Evolutionary Ecology Research, 2018, **19**: 619–637

How to get out of a maze? Stingrays (*Potamotrygon motoro*) use directional over landmark information when provided with both in a spatial task

Vera Schluessel and Christian Ober*

Institute of Zoology, Rheinische Friedrich-Wilhelms-Universität Bonn, Bonn, Germany

ABSTRACT

Background: Previous studies have shown that stingrays can exploit a variety of spatial learning strategies, including directional, landmark, and place learning. In these studies, allocentric and global strategies were preferred over egocentric information and local strategies. Elasmobranchs represent the oldest extant vertebrate group. Considering their key phylogenetic position, we wished to address the evolution of spatial strategies as well as the significance of specific strategies over others.

Aim: Investigate cue preferences in two types of maze experiments by assessing the significance of single landmarks and directional cues (both egocentric strategies) for orientation.

Organism: Freshwater stingrays (*Potamotrygon motoro*).

Methods: In two independent experiments, five juvenile stingrays were trained to find their way through a maze while being given both landmark and directional information. Transfer tests (in which conflicts between both kinds of information were created) showed which cues the rays used preferentially to reach their goal.

Results: All rays successfully learned to master the mazes in both experiments, so all were capable of learning and the application of spatial memory. Rays placed more importance on directional information than on landmark cues. This applied to abstract cues (provided in the form of cards featuring symbols) as well as 'natural' landmark cues (in the form of plants). Once rays were trained to use directional information, it was not possible to reverse their preference and get them to use landmark information instead.

Conclusion: Freshwater stingrays learn quickly and prefer directional information to single landmark cues.

Keywords: behaviour, cognition, egocentric, elasmobranchs, spatial orientation, stingrays.

Correspondence: V. Schluessel, Institute of Zoology, Rheinische Friedrich-Wilhelms-Universität Bonn, Poppelsdorfer Schloss, Meckenheimer Allee 169, 53115 Bonn, Germany. email: v.schluessel@uni-bonn.de

*This study was conducted as part of C. Ober's Bachelor thesis.

INTRODUCTION

The ability to orient spatially by memorizing and following a route has been studied in detail in various species (O'Keefe and Nadel, 1978), including fish (e.g. Rodriguez *et al.*, 1994; López *et al.*, 2000; Braithwaite and De Perera, 2006; Rodriguez *et al.*, 2006) and elasmobranchs in particular (Schluessel and Bleckmann, 2005; Fuss *et al.*, 2014a, 2014b; Schluessel *et al.*, 2015). While allocentric orientation requires detailed knowledge of the surrounding environment (i.e. the relationship of various landmarks or cues to one another and independently of one's own position), egocentric orientation as used during route learning is either based on approaching or avoiding cues (guidance strategy), or on performing body-centred turns in response to a specific cue (orientation strategy) (O'Keefe and Nadel, 1978). In its simplest form, egocentric orientation consists of a single stimulus–response association but can be extended to include an entire series of associations (Arleo and Rondi-Reig, 2007), as for example during path integration ('dead reckoning'). Here, information integrating linear and angular self-motion signals over time is collected en route and transformed into a mean vector, which is then used to return to the starting position. Both allocentric and egocentric information systems have their strengths and weaknesses and are by themselves not necessarily sufficient to allow for unambiguous spatial decision-making. Rather, they complement one another (Burgess, 2006), thereby reducing any errors from potentially disrupting spatial orientation within either system. While considerable work has been spent on training fish in various maze experiments, assessing egocentric versus allocentric abilities or preferences [for a summary on teleosts, see Rodriguez *et al.* (2006); for elasmobranchs, see Schluessel (2015)], little research has been conducted on the existence of potential preferences between various egocentric strategies in fish.

Egocentric orientation is generally acknowledged to be less flexible than allocentric orientation (O'Keefe and Nadel, 1978), as performance can easily be disrupted by habitat alterations such as landmark (cue) removal, or extensive movement throughout the environment (as one's own position changes accordingly and errors accumulate). In addition, egocentric orientation is considered to be less complex with regard to the neuronal processing of information (O'Keefe and Nadel, 1978) and, in line with this, several lesion studies in which part of the telencephalon was ablated have shown that egocentric information is processed in different neural substrates from allocentric information (e.g. Salas *et al.*, 1996; Rodriguez *et al.*, 2002; Broglio *et al.*, 2005; Fuss *et al.*, 2014a, 2014b). Additional information indicating that allocentric and egocentric information are processed independently comes from ageing studies – that is, while the performance of egocentric spatial tasks seems to be unaffected by age in several mammals including humans (e.g. Parkin *et al.*, 1996; Begega *et al.*, 2001), allocentric spatial task performance declines (Zelinski and Light, 1988; Cherry and Park, 1993; Kikushi *et al.*, 1999).

Elasmobranchs comprise an ancient phylogenetic group yet possess many of the same cognitive traits found in other vertebrates, such as teleosts, birds, and even mammals (Schluessel, 2015). Members of the family Potamotrygonidae are the only cartilaginous fish that live exclusively in freshwater habitats, with salinity less than 3 ppt (Brooks *et al.*, 1981). All 21 extant species are endemic to South American river and lake systems where they inhabit beach sands, flooded forests, as well as habitats featuring muddy or stony substrates (Rosa, 1985; Araújo *et al.*, 2004). The ocellate river stingray *Potamotrygon motoro* preys mainly on aquatic insects, molluscs, and small crustaceans (Silva and Uieda, 2007). To date, two cognition studies of *P. motoro* have assessed spatial memory and orientation strategies. In the first, it was shown *P. motoro* can use both directional and landmark information and can probably

form (visual) cognitive spatial maps of its environment (Schluessel and Bleckmann, 2005). In the second, *P. motoro* was also found to place more importance on the overall environmental or geometric arrangement of an experimental arena than on individual beacons or sets of landmarks (Schluessel *et al.*, 2015). Two additional cognition studies on members of the family Potamotrygonidae showed that they can use water jets as tools to extract food from a tube and improve efficiency by observing others – an example of social learning (Kuba *et al.*, 2010; Thonhauser *et al.*, 2013). Generally, very little is known about the visual system of *P. motoro*. It possesses a low ratio of rods to cones (Ali and Anctil, 1974), is active during the day (Velte *et al.*, 2002; V. Schluessel, personal observation), utilizes both shallow and deep-water habitats, and encounters clear as well as murky waters (Rosa, 1985; Araújo *et al.*, 2004). *Potamotrygon motoro* was recently shown to have colour vision (Seifert, 2017), with a spatial visual resolution of around 0.23 cpd [tested by discriminating between horizontally and vertically arranged black and white stripes (Alvermann, 2018)].

In the current study, freshwater stingrays (*Potamotrygon motoro*) were trained in two different maze experiments. Both tasks could be solved by using at least one of two egocentric strategies – landmark (cue or beacon) learning and/or direction learning. In transfer tests, we assessed which strategy the rays had utilized for completing the task and if only one or both strategies had been acquired. While it was expected that rays are capable of using multiple cues and strategies to learn a spatial task, previous studies have shown that one strategy is usually preferred over another, such as egocentric over allocentric strategies (Schluessel and Bleckmann, 2005), or global over local cues (Schluessel *et al.*, 2015). Cue preferences have also been shown in other fish (e.g. Rodriguez *et al.*, 1994; Odling-Smee and Braithwaite, 2003; Odling-Smee *et al.*, 2008; White and Brown, 2015a) and seem to depend largely on habitat complexity and lifestyle.

MATERIALS AND METHODS

Five female, juvenile freshwater rays (*P. motoro*) were kept in a wooden aquarium (2.1 m × 2.3 m × 0.5 m) lined with black pond foil and filled with deionized, aerated, filtered water at a temperature of 27 ± 1°C. Reef Salt (Aqua Medic) was added to the water, to maintain a conductivity between 400 and 500 μS. Food was only provided during the experimental trials. As *P. motoro* is a diurnal species, experiments were conducted during the day, from morning until early afternoon. We used a natural 12 hour light/12 hour dark cycle. All animals, ranging in disc diameter from 18 cm to 30 cm, were identifiable by phenotypic characteristics. At the start of the behavioural experiments, all animals were about one and a half years old and not sexually mature. The rays had already participated in a study assessing the importance of global versus local cues in a hole-board task (Schluessel *et al.*, 2015).

Set-up

The housing tank also served as the experimental tank. Water depth was approximately 30 cm. During experiments, the tank was divided into three compartments: a small one, in which the ray to be tested would wait for the next trial to begin, a 'waiting room' for the remaining four animals, and the experimental arena (2.0 m × 2.0 m). The floor of the tank was covered with playground sand. Two different set-ups were used.

The first set-up was a labyrinth (Experiment 1) with walls made out of Playmobil© wall elements (Fig. 1). The entrance and aisles were 20 cm wide and the walls 35 cm high. There were two routes through the maze; three animals were trained on route 1 (group 1), the other two on route 2 (group 2). In total, there were three intersections, the first of which (IS1) offered a right, left or straight-ahead choice* (hereafter the asterisk denotes the correct passage), the second (IS2) a right* (group 1) or a left* (group 2) choice, and the third (IS3) a left* (group 1) or a right* (group 2) choice. For transfer tests (see below), aisles and intersections were changed. At each turnoff, landmark cues in the form of white cards (10 cm × 8 cm) featuring a plus sign (+) or a circle (○) were positioned at the bottom of the adjacent walls (Fig. 1). The plus sign indicated the 'correct' path, while the circle pointed towards the 'incorrect' path or dead end.

The set-up used in the second experiment was a rectangular arena, divided into three equally sized compartments (Fig. 2). Each of the two dividing walls behind the entry compartment as well as the outer wall in the back of the set-up were equipped with two 'exits' featuring see-through curtains made out of soft plastic. For each set, one of the two gates was permanently closed, i.e. it was impassable (it was covered by a long curtain, the bottom of which was buried under the sand). However, it was not visible to the ray which gate was closed. The open gates (featuring shorter curtains only slightly buried under the sand) were each flanked by a single landmark in the form of a small green plastic plant, thereby 'signalling' the correct exit. The order of open gates and therefore the order of landmark cues was left, right, left. The food reward, which was provided when the ray passed though the final gate, was only detectable to the ray after making its final choice. To exclude any uncontrolled cueing and potentially disturbing external influences, the set-up was surrounded by a white pavilion. White boards covered the ceiling lamps. The divider, which separated the experimental arena from the starting compartment, contained a guillotine door, which was closed prior to a new trial. The experimenter was always situated behind the starting compartment hidden by the white pavilion. He observed experiments via the video camera on a laptop and operated the guillotine door remotely using a cable pull.

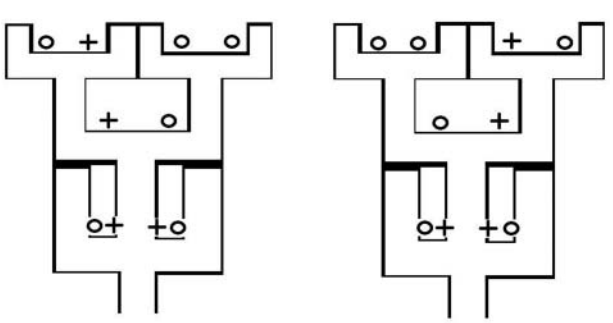

Fig. 1. Training set-up for Experiment 1. The labyrinth on the left shows the arrangement for rays #1 and #5 (straight on, left, right), while that on the right shows the arrangement for rays #2, #3, and #4 (straight on, right, left). A plus sign (+) denotes the correct passage, a circle (○) a dead end (no exit possible).

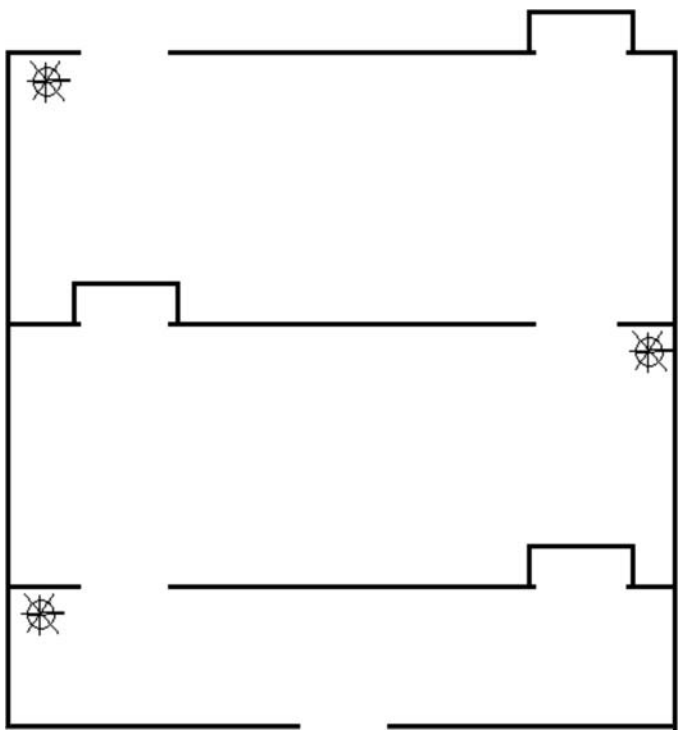

Fig. 2. Training set-up for Experiment 2. Gates marked by a landmark (green plant, depicted by the symbol) were passable, whereas alternatives were not.

Pre-training

As rays had already participated in another study (Schluessel *et al.*, 2015), no acclimatization to the experimental arena was necessary. During pre-training for Experiment 1, a small maze consisting of two Playmobil© aisles was constructed to allow familiarization with the general set-up (not in its final form). Rays had 2 days to swim freely throughout the set-up and feed in it. Similarly, in Experiment 2, animals were allowed to roam freely around the first compartment and feed in it. All gates were open during this stage (curtains in place, but all passable).

Training

Training was conducted six times a week either once or twice a day. There were ten trials per session. To begin a trial, the ray had to press against the guillotine door and then enter the arena. Trial time was defined as the time it took the ray to swim through the guillotine door (timing began when the front of the pectoral fin crossed the guillotine door) and pass though the final gate. Time was recorded using a stopwatch. Training in Experiment 1 ended when the rays reached the learning criterion, i.e. when the rays exited the maze on average within 15 seconds or less (and the longest trial time did not deviate more than 4 seconds from the session's mean trial time) in three consecutive sessions. A particular number of

'failures' was not included as a learning criterion (very few errors were actually made by rays when reaching criterion). The learning criteria for Experiment 2 were a mean trial time of 25 seconds or less, and no more than two 'failures' (entering the wrong gate at one aisle) per session over three consecutive sessions.

Inter-trial time was 45 seconds in Experiment 1 and 30 seconds in Experiment 2. To prepare the rays for subsequent transfer test trials, which were unrewarded and interspersed with regular trials over the next sessions, food was only provided in eight out of ten correct trials in all sessions following successful training. Prior to each session, it was randomly determined which trials would be unrewarded (regardless of the ray's actual choice). The first and last trial were always rewarded and there were never two unrewarded trials in a row. Not rewarding some of the regular trials was intended to prevent the rays from realizing that transfer trials were unrewarded and therefore not worth participating in. After making a choice, the ray either returned to the starting compartment by itself or was ushered back to it by the experimenter.

Transfer tests

Transfer tests were unrewarded and randomly interspersed with regular trials. Similar to regular unrewarded trials they were neither conducted at the very beginning nor at the end of a session and never preceded or followed one of the unrewarded regular trials. There were always two transfer test trials (any type) interspersed with ten regular trials per session. The purpose of the transfer trials was to elucidate which spatial strategy (direction learning or landmark cues) the rays had used to reach the goal location. A key element of the transfer tests was to create a conflict between the two strategies forcing rays to make a choice in favour of one or the other.

In Experiment 1, there were three types of transfer (T1–T3). There was no particular order in which the transfer tests were conducted, i.e. T1, T2, and T3 trials were intermixed. In T1 trials ($n = 10$ per animal), all intra-maze cues (cards with symbols) were removed to determine if the task could still be solved based on directional information alone. In T2 trials ($n = 10$ per animal), the 'positive' (+) and the alternative or 'negative' (○) stimulus cards were switched, to determine if the rays had utilized the landmark information and if it would take precedence over directional information. In the T3 trials ($n = 8$ per animal), the order of intersections was changed, i.e. IS1 (three options to choose from) and IS2 (two options to choose from) were interchanged to determine if stingrays would use landmark information in the absence of being able to apply directional information. In order to find the goal, rays now needed to use landmark cues as provided by the stimulus cards; directional information was no longer available. Ray #5 participated in very few transfer tests (i.e. T1, $n = 3$ and T2, $n = 3$) and was therefore left out of the analysis (for Experiment 1 only).

In Experiment 2, there were three types of transfer test. T1 trials were performed first, followed by T2 and T3 trials, or by T3 and T2 trials. In all T1 transfer trials, all gates were 'open', i.e. featured passable curtains. In the T1 trials ($n = 6$ per animal), landmarks (green plants) were positioned at the mirror image of their original position (right, left, right). In the T2 trials ($n = 6$ per animal), only the landmark in aisle 1 was moved to the other side (to the right), with the other two remaining in their original positions (right, left). In the T3 trials ($n = 6$ per animal), all landmarks were moved to the right side. As all of the rays exclusively chose the original left-right-left scheme during T1 trials, the first gate on the left

was closed off during T2 and T3 trials to determine whether rays would use alternative strategies if they were forced to enter an alternative gate.

Data analysis

Selected sessions (all transfer test trials) were recorded by a webcam (Logitech Webcam C210). Generalized linear mixed models (GLMM) were run in R (using individual as a random factor and type of decision as the dependent variable) to test whether rays as a group chose one orientation strategy significantly more often than another during the transfer tests. A paired *t*-test was used to compare trial times of regular and transfer test trials (all individuals grouped). For all tests, an alpha of 0.05 was considered significant. Individual performance was not statistically assessed, due to low repetitions and resulting low statistical power. Swimming tracks were created in Paint©.

RESULTS

Experiment 1

All five animals learned to swim through the maze and reached the learning criterion within 16.8 ± 5.27 sessions (mean ± SD). Figure 3 shows the learning curves for rays #1 through #5.

Transfer tests

In the T1 transfer trials ($n = 40$), in which all cards bearing symbols (landmark cues) were removed, only one error was made at IS1, by ray #3 (out of $40 \times 3 = 120$ intersection decisions; Fig. 4). All other rays swam 'correctly', i.e. according to their training scheme in all test trials. Performance in the transfer tests was therefore significantly different from chance (GLMM: $z = 3.38$, $df = 38$, $P < 0.0001$). Trial time did not change significantly when compared with that in regular trials (paired *t*-test: $t = -1.40$, $df = 4$, $P = 0.24$). Figure 4 shows the swimming tracks for all rays. Here, the removal of any landmark cues had no effect on the performance of any individual, showing that only directional information had been used.

In the T2 transfer trials ($n = 40$), in which the card symbols were exchanged, all rays used exactly the same route as before, i.e. used the same turning procedure as in the regular training trials. The exchange of landmarks had no effect on performance, which was significantly different from chance (GLMM: $z = -285.6$, $df = 38$, $P < 0.0001$). Ray #4 was the only one to make two errors (at IS2 and IS3), both of which were made within the same trial (out of $40 \times 3 = 120$ intersection decisions). Trial time did not differ significantly from regular trials (paired *t*-test: $t = -1.82$, $df = 4$, $P = 0.14$). Figure 5 shows the swimming tracks of all rays. Similar to T1 trials, the alteration of landmark cues had no effect on the performance of any individual, showing that only directional information had been used.

In the T3 transfer trials ($n = 32$), the set-up was reorganized to eliminate the option of using previously learned directional information and to assess whether rays would use landmark information under these circumstances (no other training information available). The new set-up featured a two-way intersection followed by a three-way intersection (instead of the other way around). Rays chose the 'correct path' (signalled by the '+' sign) 39 times, or in 40.63% of all trials [out of 4 (animals) × 8 (trials) × 3 (intersections) = 96 IS

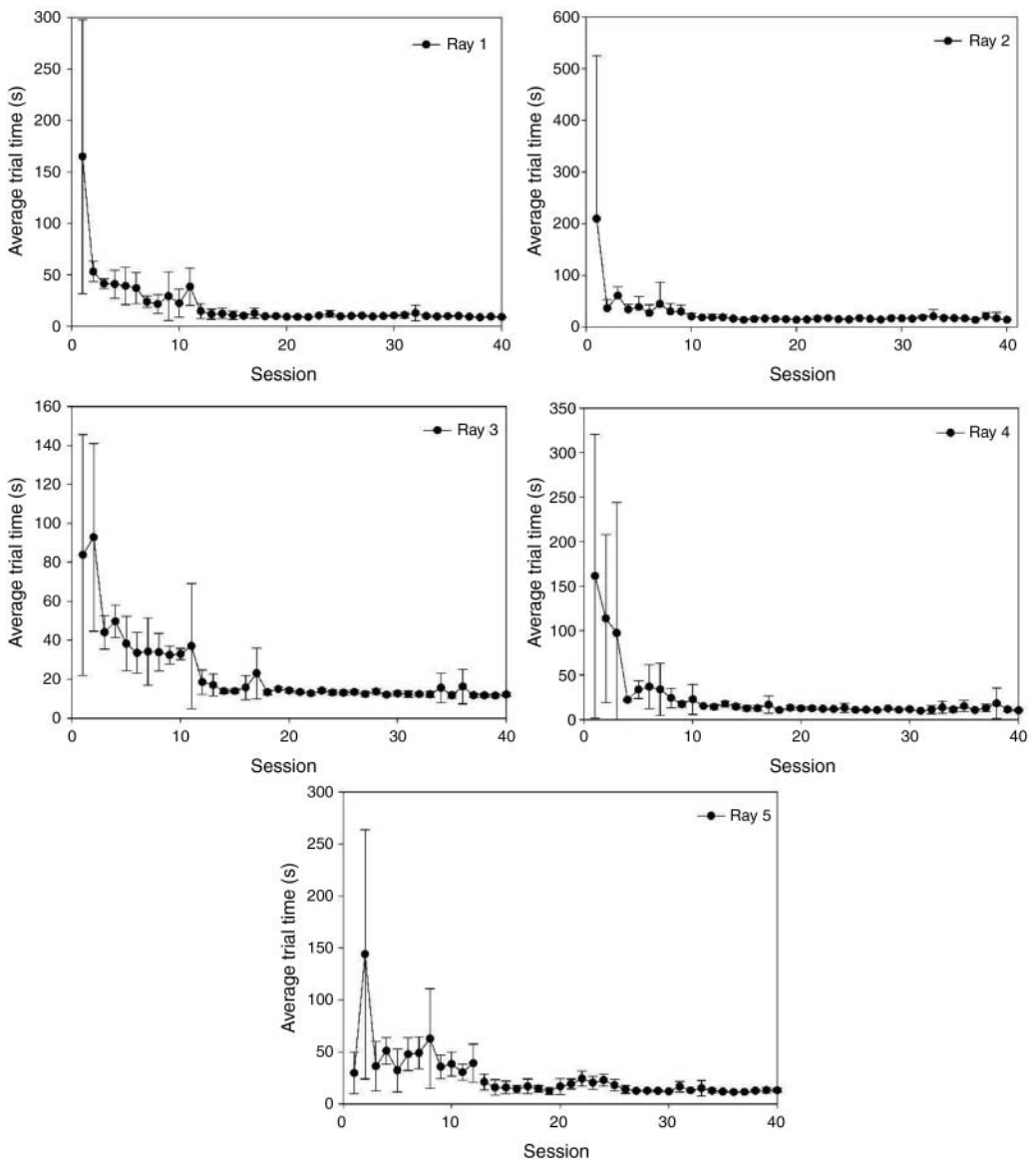

Fig. 3. Learning curves for Experiment 1. Shown is the average trial time (in seconds) ± SD per session (there were ten trials per session and up to two sessions per day).

decisions], which was not significantly more often than choosing the alternative (GLMM: $z = -0.321$, $df = 94$, $P = 0.758$). At IS1 in each trial ($n = 32$), rays made an 'incorrect' decision (i.e. the '○' sign) 93.75% of the time, which corresponded to the direction of the first turn animals had to perform during training trials (ray 1: 7 left (L)/1 right (R); ray 2: 8R; ray 4: 8R; ray 5: 8R) in all but one trial. At IS2, rays chose the 'incorrect' turn 34.37% of the time and at IS3 they chose an 'incorrect' turn 46.88% of the time. Trial time differed

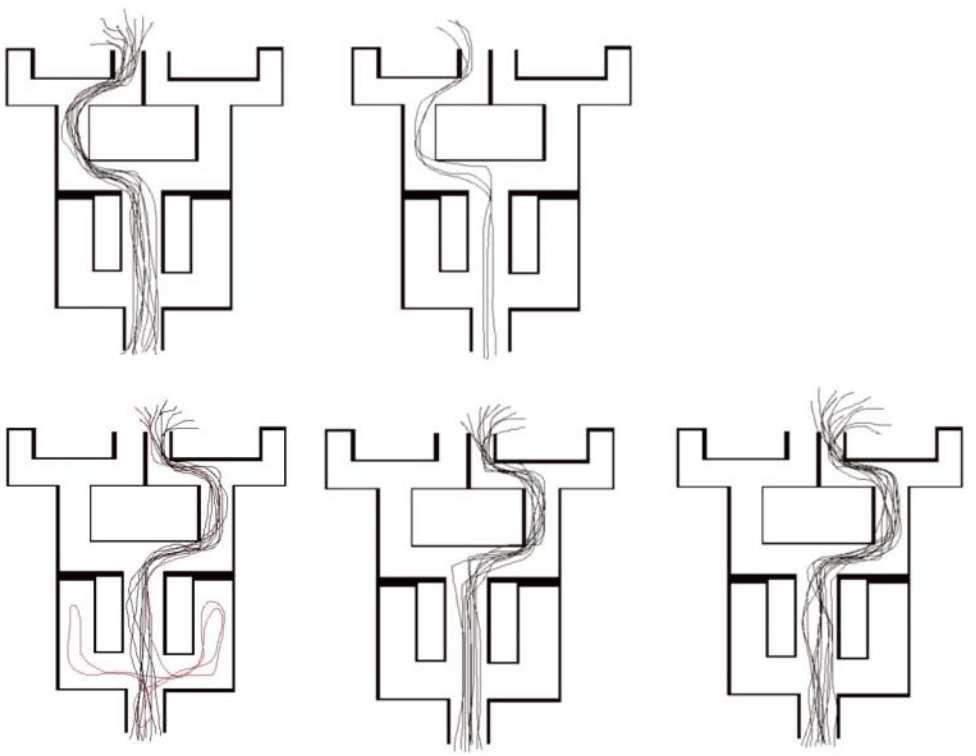

Fig. 4. Swimming tracks for T1 trials of rays #1 and #5 (top) and rays #2, #3, and #4 (bottom). Note that only first choices per intersection were considered for analysis, while each track shows the entire swimming path, in some cases including several choices per intersection.

significantly from regular trials in that the rays were much slower (paired t-test: $t = -5.86$, $df = 3$, $P = 0.01$). Figure 6 shows the swimming tracks for four of the rays (not ray #5). In line with results of the previous two transfer tests (T1 and T2), this indicates that landmark information was not applied.

Experiment 2

All five animals learned to swim through the maze (left-right-left) and reached the learning criterion within 15.2 ± 2.23 sessions (mean $\pm$ SD). Figure 7 shows the learning curves for rays #1 through #5.

In the T1 transfer trials ($n = 30$), in which all landmark cues were shifted and positioned next to the opposite gate to the training trials (mirror image position, right-left-right; Fig. 8), rays chose the training gate 97.8% (88/90) of the time and not the gate signalled by a landmark (GLMM: $z = -5.73$, $df = 88$, $P < 0.0001$). Trial time was not significantly different from that in regular trials (paired t-test: $t = -0.39$, $df = 4$, $P = 0.71$). Figure 8 shows the swimming tracks for all rays.

In the T2 transfer trials ($n = 30$), landmarks were placed in a right-right-left gate scheme (only the landmark position at the first gate differed from the training scheme; Fig. 9). The

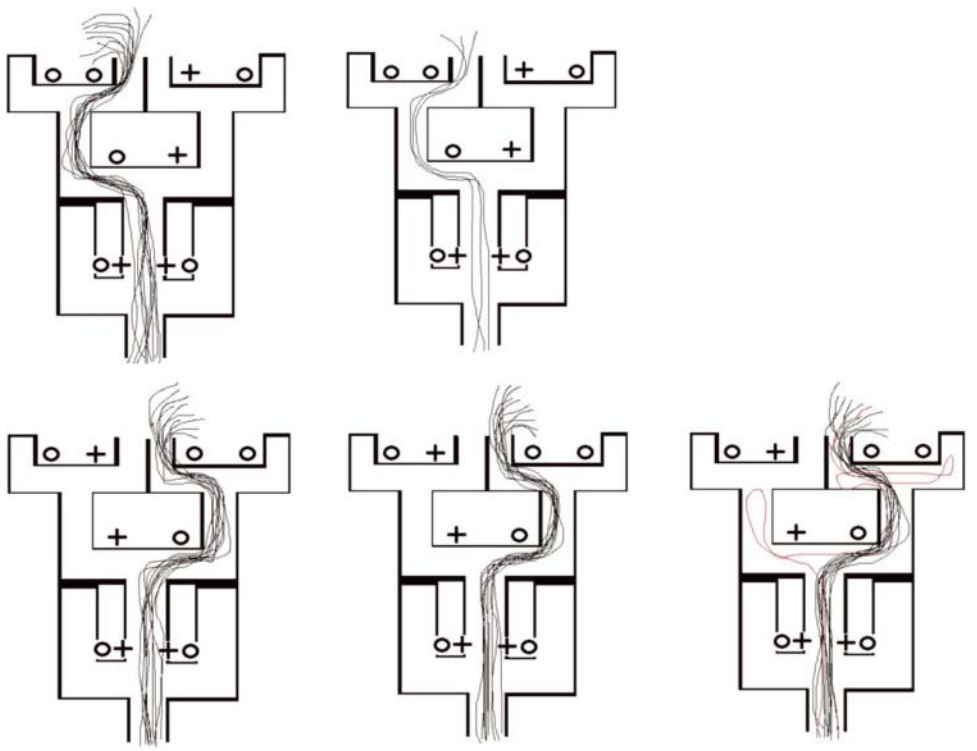

Fig. 5. Swimming tracks for T2 trials of rays #1 and #5 (top) and rays #2, #3, and #4 (bottom). Note that only first choices per intersection were considered for analysis, while each track shows the entire swimming path, in some cases including several choices per intersection.

first gate on the left (the original first training gate) was closed off. As rays were forced to exit the first gate on the 'incorrect' side, i.e. through a gate they did not want to use, these data were not used in the statistical analysis. Here, trial time is a much better indicator of performance, which was significantly different from that in regular trials, in that rays were much slower (paired *t*-test: $t = -4.89$, $df = 4$, $P = 0.01$). Most trial time was spent at the first gate (personal observation), where all rays in all trials (30/30) tried relentlessly to exit on the left side. Eventually, they did leave through the alternative gate on the right side. At the second set of gates, rays chose 18 out of 30 times (60.0%) the gate on the right side, which corresponded to the 'correct' gate during training and was also signalled by a landmark. However, this was not significantly often (GLMM: $z = -1.088$, $df = 28$, $P = 0.277$). For the last gate, rays reverted to their training regime, which again coincided with the position of the landmark. The 'correct' training gate was chosen on 25 of 30 occasions, which was significantly more often than the alternative gate (GLMM: $z = -3.285$, $df = 28$, $P = 0.001$). Figure 9 shows the swimming tracks for all rays.

In the T3 transfer trials ($n = 30$), landmarks were placed in a right-right-right gate scheme (Fig. 10). The first gate on the left (the original first training gate) was closed off again (and was thus not considered in the statistical analysis). Four out of five rays were much slower; however, the trial time (for the group) was not significantly different from that of regular

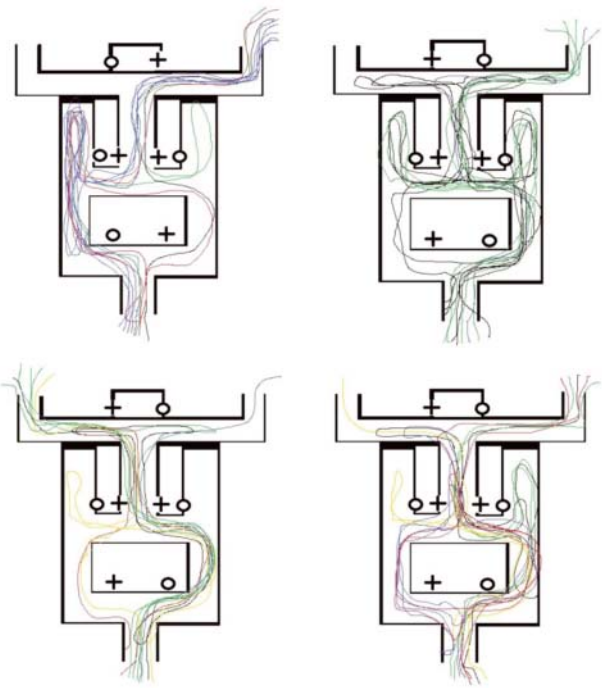

Fig. 6. Swimming tracks for T3 trials of rays #1 and #2 (top) and rays #3 and #4 (bottom). A plus sign (+) denotes the correct passage, a circle (○) a dead end. Note that only first choices per intersection were considered for analysis, while each track shows the entire swimming path, in some cases including several choices per intersection.

trials (paired *t*-test: $t = -2.43$, $df = 4$, $P = 0.11$). As in the T2 trials, most trial time was spent at the first gate (personal observation), where again the rays tried relentlessly to exit on the left side. Eventually, they left through the alternative gate on the right side. At the second gate, rays chose 18 out of 30 times the gate that corresponded to the 'correct' gate during training and which was also signalled by a landmark (GLMM: $z = -0.72$, $df = 28$, $P = 0.472$). The 'correct' training gate was again chosen on all 30 occasions at the final wall, but this time there was no landmark positioned next to it (GLMM: $z = 1.017$, $df = 28$, $P < 0.0001$). Figure 10 shows the swimming tracks for all rays.

DISCUSSION

In both experiments, rays quickly learned to find their way through a maze. Surprisingly, landmark (beacon) information was never used, whether provided in the form of symbols on cards (abstract cues) or in the form of plants (natural cues). Instead, rays in both experiments seemed to follow an orientation strategy, where they memorized a certain turn procedure, i.e. a series of turns such as right, left, right. Alterations to the training scenario and specifically closure of visited training gates during transfer tests led rays to try repeatedly and persistently to enter these gates before reluctantly using an alternative one.

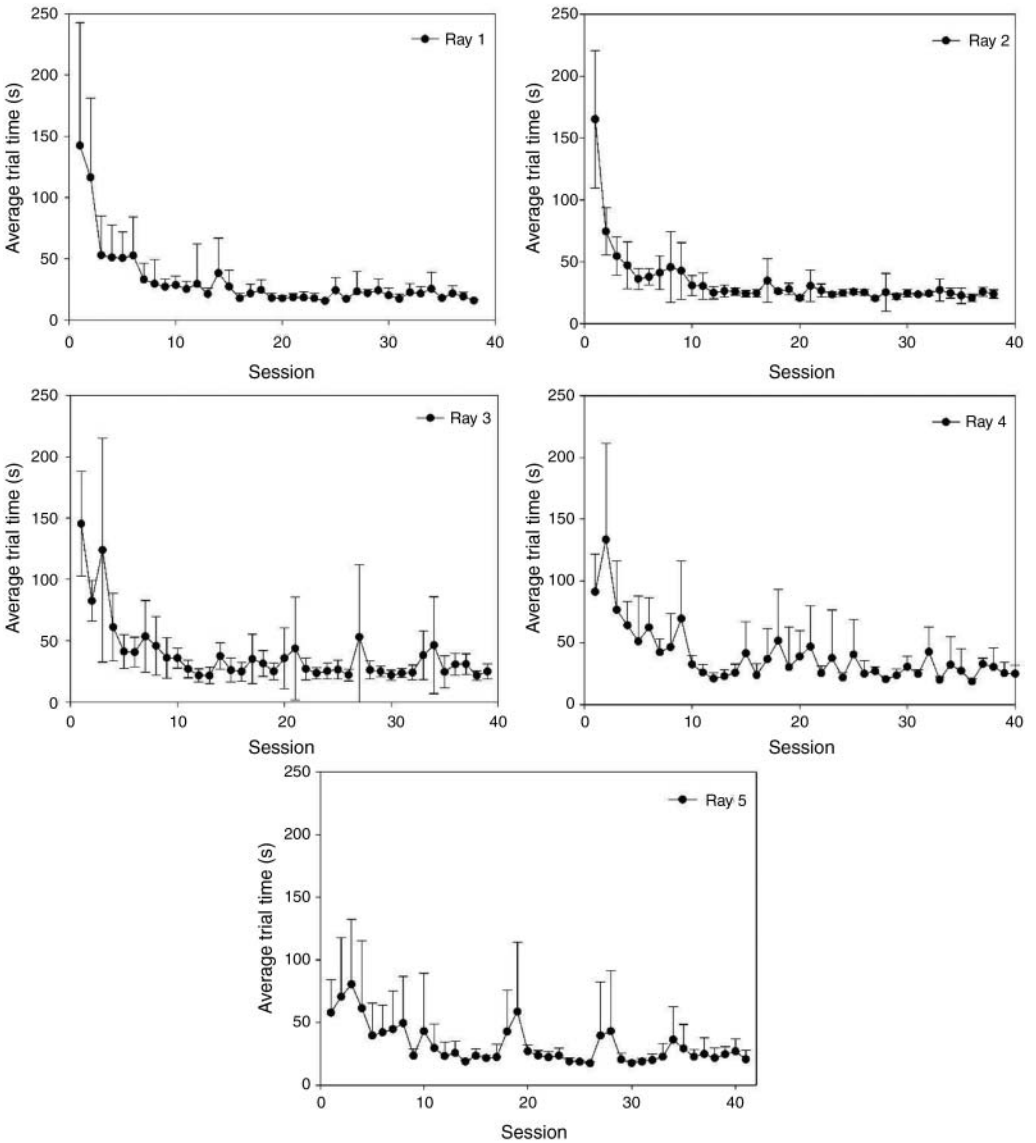

Fig. 7. Learning curves for Experiment 2. Shown is the average trial time (in seconds) ± SD per session (there were ten trials per session and up to two sessions per day).

In Experiment 1, rays learned quickly within 16 sessions (160 trials) to find the exit to the maze and retrieve a food reward. Results of T1 and T2 trials, where landmarks were repositioned or removed completely, clearly show that rays orientated exclusively according to a direction strategy and were not deterred by changes in landmark information. Finally, in the T3 trials, in which the entire set-up of the maze was changed so as to eliminate the option of using a direction strategy, the exclusive use of a direction strategy was confirmed. Rays did not rely on landmark information even under these circumstances, indicating that

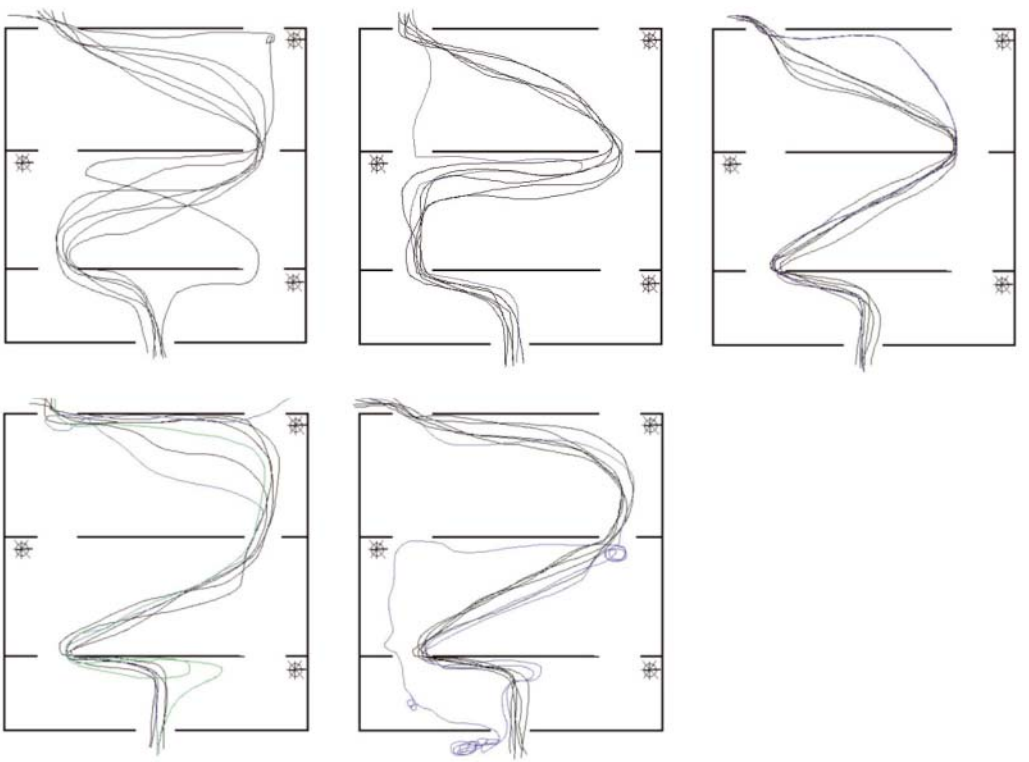

Fig. 8. Swimming tracks for T1 trials of rays #1 to #5. The symbol denotes the position of the landmark (green plant).

landmarks were not only second in importance to directional information but had in fact not been learned at all.

Considering the artificiality of the landmarks used and wanting to confirm the results in another, possibly less complex set-up, we conducted Experiment 2. Here, the correct training gates were not just signalled by cards featuring abstract symbols but by conspicuous landmarks (bright green plants). Similar to Experiment 1, rays needed on average 15 sessions (150 trials) to reach the learning criterion. Results of the T1 transfer test (mirror image position of landmarks) clearly indicate that all five individuals once more used a direction strategy. To force rays to 'reconsider' their choice and to determine whether they could be enticed to use landmark information by increasing the level of difficulty of achieving their goal using their preferred turns, the first correct training gate was closed off during the T1 and T2 transfer tests. Nonetheless, results were straightforward in showing that rays put significant effort in trying to pass through the closed off gate before eventually giving up. However, in most cases they proceeded to choose the gate that corresponded to the correct training gate, irrespective of whether a landmark was associated with it or not. This was especially clear in the T3 trials (final gate decision).

A follow-up experiment, similar to a reversal task, in which rays were trained to use landmark information exclusively by changing the set-up multiple times during a session

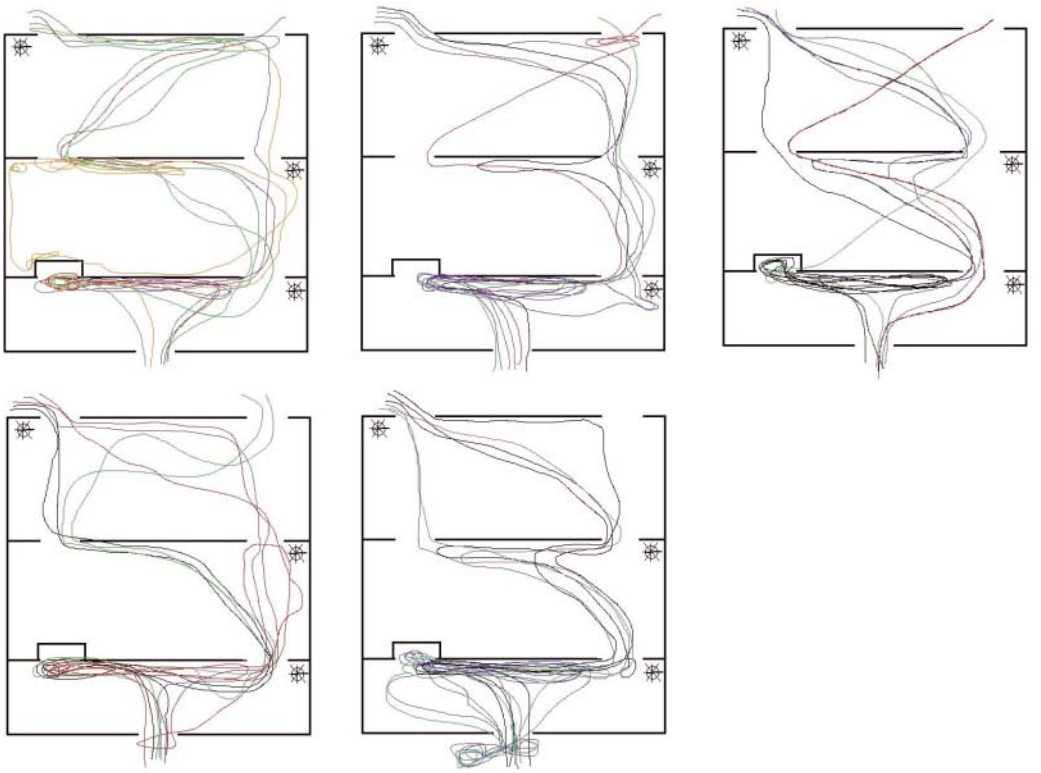

Fig. 9. Swimming tracks for T2 trials of rays #1 to #5. The symbol denotes the position of the landmark (green plant).

and by keeping only the landmark information constant, was unsuccessful for all five individuals; none reached the learning criterion, even after 30 sessions (Ober, 2015). The observed reluctance to use landmark or beacon information in this experiment was surprising and unexpected, as rays in another study were capable of using several spatial strategies (simultaneously) and were also successful in a reversal task (Schluessel and Bleckmann, 2005). It is possible that the experience the rays gained during Experiment 1 could have influenced their decision-making in Experiment 2, as well as in the reversal-learning task (results not presented here). This, however, seems unlikely in view of previous studies on *P. motoro* and other fish species and also seems ecologically not to be feasible. Guppies tested in a similar (to Experiment 1) but yet more complex maze experiment (Lucon-Xiccato and Bisazza, 2017) also ignored local visual cues (landmarks) provided in the form of colour cues; their presence or absence had no influence whatsoever on performance. In contrast to these findings, four populations of sticklebacks, tested in an experimental apparatus similar to that in Experiment 2, solved a spatial task by using either a specific sequence of turns or relying on visual information (Girvan and Braithwaite, 1998). Cue preference seemed to coincide with the ecology of each respective population, with those living in more turbid water featuring less visibility relying on turns, and those from visually more stable environments utilizing visual landmarks.

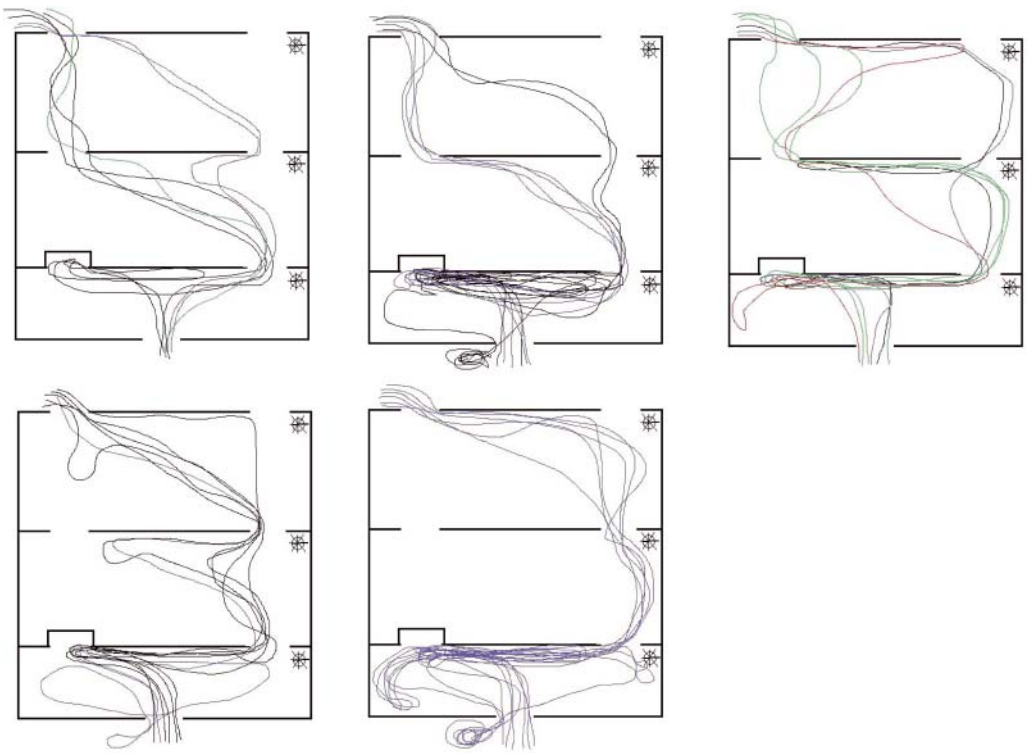

Fig. 10. Swimming tracks for T3 trials of rays #1 to #5. The symbol denotes the position of the landmark (green plant).

Results of the present study suggest a 'compartmentalized' organization with respect to landmark and directional information and thereby offer some new insights as to how orientation strategies in stingrays are structured and how flexibly they are used. Previous studies on freshwater stingrays have shown that rays can in fact use a variety of different orientation strategies (including landmark learning) that are not necessarily mutually exclusive. In a four-arm maze experiment, rays were successfully trained in both egocentric and allocentric orientation strategies (Schluessel and Bleckmann, 2005), and were found to orient primarily based on an egocentric turn strategy instead of using external (allocentric) landmark information when given a choice [ego-allocentric group (Schluessel and Bleckmann, 2005)]. This is in line with results obtained for goldfish (*Carassius auratus*) and grey bamboo sharks (*Chiloscyllium griseum*) collected under very similar experimental conditions (Rodriguez *et al.*, 1994; Fuss *et al.*, 2014a, 2014b), but contrast with those obtained for four other fish species, including goldfish (McAroe *et al.*, 2016), which either showed a place preference or no preference at all, as in the case of zebrafish. In the study by McAroe and colleagues, however, 'place preference' did not necessarily refer to an allocentrically determined goal location but one that could have merely been chosen by beacon learning (i.e. an egocentric strategy). Consequently, while the authors concluded that three of the four species preferred a place rather than a turning strategy, the results could have also been interpreted as a preference for one egocentric strategy (beacon or landmark learning) over another (direction learning). In the latter case,

results for goldfish, killifish, and Siamese fighting fish would have been of the opposite nature to those collected here. Two egocentric strategies (i.e. landmark or beacon learning vs. turn strategies) were also tested and compared between different populations of threespine stickleback (*Gasterosteus aculeatus*) (Odling-Smee and Braithwaite, 2003). Results showed that while river populations showed a significant preference for directional learning (i.e. turn preferences) across several transfer test scenarios, pond-reared threespine stickleback showed no such preference (Odling-Smee and Braithwaite, 2003). In another study, stingrays displayed a clear preference for the overall spatial arrangement of an experimental set-up, including the geometry of the experimental basin, over individual landmarks (Schluessel *et al.*, 2015). A greater relevance of geometric over non-geometric information has also been found for splitfins (Vallortigara and Sovrano, 2002; Sovrano *et al.*, 2002, 2003, 2005; Sovrano and Vallortigara, 2006) as well as several animal species other than fish. Despite preferring global to local cues, stingrays also recognized and remembered individual landmarks, with small-scale changes in the position or complete removal of landmarks having an effect on performance, i.e. search behaviour in some tasks (Schluessel *et al.*, 2015).

Cue preferences in general have been shown to occur in a wide range of taxa, including mammals (e.g. Gouteux and Thinus-Blanc, 2001; Cheng and Newcombe, 2005), birds (e.g. Kelly *et al.*, 1998; Hodgson and Healy, 2005), reptiles (e.g. López *et al.*, 2000), and fish (e.g. Sovrano *et al.*, 2002; Holbrook and Perera, 2011). In general, as observed in many studies on fish, applied or preferred spatial strategies seem to reflect environmental conditions and demands and vary with ecology and lifestyle of the respective species or individual (e.g. Hughes and Blight, 2000; Odling-Smee and Braithwaite, 2003; White and Brown, 2015a, 2015b). As mentioned previously, river populations of threespine stickleback were shown to prefer directional over landmark cues, a potentially useful adaptation in unstable habitats such as rivers, where local cues may easily or frequently disappear, become (partly) occluded or change shape (Odling-Smee and Braithwaite, 2003). The same reasoning may apply to *P. motoro*, which also inhabits river systems, in which small landmarks such as individual plants may represent unreliable cues for orientation purposes. While habitat characteristics are likely to play a significant role in shaping spatial behaviour, other factors could include genetics, age or previous experiences of other individuals.

ACKNOWLEDGEMENTS

We thank S. Braun for help with animal care, and H. Herzog and T. Thünken for help with the statistical analyses. We are specifically grateful to Frankfurt Zoo for supplying the animals used in this study. The research reported herein was performed under the guidelines established by the current German animal protection law and conform to the 'Guidelines for the use of animals in research'. This study was funded by a DFG grant to V.S. (SCHL1919/4-1).

REFERENCES

Ali, M.A. and Anctil, M. 1974. Retinas of the electric ray (*Narcine brasiliensis*) and the freshwater stingray (*Paratrygon motoro*). *Vis. Res.*, **14**: 587–588.

Alvermann, L. 2018. Amodale Vervollständigung und Visuelle Auflösung beim Pfauenaugen-stechrochen (*Potamotrygon motoro*). Unpublished BS thesis, Rheinische Friedrich-Wilhelms-Universität, Bonn, Germany.

Araújo, M.L.G., Charvet-Almeida, P., Almeida, M.P. and Pereira, H. 2004. Freshwater stingrays (Potamotrygonidae): status, conservation and challenges. In *AC 20 Informative 8*.

Arleo, A. and Rondi-Reig, L. 2007. Multimodal sensory integration and concurrent navigation strategies for spatial cognition in real and artificial organisms. *J. Integr. Neurosci.*, **6**: 327–366.

Begega, A., Cienfuegos, S., Rubio, S., Santin, J.L., Miran da, R. and Arias, J.L. 2001. Effects of ageing on allocentric and egocentric spatial strategies in the Wistar rat. *Behav. Process.*, **53**: 75–85.

Braithwaite, V.A. and De Perera, T.B. 2006. Short-range orientation in fish: how fish map space. *Mar. Freshw. Behav. Physiol.*, **39**: 37–47.

Broglio, C., Gomez, A., Duran, E., Ocana, F.M., Jimenez-Moya, F., Rodriguez, F. *et al.* 2005. Hallmarks of a common forebrain vertebrate plan: specialized pallial areas for spatial, temporal and emotional memory in actinopterygian fish. *Brain Res. Bull.*, **66**: 277–281.

Brooks, D.R., Mayes, M.A. and Thorson, T.B. 1981. Systematic review of cestodes infecting freshwater stingrays (Chondrichthyes: Potamotrygonidae) including four new species from Venezuela. *Proc. Helminthol. Soc. Wash.*, **48**: 43–64.

Burgess, N. 2006. Spatial memory: how egocentric and allocentric combine. *Trends Cogn. Sci.*, **10**: 551–557.

Cheng, K. and Newcombe, N.S. 2005. Is there a geometric module for spatial orientation? Squaring theory and evidence. *Psychon. Bull. Rev.*, **12**: 1–23.

Cherry, K.E. and Park, D.C. 1993. Individual difference and contextual variables influence spatial memory in younger and older adults. *Psychol. Aging*, **8**: 517–526.

Christofzik, N. 2016. Visuelle Wahrnehmung und Diskriminierung numerischer Informationen beim Pfauenaugenstechrochen (*Potamotrygon motoro*). Unpublished BS thesis, Rheinische Friedrich-Wilhelms-Universität, Bonn, Germany.

Fuss, T., Bleckmann, H. and Schluessel, V. 2014a. The shark *Chiloscyllium griseum* can orient using turn responses before and after partial telencephalon ablation. *J. Comp. Physiol. A: Neuroethol. Sens. Neural Behav. Physiol.*, **200**: 19–35.

Fuss, T., Bleckmann, H. and Schluessel, V. 2014b. Place learning prior to and after telencephalon ablation in bamboo and coral cat sharks (*Chiloscyllium griseum* and *Atelomycterus marmoratus*). *J. Comp. Physiol. A: Neuroethol. Sens. Neural Behav. Physiol.*, **200**: 37–52.

Girvan, J.R. and Braithwaite, V.A. 1998. Population differences in spatial learning in three-spined sticklebacks. *Proc. R. Soc. Lond. B: Biol. Sci.*, **265**: 913–918.

Gouteux, S. and Thinus-Blanc, C. 2001. Rhesus monkeys use geometric and nongeometric information during a reorientation task. *J. Exp. Psychol.*, **130**: 505–519.

Hodgson, Z.G. and Healy, S.D. 2005. Preference for spatial cues in a non-storing songbird species. *Anim. Cogn.*, **8**: 211–214.

Holbrook, R.I. and Perera, T.B. 2011. Three-dimensional spatial cognition: information in the vertical dimension overrides information from the horizontal. *Anim. Cogn.*, **14**: 613–619.

Hughes, R.N. and Blight, C.M. 2000. Two intertidal fish species use visual association learning to track the status of food patches in a radial maze. *Anim. Behav.*, **59**: 613–621.

Kellogg, W.N. and Gavin, J. 1960. Maze-learning in the guppy. *Psychol. Rep.*, **6**: 445–446.

Kelly, D.M., Spetch, M.L. and Heth, C.D. 1998. Pigeons' (*Columba livia*) encoding of geometric and featural properties of a spatial environment. *J. Comp. Psychol.*, **112**: 259–269.

Kikusui, T., Tonohiro, T. and Kaneko, T. 1999. Age-related working memory deficits in the allocentric place discrimination task: possible involvement in cholinergic dysfunction. *Neurobiol. Aging*, **20**: 629–636.

Kuba, M.J., Byrne, R.A. and Burghard, G.M. 2010. A new method for studying problem solving and tool use in stingrays (*Potamotrygon castexi*). *Anim. Cogn.*, **13**: 507–513.

López, J.C., Rodríguez, F., Gómez, Y., Vargas, J.P., Broglio, C. and Salas, C. 2000. Place and cue learning in turtles. *Anim. Learn. Behav.*, **28**: 360–372.

Lucon-Xiccato, T. and Bisazza, A. 2017. Complex maze learning by fish. *Anim. Behav.*, **125**: 69–75.

McAroe, C.L., Craig, C.M. and Holland, R.A. 2016. Place versus response learning in fish: a comparison between species. *Anim. Cogn.*, **19**: 153–161.

Ober, C. 2015. Räumliche Orientierung und Landmarkenlernen beim Pfauenaugenstechrochen Potamotrygon motoro. Bachelor thesis, University of Bonn, Germany.

Odling-Smee, L. and Braithwaite, V.A. 2003. The influence of habitat stability on landmark use during spatial learning in the three-spined stickleback. *Anim. Behav.*, **65**: 701–707.

Odling-Smee, L.C., Boughman, J.W. and Braithwaite, V.A. 2008. Sympatric species of threespine stickleback differ in their performance in a spatial learning task. *Behav. Ecol. Sociobiol.*, **62**: 1935–1945.

O'Keefe, J. and Nadel, L. 1978. *The Hippocampus as a Cognitive Map*. Oxford: Clarendon Press.

Parkin, A.J., Walter, B.M. and Hunkin, N.M. 1996. Relationships between normal aging, frontal lobe function, and memory for temporal and spatial information. *Neuropsychology*, **9**: 304–312.

Rodriguez, F., Duràn, E., Vargas, J.P., Torres, B. and Salas, C. 1994. Performance of goldfish trained in allocentric and egocentric maze procedures suggests the presence of a cognitive mapping system in fishes. *Anim. Learn. Behav.*, **22**: 409–420.

Rodriguez, F., Lopez, J.C., Vargas, J.P., Gomez, Y., Broglio, C. and Salas, C. 2002. Conservation of spatial memory function in the pallial forebrain of reptiles and ray-finned fishes. *J. Neurosci.*, **22**: 2894–2903.

Rodriguez, F., Broglio, C., Durán, E., Gómez, Y. and Salas, C. 2006. Neural mechanisms of learning in teleost fishes. In *Fish Cognition and Behaviour* (C. Brown, K. Laland and J. Krause, eds.), pp. 243–277. Oxford: Blackwell.

Rosa, R.S. 1985. A systematic revision of the South American freshwater stingrays (Chondrichthyes: Potamotrygonidae). Unpublished PhD dissertation, The College of William and Mary, Williamsburg, VA.

Salas, C., Broglio, C., Rodriguez, F., Lopez, J.C., Portavella, M. and Torres, B. 1996. Telencephalic ablation in goldfish impairs performance in a 'spatial constancy' problem but not in a cued one. *Behav. Brain Res.*, **79**: 193–200.

Schluessel, V. 2015. Who would have thought that 'Jaws' also has brains? Cognitive functions in elasmobranchs. *Anim. Cogn.*, **18**: 19–37.

Schluessel, V. and Bleckmann, H. 2005. Spatial memory and orientation strategies in the elasmobranch *Potamotrygon motoro*. *J. Comp. Physiol. A: Neuroethol. Sens. Neural Behav. Physiol.*, **191**: 695–706.

Schluessel, V. and Bleckmann, H. 2012. Spatial learning and memory retention in the grey bamboo shark (*Chiloscyllium griseum*). *J. Zool.*, **115**: 346–353.

Schluessel, V., Herzog, H. and Scherpenstein, M. 2015. Seeing the forest before the trees – spatial orientation in freshwater stingrays (*Potamotrygon motoro*) in a hole-board task. *Behav. Process.*, **119**: 105–115.

Seifert., F.D. 2017. Farbwahrnehmung beim Pfauenaugenstechrochen (*Potamotrygon motoro*). Unpublished BS thesis, Rheinische Friedrich-Wilhelms-Universität, Bonn, Germany.

Silva, T.B. and Uieda, V.S. 2007. Preliminary data on the feeding habits of the freshwater stingrays *Potamotrygon falkneri* and *Potamotrygon motoro* (Potamotrygonidae) from the Upper Paraná River basin, *Brazil. Biota Neotrop.*, **7**(1).

Sovrano, V.A. and Vallortigara, G. 2006. Dissecting the geometric module: a sense linkage for metric and landmark information in animals' spatial reorientation. *Psychol. Sci.*, **17**: 616–621.

Sovrano, V.A., Bisazza, A. and Vallortigara, G. 2002. Modularity and spatial reorientation in a simple mind: encoding of geometric and non-geometric properties of a spatial environment by fish. *Cognition*, **85**: B51–B59.

Sovrano, V., Bisazza, A. and Vallortigara, G. 2003. Modularity as a fish (*Xenotoca eiseni*) views it: conjoining geometric and non-geometric information for spatial reorientation. *J. Exp. Psychol. Anim. Behav. Process.*, **29**: 199–210.

Sovrano, V.A., Bisazza, A. and Vallortigara, G. 2005. Animals' use of landmarks and metric information to reorient: the effects of the size of the experimental space. *Cognition*, **97**: 121–133.

Thonhauser, K.E., Gutnick, T., Byrne, R.A., Kral, K., Burghardt, G.M. and Kuba, M. 2013. Social learning in cartilaginous fish (stingrays *Potamotrygon falkneri*). *Anim. Cogn.*, **16**: 927–932.

Vallortigara, G. and Sovrano, V.A. 2002. Conjoining information from different modules: a comparative perspective. *Behav. Brain Sci.*, **25**: 701–702.

Velte, F., Fleissner, G. and Wicker, R. 2002. Untersuchungen zur Chronoethologie des Pfauenaugenstechrochens (*Potamotrygon motoro*) – erste Ergebnisse. *Verhandlungen der Gesellschaft für Ichthyologie*, **3**: 119–129.

White, G.E. and Brown, C. 2015a. Cue choice and spatial learning ability are affected by habitat complexity in intertidal gobies. *Behav Ecol.*, **26**: 178–184.

White, G.E. and Brown, C. 2015b. Microhabitat use affects goby (Gobiidae) cue choice in spatial learning task. *J. Fish Biol.*, **86**: 1305–1318.

Zelinski, E.M. and Light, L.L. 1988. Young and older adults' use of context in spatial memory. *Psychol. Aging*, **3**: 99–101.