Simon, P., Dupuis, R., & Costentin, J. (1994). Thigmotaxis as an index of anxiety in mice. Influence of dopaminergic transmissions. *Behavioural Brain Research*, *61*(1), 59–64. https://doi.org/10.1016/0166-4328(94)90008-6

230. Sison, M., & Gerlai, R. (2010). Associative learning in zebrafish (*Danio rerio*) in the plus maze. *Behavioural Brain Research*, *207*(1), 99–104. https://doi.org/10.1016/j.bbr.2009.09.043

231. Smeets, W. J., Marin, O., & Gonzalez, A. (2000). Evolution of the basal ganglia: new perspectives through a comparative approach. *Journal of Anatomy*, *196*(4), 501–517. https://doi.org/10.1046/j.1469-7580.2000.19640501.x

232. Solstad, T., Boccara, C. N., Kropff, E., Moser, M. B., & Moser, E. I. (2008). Representation of geometric borders in the entorhinal cortex. *Science*, *322*(5909), 1865–1868. https:// doi/10.1126/science.1166466

233. Song, J., Yan, H. Y., & Popper, A. N. (1995). Damage and recovery of hair cells in fish canal (but not superficial) neuromasts after gentamicin exposure. *Hearing Research*, *91*(1–2), 63–71. https://doi.org/10.1016/0378-5955(95)00170-0

234. Sovrano, V. A., Albertazzi, L., & Rosa Salva, O. (2015). The Ebbinghaus illusion in a fish (*Xenotoca eiseni*). *Animal Cognition*, *18*(2), 533–542. https://doi.org/10.1007/s10071-014-0821-5

235. Sovrano, V. A., Baratti, G, & Lee S. A (2020*a*). The role of learning and environmental geometry in landmark-based spatial reorientation of fish (*Xenotoca eiseni*). *PloS one*, *15*(3), e0229608. https://doi.org/10.1371/journal.pone.0229608

236. Sovrano, V. A., Baratti, G., & Potrich, D. (2018). A detour task in four species of fishes. *Frontiers in Psychology*, *9*, 2341. https://doi.org/10.3389/fpsyg.2018.02341

237. Sovrano, V. A., Baratti, G., Potrich, D., & Bertolucci, C. (2020*b*). The geometry as an eyed fish feels it in spontaneous and rewarded spatial reorientation tasks. *Scientific Reports*, *10*(1), 1–14. https://doi.org/10.1038/s41598-020-64690-1

238. Sovrano, V. A., Baratti, G., Potrich, D., Rosà, T., & Mazza, V. (2022*a*). “Classifying-together” phenomenon in fish (*Xenotoca eiseni*): Simultaneous exposure to visual stimuli impairs subsequent discrimination learning. *PloS one*, *17*(8), e0272773. https://doi.org/10.1371/journal.pone.0272773

239. Sovrano, V. A., & Bisazza, A. (2008). Recognition of partly occluded objects by fish. *Animal Cognition*, *11*(1), 161–166. https://doi.org/10.1007/s10071-007-0100-9

240. Sovrano, V. A., & Bisazza, A. (2009). Perception of subjective contours in fish. *Perception*, *38*(4), 579–590. https://doi.org/10.1068/p6121

241. Sovrano, V. A., Bisazza, A., & Vallortigara, G. (2001). Lateralization of response to social stimuli in fishes: a comparison between different methods and species. *Physiology & Behavior*, *74*(1-2), 237–244. https://doi.org/10.1016/S0031-9384(01)00552-2

242. Sovrano, V. A., Bisazza, A., & Vallortigara, G. (2002). Modularity and spatial reorientation in a simple mind: Encoding of geometric and nongeometric properties of a spatial environment by fish. *Cognition*, *85*(2), B51–B59. https://doi.org/10.1016/S0010-0277(02)00110-5

243. Sovrano, V. A., Bisazza, A., & Vallortigara, G. (2003). Modularity as a fish (*Xenotoca eiseni*) views it: conjoining geometric and nongeometric information for spatial reorientation. *Journal of Experimental Psychology:*

Animal Behaviour Processes, *29*(3), 199–210. https://doi.org/10.1037/0097-7403.29.3.199

244. Sovrano, V. A., Bisazza, A., & Vallortigara, G. (2005*a*). Animals' use of landmarks and metric information to reorient: Effects of the size of the experimental space. *Cognition*, *97*(2), 121–133. https://doi.org/10.1016/j.cognition.2004.08.003

245. Sovrano, V. A., Bisazza, A., & Vallortigara, G. (2007). How fish do geometry in large and in small spaces. *Animal Cognition*, *10*(1), 47–54. https://doi.org/10.1007/s10071-006-0029-4

246. Sovrano, V. A., & Chiandetti, C. (2017). Reorientation ability in redtail splitfin (*Xenotoca eiseni*): Role of environmental shape, rearing in group and exposure time. *Biological Communications*, *62*(1), 48–56. https://dx.doi.org/10.21638/11701/spbu03.2017.106

247. Sovrano, V. A., Dadda, M., & Bisazza, A. (2005*b*). Lateralized fish perform better than nonlateralized fish in spatial reorientation tasks. *Behavioural Brain Research*, *163*(1), 122–127. https://doi.org/10.1016/j.bbr.2005.04.012

248. Sovrano, V. A., Da Pos, O., & Albertazzi, L. (2016). The Müller-Lyer illusion in the teleost fish *Xenotoca eiseni*. *Animal Cognition*, *19*(1), 123–132. https://doi.org/10.1007/s10071-015-0917-6

249. Sovrano, V. A., Potrich, D., Foà, A., & Bertolucci, C. (2018*a*). Extra-visual systems in the spatial reorientation of cavefish. *Scientific Rep*orts, *8*(1), 17698, 1-9. https://doi.org/10.1038/s41598-018-36167-9

250. Sovrano, V. A., Potrich, D., & Vallortigara, G. (2013). Learning of geometry and features in bumblebees (*Bombus terrestris*). *Journal of Comparative Psychology*, *127*(3), 312–318. https://doi.org/10.1037/a0032040

251. Sovrano, V. A., Quaresmini, C., & Stancher, G. (2018*b*). Tortoises in front of mirrors: Brain asymmetries and lateralized behaviours in the tortoise (*Testudo hermanni*). *Behavioural Brain Research*, *352*, 183–186. https://doi.org/10.1016/j.bbr.2017.06.021

252. Sovrano, V. A., Rainoldi, C., Bisazza, A., & Vallortigara, G. (1999). Roots of brain specializations: preferential left-eye use during mirror-image inspection in six species of teleost fish. *Behavioural Brain Research*, *106*(1-2), 175–180. https://doi.org/10.1016/S0166-4328(99)00105-9

253. Sovrano, V. A., Rigosi, E., & Vallortigara, G. (2012). Spatial reorientation by geometry in bumblebees. *PloS one*, *7*, e37449. https://doi.org/10.1371/journal.pone.0037449

254. Sovrano, V. A., & Vallortigara, G. (2006). Dissecting the geometric module: A sense linkage for metric and landmark information in animals' spatial reorientation. *Psychological Science*, *17*(7), 616–621. https://doi.org/10.1111/j.1467-9280.2006.01753.x

255. Sovrano, V. A., Vicidomini, S., Potrich, D., Miletto Petrazzini, M. E., Baratti, G., & Rosa-Salva, O. (2022*b*). Visual discrimination and amodal completion in zebrafish. *Plos one*, *17*(3), e0264127. https://doi.org/10.1371/journal.pone.0264127

256. Spelke, E. S., Lee, S. A., & Izard, V. (2010). Beyond core knowledge: Natural geometry. *Cognitive Science*, *34*(5), 863−884. https://doi.org/10.1111/j.1551-6709.2010.01110.x

257. Spence, R., Gerlach, G., Lawrence, C., & Smith, C. (2008). The behaviour and ecology of the zebrafish, *Danio rerio*. *Biological Reviews*, *83*(1), 13–34. https://doi.org/10.1111/j.1469-185X.2007.00030.x

258. Spence, R., Magurran, A. E., & Smith, C. (2011). Spatial cognition in zebrafish: the role of strain and rearing environment. *Animal Cognition*, *14*(4), 607–612. https://doi.org/10.1007/s10071-011-0391-8

259. Spence, R., & Smith, C. (2006). Mating preference of female zebrafish, *Danio rerio*, in relation to male dominance. *Behavioral Ecology*, *17*(5), 779–783. https://doi.org/10.1093/beheco/arl016

260. Spotila, J. R., Terpin, K. M., Koons, R. R., & Bonati, R. L. (1979). Temperature requirements of fishes from eastern Lake Erie and upper Niagara River. *Environmental Biology of Fishes*, 4, 281–307.

261. Stancher, G., Clara, E., Regolin, L., & Vallortigara, G. (2006). Lateralized righting behavior in the tortoise (*Testudo hermanni*). *Behavioural Brain Research*, *173*(2), 315–319. https://doi.org/10.1016/j.bbr.2006.06.023

262. Stancher, G., Sovrano, V. A., Potrich, D., & Vallortigara, G. (2013). Discrimination of small quantities by fish (redtail splitfin, *Xenotoca eiseni*). *Animal Cognition*, *16*(2), 307–312. https://doi.org/10.1007/s10071-012-0590-y

263. Stubbs, D. (1989): *Testudo hermanni*. In: R. I. Swingland & M. W. Klemens (Eds.), *The Conservation Biology of Tortoises* (pp. 34–36). IUCN 5, Gland, Switzerland.

264. Stubbs, D., & Swingland, I. R. (1985). The ecology of a Mediterranean tortoise (*Testudo hermanni*): a declining population. *Canadian Journal of Zoology*, *63*(1), 169–180. https://doi.org/10.1139/z85-026

265. Stürzl, W., Cheung, A., Cheng, K., & Zeil, J. (2008). Information content of panoramic images: I. Rotational errors and the similarity of views in rectangular arenas. *Journal of Experimental Psychology: Animal Behavior Processes*, *34*(1), 1–14. https://doi.org/10.1037/0097-7403.34.1.1

266. Sutherland, L., Holbrook, R. I., & Burt De Perera, T. (2009). Sensory system affects orientational strategy in a short-range spatial task in blind and eyed morphs of the fish, *Astyanax fasciatus*. *Ethology*, *115*(5), 504–510. https://doi.org/10.1111/j.1439-0310.2009.01630.x

267. Teyke, T. (1985). Collision with and avoidance of obstacles by blind cave fish *Anoptichthys jordani* (Characidae). *Journal of Comparative Physiology A*, *157*(6), 837–843. https://doi.org/10.1007/BF01350081

268. Teyke, T. (1989). Learning and remembering the environment in the blind cave fish *Anoptichthys jordani*. *Journal of Comparative Physiology A*, *164*(5), 655–662. https://doi.org/10.1007/BF00614508

269. Teyke, T., & Schaerer, S. (1994). Blind Mexican cave fish (*Astyanax hubbsi*) respond to moving visual stimuli. *Journal of Experimental Biology*, *188*(1), 89–101. https://doi.org/10.1242/jeb.188.1.89

270. Thorndike, E. L. (1927). The law of effect. *The American journal of Psychology*, *39*(1/4), 212–222. https://doi.org/10.2307/1415413

271. Tinbergen, N. (1932). Über die Orientierung des Bienenwolfes (Philanthus triangulum Fabr.). *Zeitschrift für Vergleichende Physiologie*, *16*(3), 305–334. https://doi.org/10.1007/BF00339640

272. Tinbergen, N. (1951). *The study of instinct.* Oxford: Oxford University Press.

273. Tolman, E. C., & Honzik, C. H. (1930*a*). Degrees of hunger, reward and non-reward, and maze learning in rats. *University of California Publications in Psychology*, *4*, 241–256.

274. Tolman, E. C., & Honzik, C. H. (1930*b*). Introduction and removal of reward, and maze performance in rats. *University of California Publications in Psychology*, *4*, 257–275.

275. Tolman, E. C., Ritchie, B. F., & Kalish, D. (1946). Studies in spatial learning. II. Place learning versus response learning. *Journal of Experimental Psychology*, *36*(3), 221–229. https://doi.org/10.1037/h0060262

276. Tommasi, L., Chiandetti, C., Pecchia, T., Sovrano, V. A., & Vallortigara, G. (2012). From natural geometry to spatial cognition. *Neuroscience & Biobehavioral Reviews*, *36*(2), 799–824. https://doi.org/10.1016/j.neubiorev.2011.12.007

277. Tommasi, L., & Polli, C. (2004). Representation of two geometric features of the environment in the domestic chick (*Gallus gallus*). *Animal Cognition*, *7*(1), 53−59. https://doi.org/10.1007/s10071-003-0182-y

278. Tommasi, L., & Vallortigara, G. (2000). Searching for the center: spatial cognition in the domestic chick (*Gallus gallus*). *Journal of Experimental Psychology: Animal Behavior Processes*, *26*(4), 477–486. https://doi.org/10.1037/0097-7403.26.4.477

279. Tonn, W. M., Paszkowski, C. A., & Holopainen, I. J. (1991). Selective piscivory by perch: effects of predator size, prey size, and prey species. *Verhandlungen der Internationale Vereinigung Limnologie*, 24, 2406–2411.

280. Treit, D., & Fundytus, M. (1988). Thigmotaxis as a test for anxiolytic activity in rats. *Pharmacology Biochemistry and Behavior*, *31*(4), 959–962. https://doi.org/10.1016/0091-3057(88)90413-3

281. Truppa, V., Sovrano, V. A., Spinozzi, G., & Bisazza, A. (2010). Processing of visual hierarchical stimuli by fish (*Xenotoca eiseni*). *Behavioural Brain Research*, *207*(1), 51–60. https://doi.org/10.1016/j.bbr.2009.09.039

282. Twyman, A., Friedman, A., & Spetch, M. L. (2007). Penetrating the geometric module: catalyzing children's use of landmarks. *Developmental Psychology*, *43*(6), 1523–1530. https://doi.org/10.1037/0012-1649.43.6.1523

283. Twyman, A. D., Holden, M. P., & Newcombe, N. S. (2018). First direct evidence of cue integration in reorientation: A new paradigm. *Cognitive Science*, *42*(3), 923−936. https://doi.org/10.1111/cogs.12575

284. Twyman, A. D., & Newcombe, N. S. (2010). Five reasons to doubt the existence of a geometric module. *Cognitive Science*, *34*(7), 1315–1356. https://doi.org/10.1111/j.1551-6709.2009.01081.x

285. Vallortigara, G. (2009). Animals as natural geometers. In L. Tommasi, M. A. Peterson, & L. Nadel (Eds.), *Cognitive Biology* (pp. 83–104). MIT Press. https://doi.org/10.7551/mitpress/9780262012935.003.0081

286. Vallortigara, G. (2012). Core knowledge of object, number, and geometry: A comparative and neural approach. *Cognitive Neuropsychology*, *29*(1–2), 213–236. https://doi.org/10.1080/02643294.2012.654772

287. Vallortigara, G. (2018). Comparative cognition of number and space: the case of geometry and of the mental number line. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *373*(1740), 20170120. https://doi.org/10.1098/rstb.2017.0120

288. Vallortigara, G., Zanforlin, M., & Pasti, G. (1990). Geometric modules in animals' spatial representations: a test with chicks (*Gallus gallus domesticus*). *Journal of Comparative Psychology*, *104*(3), 248–254. https://doi.org/10.1037/0735-7036.104.3.248

289. Van Bergeijk, W. A. (1967). The evolution of vertebrate hearing. In W. D. Neff (Ed.), *Contributions to sensory physiology*, vol. 2 (pp. 1–49). New York: Academic Press. https://doi.org/10.1016/B978-1-4831-6749-7.50007-6

290. Van Dijk, P.P., Corti, C., Mellado, V. P. & Cheylan, M. (2004). *Testudo hermanni* (errata version published in 2020). *The IUCN Red List of Threatened Species 2004*: e.T21648A176604335. https://dx.doi.org/10.2305/IUCN.UK.2004.RLTS.T21648A176604335.en

291. Van Trump, W. J., Coombs, S., Duncan, K., & McHenry, M. J. (2010). Gentamicin is ototoxic to all hair cells in the fish lateral line system. *Hearing Research*, *261*(1–2), 42–50. https://doi.org/10.1016/j.heares.2010.01.001

292. Vargas, J. P., Bingman, V. P., Portavella, M., & López, J. C. (2006). Telencephalon and geometric space in goldfish. *European Journal of Neuroscience*, *24*(10), 2870–2878. https://doi.org/10.1111/j.1460-9568.2006.05174.x

293. Vargas, J. P., López, J. C., Salas, C., & Thinus-Blanc, C. (2004). Encoding of geometric and featural spatial information by goldfish (*Carassius auratus*). *Journal of Comparative Psychology*, *118*(2), 206–216. https://doi.org/10.1037/0735-7036.118.2.206

294. Vargas, J. P., Portavella, M., Quintero, E., & López, J. C. (2011). Neural basis of the spatial navigation based on geometric cues. *Behavioural Brain Research*, *225*(1), 367–372. https://doi.org/10.1016/j.bbr.2011.07.027

295. Vernier, P., Kyzar, E. J., Maximino, C., Tierney, K., Gebhardt, M., Lange, M., ... & Kalueff, A. V. (2012). Time to recognize zebrafish ‘affective’ behavior. *Behaviour*, *149*(10–12), 1019–1036. https://doi.org/10.1163/1568539X-00003030

296. Von Campenhausen, C., Riess, I., & Weissert, R. (1981). Detection of stationary objects by the blind Cave Fish *Anoptichthys jordani* (Characidae). *Journal of Comparative Physiology*, *143*(3), 369–374. https://doi.org/10.1007/BF00611175

297. Von der Emde, G., & Bleckmann, H. (1998). Finding food: senses involved in foraging for insect larvae in the electric fish *Gnathonemus petersii*. *Journal of Experimental Biology*, *201*(7), 969–980. https://doi.org/10.1242/jeb.201.7.969

298. Wang, R. F., Hermer, L., & Spelke, E. S. (1999). Mechanisms of reorientation and object localization by children: a comparison with rats. *Behavioral neuroscience*, *113*(3), 475–485. https://doi.org/10.1037/0735-7044.113.3.475

299. Wang, R. F., & Spelke, E. S. (2003). Comparative approaches to human navigation. In K. J. Jeffery (Ed.), *The neurobiology of spatial behaviour* (pp. 119–143). Oxford: Oxford University Press.

300. Webb, J. F. (1989). Gross morphology and evolution of the mechanoreceptive lateral-line system in teleost fishes (Part 1 of 2). *Brain, Behavior and Evolution*, *33*(1), 34–43. https://doi.org/10.1159/000115896

301. Webb, J. F. (1989). Developmental constraints and evolution of the lateral line system in teleost fishes. In S. Coombs, P. Görner, & H. Münz (Eds.),

The mechanosensory lateral line (pp. 79–97). New York: Springer. https://doi.org/10.1007/978-1-4612-3560-6_4

302. Weissert, R., & Von Campenhausen, C. (1981). Discrimination between stationary objects by the blind cave fish *Anoptichthys jordani* (Characidae). *Journal of Comparative Physiology*, *143*(3), 375–381. https://doi.org/10.1007/BF00611176

303. Wiener, J., Shettleworth, S., Bingman, V. P., Cheng, K., Healy, S., Jacobs, L. F., Jeffery, K. J. (2011) Animal Navigation—A Synthesis. In R. Menzel & J. Fisher (Eds.), *Animal Thinking: Contemporary Issues in Comparative Cognition* (pp. 51–78). MIT Press: Cambridge, MA, USA.

304. Wilkinson A., Glass E. (2018) Cold-blooded cognition: How to get a tortoise out of its shell. In F. Amici, & N. Bueno (Eds.), *Animal cognition*. Cambridge, UK: Cambridge University Press

305. Wilkinson, A., Kuenstner, K., Mueller, J., & Huber, L. (2010). Social learning in a non-social reptile (*Geochelone carbonaria*). *Biology Letters*, *6*(5), 614–616. https://doi.org/10.1098/rsbl.2010.0092

306. Williams, F. E., White, D., & Messer Jr, W. S. (2002). A simple spatial alternation task for assessing memory function in zebrafish. *Behavioural Processes*, *58*(3), 125–132. https://doi.org/10.1016/S0376-6357(02)00025-6

307. Wilson, P. N., & Alexander, T. (2008). Blocking of spatial learning between enclosure geometry and a local landmark. *Journal of Experimental Psychology: Learning, Memory, and Cognition*, *34*(6), 1369–1376. https://doi.org/10.1037/a0013011

308. Wiltschko, R., & Wiltschko, W. (2003). Avian navigation: from historical to modern concepts. *Animal Behaviour*, *65*(2), 257–272. https://doi.org/10.1006/anbe.2003.2054

309. Windsor, S. P., Norris, S. E., Cameron, S. M., Mallinson, G. D., & Montgomery, J. C. (2010). The flow fields involved in hydrodynamic imaging by blind Mexican cave fish (*Astyanax fasciatus*). Part I: open water and heading towards a wall. *Journal of Experimental Biology*, *213*(22), 3819–3831. https://doi.org/10.1242/jeb.040741

310. Windsor, S. P., Tan, D., & Montgomery, J. C. (2008). Swimming kinematics and hydrodynamic imaging in the blind Mexican cave fish (*Astyanax fasciatus*). *Journal of Experimental Biology*, *211*(18), 2950–2959. https://doi.org/10.1242/jeb.020453

311. Wong, K., Elegante, M., Bartels, B., Elkhayat, S., Tien, D., Roy, S., ... & Kalueff, A. V. (2010). Analyzing habituation responses to novelty in zebrafish (*Danio rerio*). *Behavioural Brain Research*, *208*(2), 450–457. https://doi.org/10.1016/j.bbr.2009.12.023

312. Wystrach, A. (2009). Ants in rectangular arenas: a support for the global matching theory. *Communicative & Integrative Biology*, *2*(5), 388–390. https://doi.org/10.4161/cib.2.5.8717

313. Wystrach, A., & Beugnon, G. (2009). Ants learn geometry and feature. *Current Biology*, *19*(1), 61–66. https://doi.org/10.1016/j.cub.2008.11.054

314. Wystrach, A., Beugnon, G., & Cheng, K. (2011*a*). Landmarks or panoramas: What do navigating ants attend to for guidance? *Frontiers in Zoology*, *8*(1), 21. https://doi.org/10.1186/1742-9994-8-21

315. Wystrach, A., Cheng, K., Sosa, S., & Beugnon, G. (2011*b*). Geometry, features, and panoramic views: Ants in rectangular arenas. *Journal of Experimental Psychology, Animal Behavior Processes*, *37*(4), 420–435. https://doi.org/10.1037/a0023886

316. Yoshizawa, M., Gorički, Š., Soares, D., & Jeffery, W. R. (2010). Evolution of a behavioral shift mediated by superficial neuromasts helps cavefish find food in darkness. *Current Biology*, *20*(18), 1631–1636. https://doi.org/10.1016/j.cub.2010.07.017

317. Yoshizawa, M., Yamamoto, Y., O'Quin, K. E., & Jeffery, W. R. (2012). Evolution of an adaptive behavior and its sensory receptors promotes eye regression in blind cavefish. *BMC Biology*, *10*(1), 108. https://doi.org/10.1186/1741-7007-10-108

Supplementary Materials

Study	Experiment	Statistics
No. 1 – Conspicuous landmark (blue wall) in zebrafish	Experiment 1: *Long blue wall landmark* Experiment 2: *Short blue wall landmark*	• One-way ANOVA (trials × wall length; trials × wall distance) • Repeated measure ANOVA (corner × wall length × wall distance) on first/total choices [%] in learning session • Paired samples *t*-test (A vs. B vs. C vs. D) on first/total choices [%] in learning session • Paired samples *t*-test (AC vs. BD) on first/total choices [%] in geometric test (test 1) • Repeated measure ANOVA (corner) on first/total choices [%] in affine transformation (test 2) • Paired samples *t*-test (A vs. B vs. C vs. D) on first/total choices [%] in affine transformation (test 2)
No. 2 – Local landmarks (corner panels) in zebrafish	Experiment 1: *Landmark-use in a rectangular opaque arena*	• Repeated measure ANOVA (corner × panel) on first/total choices [%] in learning session • Paired samples *t*-test (A vs. B vs. C vs. D) on first/total choices [%] in learning session • Repeated measure ANOVA (corner) on first/total choices [%] in affine transformation (test 1) • Paired samples *t*-test (A vs. B vs. C vs. D) on first/total choices [%] in affine transformation (test 1) • Repeated measure ANOVA (corner) on first/total choices [%] in diagonal transposition (test 2) • Paired samples *t*-test (A vs. B vs. C vs. D) on first/total choices [%] in diagonal transposition (test 2)

		• Paired samples *t*-test (AC vs. BD) on first/total choices [%] in partial removal of panels (test 3) • Paired samples *t*-test (AC vs. BD) on first/total choices [%] in total removal of panels (test 4)
	Experiment 2: *Landmark-use in a large square opaque arena*	• No tests used
	Experiment 3: *Landmark-use in a small square opaque arena*	• No tests used
	Experiment 4: *Landmark-use in a large square transparent arena*	• Repeated measure ANOVA (corner × panel) on first/total choices [%] in learning session • Paired samples *t*-test (A vs. B vs. C vs. D) on first/total choices [%] in learning session

No. 3 – environmental geometry in tortoises	Experiment 1	• Paired samples *t*-test ($C_{1,2}$ vs. $X_{1,2}$) on total choices [prop] in learning + validation session • Paired samples *t*-test ($C_{1,2}$ wall-following vs. centre-to-corner) on motion strategy in learning + validation session • Paired samples *t*-test ($C_{1,2}$ wall-following left vs. right) on motion direction in learning + validation session • Repeated measure ANOVA (zone) on time spent [%] in learning + validation session • Paired samples *t*-test (perimetrical vs. diagonal vs. neutral) on time spent [%] in learning + validation session • One-sample *t*-test on motion strategy (wall-following vs. centre-

		to-corner) and direction (left vs. right) in learning + validation session

No. 4 – Conspicuous landmark (blue wall) in tortoises	Experiment 1: *Blue wall landmark near the correct corner* Experiment 2: *Blue wall landmark far from the correct corner*	• One-way ANOVA (trials × wall distance) • Repeated measure ANOVA (corner × wall distance) on total choices [prop] in learning + validation session • Paired samples *t*-test (A vs. B vs. C vs. D) on total choices [prop] in learning + validation session • Repeated measure ANOVA (motion strategy × wall distance) on total choices [prop] in learning + validation session • Repeated measure ANOVA (motion direction × wall distance) on total choices [prop] in learning + validation session • One-sample *t*-test on motion strategy (wall-following vs. centre-to-corner) and direction (left vs. right) in learning + validation session

No. 5 – Nonvisual environmental geometry in zebrafish, redtail splitfin fish, and goldfish	Experiment 1: *Social-cued memory task with no experience*	• Repeated measure ANOVA (time × geometry × species) on first/total choices [f]
	Experiment 2: *Social-cued memory task with short experience*	• Repeated measure ANOVA (time × geometry × species) on first/total choices [f] • Paired samples Wilcoxon test (CR vs. NF) on first choices [f]
	Experiment 3: *Rewarded exit task with extensive experience*	• One-way ANOVA (trials × species) • Repeated measure ANOVA (geometry × species) on first/total choices [f] in learning session

		• Paired samples Wilcoxon test (C_1 vs. C_2; X_1 vs. X_2) on first choices [f] in learning session • Paired samples *t*-test (C_1 vs. C_2; X_1 vs. X_2) on total choices [f] in learning session • Repeated measure ANOVA (geometry × species) on first/total choices [f] in geometric test • Paired samples Wilcoxon test (C_1 vs. C_2; X_1 vs. X_2) on first choices [f] in geometric test • Paired samples *t*-test (C_1 vs. C_2; X_1 vs. X_2) on total choices [f] in geometric test • Repeated measure ANOVA (time × geometry × species) on first/total choices [f]
No. 6 – Isolated environmental geometric cues in zebrafish	Experiment 1: *Use of boundary distance*	• Unpaired samples *t*-test (diagonal 1 vs. diagonal 2) on trials to learn • Paired samples *t*-test ($C_{1,2}$ vs. $X_{1,2}$) on first/total choices [%] in learning session • Paired samples *t*-test (C_1 vs. C_2; X_1 vs. X_2) on first/total choices [%] in learning session • Paired samples *t*-test ($C_{1,2}$ vs. $X_{1,2}$) on first/total choices [%] in validation session • Paired samples *t*-test (C_1 vs. C_2; X_1 vs. X_2) on first/total choices [%] in validation session
	Experiment 2: *Use of freestanding corners*	• Unpaired samples *t*-test (diagonal 1 vs. diagonal 2) on trials to learn • Paired samples *t*-test ($C_{1,2}$ vs. $X_{1,2}$) on first/total choices [%] in learning session • Paired samples *t*-test (C_1 vs. C_2; X_1 vs. X_2) on first/total choices [%] in learning session

		• Paired samples *t*-test ($C_{1,2}$ vs. $X_{1,2}$) on first/total choices [%] in validation session • Paired samples *t*-test (C_1 vs. C_2; X_1 vs. X_2) on first/total choices [%] in validation session
	Experiment 3: *Use of boundary length*	• Unpaired samples *t*-test (diagonal 1 vs. diagonal 2) on trials to learn • Paired samples *t*-test ($C_{1,2}$ vs. $X_{1,2}$) on first/total choices [%] in learning session • Paired samples *t*-test (C_1 vs. C_2; X_1 vs. X_2) on first/total choices [%] in learning session • Paired samples *t*-test ($C_{1,2}$ vs. $X_{1,2}$) on first/total choices [%] in validation session • Paired samples *t*-test (C_1 vs. C_2; X_1 vs. X_2) on first/total choices [%] in validation session
	Experiment 4: *Control*	• Repeated measure ANOVA (geometry × session × diagonal) on first/total choices [%]
No. 7 – 3D outside landmark (blue cylinder) in zebrafish	Experiment 1: *Proximal Short* Experiment 2: *Proximal Long* Experiment 3: *Distal Short* Experiment 4: *Distal Long*	• Repeated measure ANOVA (session × corner × condition) on total choices [f] in the last four training sessions • Repeated measure ANOVA (session × corner) on total choices [f] in the last four training sessions, in the four conditions separately • Paired samples *t*-test (C vs. N vs. R vs. F) on total choices [f] in the last four training sessions, in the four conditions separately • Repeated measure ANOVA (session × corner × condition) on total choices [f] in the last two training sessions • Repeated measure ANOVA (session × corner) on total choices

	[f] in the last two training sessions, in the four conditions separately • Paired samples *t*-test (C vs. N vs. R vs. F) on total choices [f] in the last two training sessions, in the four conditions separately • Repeated measure ANOVA (session × diagonal × condition) on total choices [f] over twenty-five training sessions, for unsuccessful fish • Paired samples *t*-test (CR vs. NF) on total choices [f] over twenty-five training sessions • Repeated measure ANOVA (session × diagonal × condition) on total choices [f] in the last two sessions before learning for successful fish and the last two training sessions for unsuccessful fish, in the four conditions separately • Paired samples *t*-test (CR vs. NF) on total choices [f] in the last two sessions before learning for successful fish and the last two training sessions for unsuccessful fish, in the four conditions separately • Repeated measure ANOVA (session × diagonal × condition) on total choices [f] in the last two sessions before learning for successful fish, after collapsing the three conditions in which fish learned • Paired samples *t*-test (CR vs. NF) on total choices [f] in the last two sessions before learning for successful fish, after collapsing the three conditions in which fish learned

		• Unpaired samples *t*-test (proximal short vs. proximal long) on trials to learn • Repeated measure ANOVA (session × corner × condition) on total choices [f] in learning and validation session, after collapsing the three conditions in which fish learned • Paired samples *t*-test (C vs. N vs. R vs. F) on total choices [f] in learning and validation session separately • Repeated measure ANOVA (session × condition) on latency times [s] in the first and last training sessions • Paired samples *t*-test (first vs. last training sessions) on latency times [s] in the four conditions separately • Repeated measure ANOVA (session × strategy × condition) on C total choices [f] in the last two training sessions • Repeated measure ANOVA (session × direction × condition) on C wall-following total choices [f] in the last two training sessions

No. 8 – Lateral line pharmacological ablation in zebrafish	Experiment 1: *Protocol 1, 10 mg/L gentamicin*	• Repeated measure ANOVA (geometry × session) on total choices [prop]
	Experiment 2: *Protocol 1, 20 mg/L gentamicin*	• Repeated measure ANOVA (geometry × session) on total choices [prop]
	Experiment 3: *Protocol 2, 20 mg/L gentamicin*	• Repeated measure ANOVA (treatment × geometry × session) on total choices [prop]

	Experiment 4: *Protocol 1, 40 mg/L gentamicin*	• Repeated measure ANOVA (geometry × session) on total choices [prop]
	Experiment 5: *Protocol 2, 40 mg/L gentamicin*	• Repeated measure ANOVA (treatment × geometry × session) on total choices [prop]
No. 8 – Lateral line pharmacological ablation in zebrafish	Experiments 1-5	• Repeated measure ANOVA (geometry × session × protocol) on total choices [prop] after collapsing the five experiments • Repeated measure ANOVA (geometry × session × dosage) on total choices [prop] after collapsing the five experiments • Repeated measure ANOVA (strategy × session × protocol) on $C_{1,2}$ total choices [prop] • Repeated measure ANOVA (strategy × session × dosage) on $C_{1,2}$ total choices [prop] • Repeated measure ANOVA (direction × session × condition) on $C_{1,2}$ wall-following total choices [prop] • Repeated measure ANOVA (direction × session × dosage) on $C_{1,2}$ wall-following total choices [prop] • One-sample *t*-test on motion strategy (wall-following vs. centre-to-corner) and direction (left vs. right) in learning and validation session
No. 8 – Lateral line pharmacological ablation in zebrafish	Control experiments	• Paired samples *t*-test ($C_{1,2}$ vs. $X_{1,2}$) on total choices in learning + validation session, in the four experiments separately • Paired samples *t*-test (motion strategy: wall-following vs. centre-to-corner) on $C_{1,2}$ total choices [prop] in learning + validation

	session, in experiments 1-4 separately • Paired samples *t*-test (motion direction: left vs. right) on $C_{1,2}$ total choices [prop] in learning + validation session, in experiment 1 (rectangular opaque arena)

Suppl_1: Summarizing table detailing all the statistic tests performed for each study and experiment.