



Reorientation ability in redbtail splitfin (*Xenotoca eiseni*): Role of environmental shape, rearing in group and exposure time

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Abstract

When passively disoriented in an enclosed space, animals use the geometry of the environment (angular cues and metrically distinct surfaces) to find a position. Whether the ability to deal with geometry is a mechanism available at birth, with little influence of previous experience with the same kind of information, is still debated. We reared fish (*Xenotoca eiseni*) in tanks of different shape (circular or rectangular) either singly or in group and tested at different ages (at one week or one, five or ten months). Fish were trained to reorient in an enclosure with a distinctive geometry (a rectangular arena) and a blue wall providing non-geometric, featural information. Then, they were tested after an affine transformation that created conflict between geometric and non-geometric information as learned during training. We found that all fish, since one-week old, use significantly more the geometry of the enclosure for reorientation independently from the experience in circular or rectangular tanks. At one month of age, we observed a modulatory effect of rearing experience during learning with an advantage of individuals reared singly in rectangular cages, but no difference was evident at test. Furthermore, such effect on learning propensity disappeared later in development, i.e., when fish were trained at five or ten months of age. These results confirm that the use of geometric information provided by the shape of an enclosure is spontaneous and inborn, and that a modulatory effect of experience can appear briefly during ontogeny, but experience is not essentially needed to deal with geometry.

Keywords: Redtail splitfin *Xenotoca eiseni*, spatial cognition, reorientation, geometric module, experience, rearing

Introduction

Animals are able to encode and use the geometric properties of an enclosed rectangular space to recover their sense of place in order to find a target position (rats: Cheng, 1986; humans: Hermer and Spelke, 1994; monkeys: Gouteux et al., 2001; chicks: Vallortigara et al., 2004; pigeons: Kelly et al., 1998; fish: Sovrano et al., 2002; 2003; ants: Wystrach and Beugnon, 2009; bumblebees: Sovrano et al., 2012). The ability to rely on the enclosure shape seems to occur spontaneously even in the presence of featural information (e.g., a distinctive coloured wall) that would support an easier disambiguation of the two geometrically equivalent positions by breaking the symmetry of the space (review in Cheng and Newcombe, 2005).

The role of experience with angled surfaces of different lengths on the predominant use of geometry has been questioned. Studies on humans showed that the use of geometry is possible even in the absence of specific formal training with spatial concepts (Dehaene et al., 2006; Izard et al., 2011). However, cross-species comparisons resulted in partial contrasting evidence. Wild-caught mountain chick-

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adees (*Poecile gambeli*) do not encode the geometry of an enclosure when salient features are present near the target (Gray et al., 2005). Reliance on featural, over geometric, information in wild-caught birds may be imputed to their little experience with incidental surfaces of different length in small enclosures, geometric cues that are instead well-experienced by animals reared in the laboratory. The comparison of wild-caught and hand-reared individuals of two species of one genus [mountain chickadees (*Poecile gambeli*) and black-capped chickadees (*Poecile atricapillus*)] showed that both groups of birds make consistent use of geometry independently from experience (Batty et al., 2009), although mountain chickadees seem to rely less on geometry. This result is probably pinpointing a species-specific difference in the reliance on diverse information rather than an effect of previous experience with geometry *per se*. Indeed, Clark's nutcrackers show species-specific sensitivity to geometric cues in comparison with other avian species (Reichert et al., 2015). In this species, the order in which a specific kind of information (geometric or non-geometric) is experienced can modulate the reliance on geometry. If the birds learn to locate a corner on the basis of the arrangement of the surfaces and then on the basis of the features, they use equally both geometric and non-geometric cues at test, but if geometry is learned first, it is then weighed more than non-geometric information and used preferentially for reorientation.

Another series of studies tried to tackle the issue by providing laboratory animals with controlled rearing experiences. Such manipulation was operated in order to rigorously check the kind of exposure to geometric information the animals experienced before attending the test. Newborn domestic chicks (*Gallus gallus*) have been reared, soon after hatching in the dark, in either a circular or a rectangular cage, i.e., in the absence or in the presence of geometry as conveyed by the surfaces of the raising cages. Both circular- and rectangular-reared chicks proved identically able in heading toward the correct location by encoding and using the geometry of the enclosure (Chiandetti and Vallortigara, 2008), supporting the hypothesis that the use of geometry may be predisposed and little affected by experience. In a similar vein, Brown et al. (2007) reared the convict cichlid fish (*Archocentrus nigrofasciatus*) in circular or rectangular tanks and showed that both groups of fish were identically able to use geometric information to reorient. When geometry and features were set in conflict, with a displacement of features from training to test providing incongruent information about the target position (affine transformation), circular- but not rectangular-raised fish made less use of geometric information. Although the geometry is spontaneously used to reorient independently from specific exposure to angular geometric cues during rearing, the rearing environment in fish can affect the ability to navigate by landmarks in adulthood. Indeed, when

this same test situation was replicated with chicks, both circular- and rectangular-reared chicks showed no difference in choices (Chiandetti and Vallortigara, 2010). Also, when chicks are tested immediately after hatching with an imprinting procedure that prevents the need to train the animals, hence incidental exposure to geometry is avoided and direct testing is performed, they show a spontaneous use of the geometry of the enclosure (Chiandetti et al., 2015). Since chicks belong to precocial species, whereas convict cichlid fish need some parental care (after hatching they spend another 3 days before reaching complete development and starting free-swimming), a species-specific difference may account for the discrepancy of results between fish and chicks and the same species-specificity would hold true for the difference observed in chickadees as well (Vallortigara et al., 2009).

One possible complementary explanation regards the different rearing conditions between chicks and fish. Chicks were reared singly for 3 days before training and testing or tested soon after hatching, whereas fish were reared in groups for the extended period of 4 months. Living with companions for a prolonged period may have favoured the use of the location of individual conspecifics as featural cues for spatial orientation or may have partially occluded the shape of the rearing enclosure.

Twyman et al. (2012) investigated the influence of age by comparing reorienting abilities in young and adult mice housed in circular or rectangular cages. The two groups of animals displayed no difference in mastering the reorientation task in a rectangular environment and used preferentially the geometry of the arena to get their bearings, but young mice tended to use featural information more than adult mice when different colored cues were placed at the corners. In this experiment with mice, the characteristic of being an altricial species was assorted with the amount of time spent experiencing the environmental features and, moreover, some time (from one to three months) in regular rectangular environments was experienced by all animals before the experiment started, thus resulting in a spurious manipulation.

To find the key to the problem, we tested a species of fish, the redbait splitfin (*Xenotoca eiseni*) after rearing single individuals or groups of animals either in circular or rectangular tanks for different time windows. This South American Goodeidae is a livebearer fish, meaning that the fries are free-swimming at birth (for reviews on the biology of the species see: Fitzsimons, 1972; Meyer et al., 1985; Schubert, 1990). In this feature, they are very similar to the precocious domestic chicks that are independent of any parental care after hatching and freely explore the surroundings. The redbait splitfin has been tested in several neurocognitive domains, showing a striking continuity with other species for instance in functional visual perception (Sovrano and Bisazza, 2008; 2009) and numerical appreciation (Stancher et al., 2013). This spe-

cies of fish has also already proved to be successful in the geometric module task (Sovrano et al., 2002; 2003; 2005; 2007; Lee et al., 2012) in a way similar to that of all other species of animals tested insofar, hence here it is amenable to a specific focus on the role of experience. We manipulated the shape of the rearing environment as well as the amount of time spent in it and the living condition, alone or in group, and tested the animals in the affine transformation task. The shape of the tank, the density of animals inhabiting the same tank and the time period spent experiencing those rearing conditions could have been crucial in influencing the use of geometric information at test in previous works and, despite this, such factors have never been systematically controlled for.

Materials and methods

Subjects and Rearing Conditions — Subjects were overall 45 juvenile redbait splitfin (*Xenotoca eiseni*) reared individually (Experiment 1) or in group (Experiment 2) either in circular or rectangular environments, and tested at different ages (one week, one month, five months, ten months).

Individual rearing: As soon as the fries hatched, they were singly transferred either in circular (8 cm in diameter, 4 cm in height) or rectangular (12 × 8 × 4.5 cm) aluminum containers punctured on the bottom and in the lower part of the side walls by means of a needle to allow the water circulation. Up to five small containers were maintained in two larger plastic single tanks (49 × 31 × 13 cm), thus having a common filter and a common heater in 7 liters of water. The larger tanks were equipped with a large opaque plastic cover to prevent exposure to any external clues. Assignment of 17 fries to either circular (n=8) or rectangular (n=9) containers was random; circular- and rectangular-reared fish were trained and tested when they were 1 week (respectively, circular: n=4; rectangular: n=4) or 1 month old (respectively, circular: n=4; rectangular: n=5). This species is highly gregarious and the rearing in isolation is an experiential condition that can be conducive to the development of anti-social behaviours, symptomatic of stressful rearing conditions. Hence, we limited the test of individuals raised alone to one month of age to reduce any detrimental effect, in line with animal welfare regulation.

Group rearing: Immediately after birth, groups of 5 fries were transferred either in a circular (18 cm in diameter, 16 cm in height) or in a rectangular container (22 cm long, 13.5 cm wide and 16 cm high), with a white uniform ground. Pairs of containers were inserted into a single bigger tank (49 × 31 × 13 cm) providing 16 liters of water and, for other aspects, as described for the individual rearing condition. Assignment of 28 fries to either circular (n=14) or rectangular (n=14) containers was random; fish were trained and tested when they

were 1 month old (respectively, circular: n=5; rectangular: n=5), 5 months (respectively, circular: n=6; rectangular: n=6) and 10 months (respectively, circular: n=3; rectangular: n=3) old. Immediately after hatching, group rearing is very limitative for laboratory experiments because the very high competition for the food prevents a homogeneous growth that results in some of the fish significantly smaller than others. Fries compete for food resources even when the group is calibrated in density with respect to the size of the environment. For this reason, we started the testing with one-month-old animals, when the delay in growth, the lack of homogeneity and the aggression within the group had rather decreased.

Fish were fed once a day with dry food (Duplarin® for fries 1 week and 1 month old and GVG-Mix SERA® after 2 months from birth), after removing any organic waste products from the animal or from food scraps.

No vegetation was inserted in the rearing aquaria in order to maximize the visual processing of the shape of the rearing environment. However, no assaults or fights were witnessed between the individuals reared in groups and all fish were intact during the whole experimental procedure.

Apparatus — The apparatus was identical to the small-size apparatus used in Sovrano et al. (2005; 2007) and consisted of a rectangular tank (15 cm long, 7 cm wide and 16 cm high) inserted in a larger tank (54 × 32 × 23 cm) so as to create an annular region with vegetation, food and another conspecific that provided motivation for social reinstatement (Sovrano et al., 1999; 2001; Bisazza et al., 2001). The water in the tank was 6 cm high. The apparatus was placed in a darkened room and lit centrally from above with a 100 W light bulb. A video camera was fixed on the top of the lamp in order to be able to entirely frame all the four corners. In each corner of the apparatus there was a small tunnel (2.5 cm long, 2 cm wide and 3 cm high, located 4.5 cm from the floor of the tank) of white plastic material (Poliplak®), allowing the fish passing through it to rejoin the annular region and the conspecific in the outer tank. At the end of each tunnel, there was a door (2.5 × 3.5 cm) made of a sheet of an opaque plastic material on the top and of a transparent very flexible plastic material on the bottom (1 × 2 cm) that could be easily pushed and bent by the fish with its snout. During training, with a blue wall disposed on a short wall of the tank, only one door could be opened, the others being blocked. For the blocked doors the flexible plastic material was completely glued to the outer walls of the tunnel so that it could not be opened. For the correct door, in contrast, the flexible plastic material was glued to the wall of the tunnel only on the top-side so that the fish could easily bend it by pressing on it with the snout. The four doors were visually identical. During the affine transformation test, all the four doors were closed in the same way in order to avoid any dif-

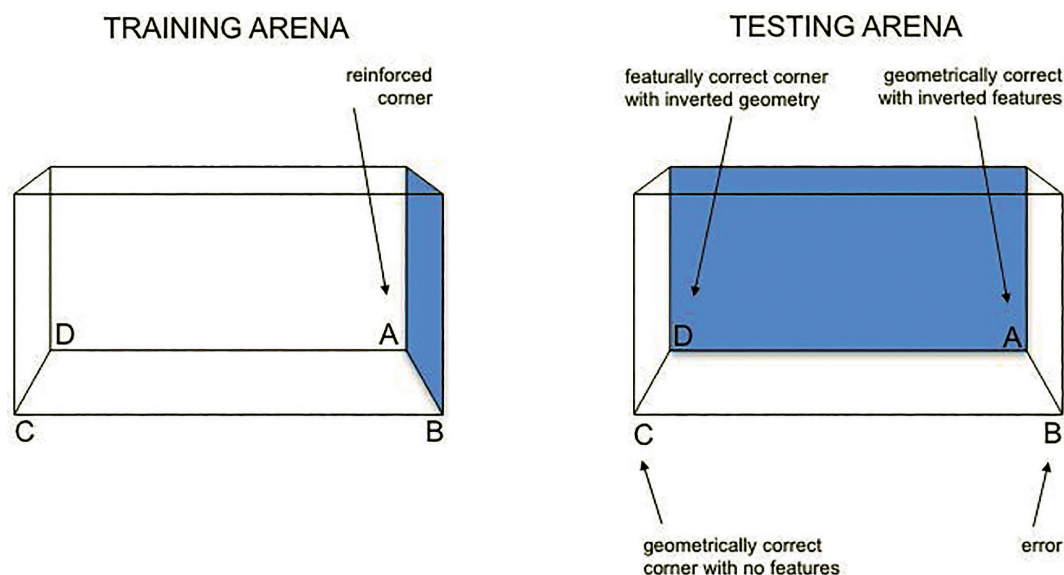


Fig. 1. Schematic 3D representation of the rectangular white arena with the blue coloured feature placed on a short wall for training (left-most) and the result of the affine transformation with the displacement of the featural information from the short to the long wall at test (right-most). With respect to the reinforced corner during the training (corner A), the affine transformation makes corner A geometrically correct but with inverted arrangement of colour features; corner D maintains the featural left-right arrangement but has no correct metric, whereas corner C is geometrically correct but without features and corner B has neither correct colour nor correct metric.

ferential reinforcement (extinction procedure). Choices for each door were clearly identifiable from the video recordings, and considered as valid only when the whole body of the fish disappeared within the corridor.

Procedure — Experiments comprised two phases: training and affine transformation test. During training, following an operant conditioning paradigm with food and vegetation in the annular region acting as the reinforcer, fish were given daily session of 10 trials until reaching the learning criterion (established as at least 60 % of choices for the correct corner in a single session and in the choice for the correct corner for a number of times at least double compared to each other corner). In each trial, the fish was brought from the annular region of the bigger tank by gently inserting it into a transparent plastic cylinder (6 cm in diameter; without top and bottom) placed in the center of the inner tank. After 10 seconds the cylinder was removed by lifting it gently, thus leaving the fish in the middle of the test tank. In each trial, the number of choices for the four doors was scored, until the fish was able to exit in the annular region; inter-trials interval, during which the fish was allowed to remain in the annular region (reinforcement time), was 10 minutes if the correct corner was identified at the first attempt and 3 minutes when this occurred after two or more attempts. After that, the fish was placed in a closed, opaque container (13 cm in diameter, 7 cm in height), passively disoriented on a rotating device and the entire apparatus was rotated 90 degrees in order to eliminate the use of compass and inertial information before starting another trial. Number of choices for the four corners, i.e. total number of choices per fish summed over the session of 10 tri-

als was used as individual data. Note that, during training, multiple choices for the correct corner could occur, either because fish explored the door without actually getting out or because not enough strength was exerted. The day after fish had reached the learning criterion, they were given two further trials to reinstate motivation.

During the affine transformation test, the blue wall was moved from the short to the long wall (as shown in Fig. 1) and fish were given a single session of 8 trials with all four doors blocked (extinction procedure). A training trial was mixed every two test trials to keep the motivation high. The procedure was exactly the same as in the training phase. The behavior of fish was observed for two minutes or until the completion of at least one valid choice, in any case for a maximum time of 20 minutes. During test, all inter-trials intervals were 8 minutes. An inter-rater reliability criterion (Caro et al., 1979) was applied in the re-coding of a subset of 10 % of different videos ($p < 0.001$, Pearson's correlation between the ratio calculated on the original coding and on the *de novo* coding performed by an experimenter blind on the rearing condition of the fish). Data were analyzed with the IBM SPSS Statistics 20 software package.

EXPERIMENT 1 — INDIVIDUAL REARING

In this experiment, we reared each newly hatched fish in a separate tank of either rectangular or circular shape and tested it either one week or one month after for its reorientation ability in the geometric module task with a blue wall (adjacent to the short wall during the training

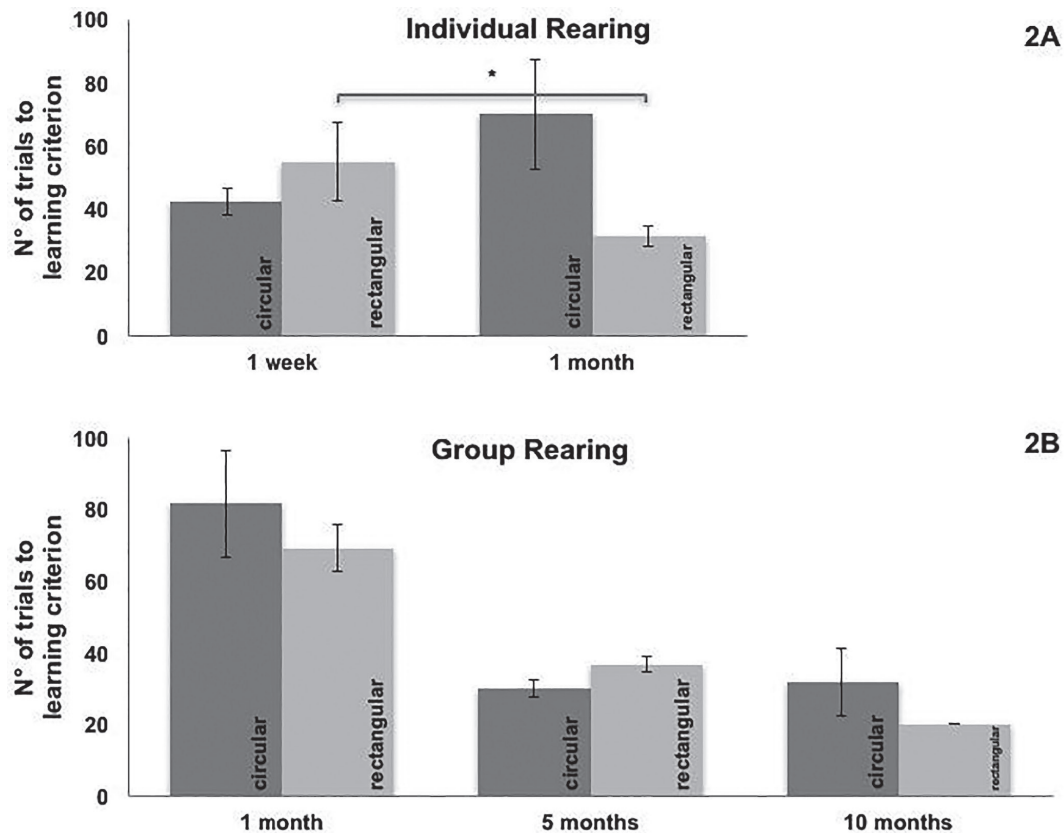


Fig. 2. 2A: Mean of learning trials to criterion (with SEM) for Experiment 1 — Individual Rearing. 2B: Mean of learning trials to criterion (with SEM) for Experiment 2 — Group Rearing. * $p < 0.05$

and, after an affine transformation, adjacent to the long wall during the test).

Results — The number of trials needed to reach the learning criterion was entered in a multivariate ANOVA with Type of rearing (circular, rectangular) and Age (1 week, 1 month) as between subjects factors. The ANOVA revealed no significant main effects (Type of rearing: $F(1,17) = 1.577$, $p = 0.231$; Age: $F(1,17) = 0.031$, $p = 0.862$) and a significant Age $\times$ Type of rearing interaction ($F(1,17) = 6.000$, $p = 0.029$) with rectangular-reared animals learning faster at 1 month than when 1 week old ($t(7) = 2.057$, $p = 0.039$ one-tail Independent t-Test) as shown in Fig. 2.

Results of affine transformation test are reported in Fig. 3. Data were analyzed with an ANOVA with Type of rearing (circular, rectangular) and Age (1 week, 1 month) as between subjects factors and Corners (A, B, C, D) as within subjects factor. The ANOVA, Greenhouse-Geisser corrected for Sphericity, showed only a significant main effect of Corners ($F(1.813,39) = 21.059$, $p < 0.001$). There were no other statistically significant effects (Age: $F(1,13) = 2.654$, $p = 0.127$; Type of rearing: $F(1,13) = 0.166$, $p = 0.690$) or interactions (Age $\times$ Type of rearing: $F(1,13) = 0.373$, $p = 0.552$; Corner $\times$ Age: $F(1.813,39) = 0.829$, $p = 0.438$; Corner $\times$ Type of rearing:

$F(1.813,39) = 0.256$, $p = 0.755$; Corner $\times$ Age $\times$ Type of rearing: $F(1.813,39) = 1.034$, $p = 0.365$).

All fish trained to find the correct corner (A) adjacent to the blue wall, regardless of the age and the type of rearing, when tested after the affine transformation preferentially choose the corner C, the equivalent rotational of the corner reinforced during training, which preserves all the geometrical properties, but not the presence of the featural information (C vs A, C vs B and C vs D, all $p < 0.017$ Bonferroni corrected).

EXPERIMENT 2 — GROUP REARING

In this experiment, we reared newly hatched fish in groups in separate tanks of either rectangular or circular shape and tested them singly either one, five or ten months after for their reorientation ability, as in the previous experiment, in the geometric module task with a blue wall (adjacent to the short wall during the training and, after an affine transformation, adjacent to the long wall during the test).

Results — The number of trials to reach the learning criterion was entered in a multivariate ANOVA with the Type of rearing (circular, rectangular) and Age (1, 5 and 10 months) as between subjects factors. The ANOVA showed only a significant main effect of Age

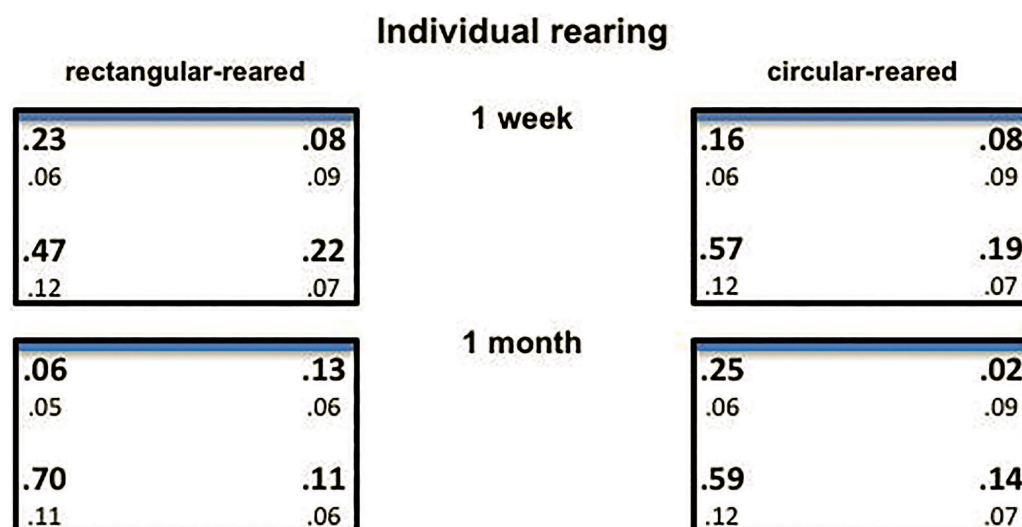


Fig. 3. Mean proportions of choices at test for each corner (in bold, with SEM below) for Experiment 1 — Individual Rearing. Results are plotted on a 2D schema (the blue line represents the blue wall) separately for fries that were one-week or one-month old and reared in rectangular or circular tanks.

($F(1,22)=22.716$, $p<0.001$), due to the fact that animals tested after 1 month differ from both animals tested at 5 and 10 month, but 5 and 10 months old individuals performed analogously (LSD, 1 vs 5 months $p<0.001$; 1 vs 10 months $p<0.001$; 5 vs 10 months $p=0.385$), showing a progressive reduction in the number of trials to reach the learning criterion with increasing age but only up to maturity, i.e., 5 months (Fig. 2). There were no other statisti-

cally significant effects (Type of rearing: $F(1,22)=0.756$, $p=0.394$; Age $\times$ Type of rearing: $F(2,22)=1.059$, $p=0.364$).

Results of the affine transformation test are reported in Fig. 4. Data were analyzed with an ANOVA with Type of rearing (circular, rectangular) and Age (1, 5 or 10 months) as between subjects factors and Corners (A, B, C, D) as a within subjects factor. The ANOVA showed only a main significant effect of Corners



Fig. 4. Mean proportions of choices at test for each corner (in bold, with SEM below) for Experiment 2 — Group Rearing. Results are plotted on a 2D schema (the blue line represents the blue wall) separately for fish that were one-, five- or ten-months old, reared in rectangular or circular tanks.

($F(3,66) = 11.537$, $p < 0.001$); all others main effects and interactions were not significant (Age: $F(2,22) = 2.357$, $p = 0.118$; Type of rearing: $F(1,22) = 2.095$, $p = 0.162$; Age $\times$ Type of rearing: $F(2,22) = 2.357$, $p = 0.118$; Corners $\times$ Age: $F(6,66) = 1.434$, $p = 0.215$; Corners $\times$ Type of rearing: $F(3,66) = 0.749$, $p = 0.527$; Corners $\times$ Age $\times$ Type of rearing: $F(6,66) = 1.138$, $p = 0.350$).

Overall, fish trained to find the correct corner (A) adjacent to the blue wall, regardless of the age and the type of rearing, when tested after the affine transformation preferentially choose the corner C (C vs A, C vs B and C vs D, all $p < 0.017$ Bonferroni corrected).

ONE-MONTH OLDS DIRECT COMPARISON

We run a further analysis to directly compare the effects of circular vs. rectangular and individual vs. group rearing on fish performance at 1 month of age.

The ANOVA with Type of rearing (circular, rectangular) and Condition (individual, group) as between-subject factors showed heterogeneity of both Type of rearing ($F(1,19) = 4.962$, $p = 0.042$) and Condition ($F(1,19) = 4.657$, $p = 0.048$) but showed no significant interaction ($F(1,19) = 1.319$, $p = 0.269$).

Circular reared animals needed basically more trials to learn the task ($t(17) = 2.047$, $p = 0.056$, Independent Samples t-Test) irrespectively of whether individually or group reared. Individually-reared animals needed significantly fewer trials to learn the task than group-reared fish ($t(17) = -2.117$, $p = 0.049$, Independent Samples t-Test).

At test, the ANOVA with Type of rearing (circular, rectangular) and Condition (individual, group) as between subjects factors and Corners (A, B, C, D) as a within subjects factor showed a main significant effect of Corners ($F(3,45) = 17.312$, $p < 0.001$), Condition ($F(1,15) = 8.847$, $p = 0.011$) and a significant interaction Corners $\times$ Condition ($F(3,45) = 2.887$, $p = 0.046$). All other main effects and interactions were not significant (Type of rearing: $F(1,15) = 1.299$, $p = 0.272$; Condition $\times$ Type of rearing: $F(1,15) = 0.786$, $p = 0.389$; Corners $\times$ Type of rearing: $F(3,45) = 1.275$, $p = 0.295$; Corners $\times$ Condition $\times$ Type of rearing: $F(3,45) = 0.243$, $p = 0.866$).

All fish trained to find the correct corner (A) adjacent to the blue wall, regardless of the type of rearing, when tested after the affine transformation preferentially choose the corner C (C vs A, C vs B and C vs D, all $p < 0.017$ Bonferroni corrected). However, group-reared fish choose significantly less corner C ($t(17) = 2.339$, $p = 0.032$, Independent Samples t-Test).

General discussion

Our work was aimed at investigating the role of rearing factors on *Xenotoca eiseni*'s ability to reorient in a rectangular enclosure. We controlled for the shape of the living envi-

ronment, for the conditions in which the fish were raised and the amount of time of experiencing such rearing, i.e. we compared the performance of fish reared in geometrically rich (rectangular) or poor (circular) tanks, and living singly or in group for 1 week or up to 10 months.

We found that fish at one week of age, reared singly, preferentially rely on the geometry of the enclosure to reorient, independently from the experience of living in circular or rectangular tanks. Indeed, the training condition provided both geometrical and featural cues, and learning of the task required the fish to conjoin the features and geometry to find the single correct corner; by putting the two cues in conflict with the affine transformation test, we provide the animal with no unequivocal solution but alternative choices are made available depending on which information is weighted as more relevant. Our results replicate the findings already obtained with *X. eiseni* (Sovrano et al., 2007) in which animals made a predominant use of geometry but here we demonstrate that this ability is available already at the early age of one week after hatching, similarly to what observed in precocial domestic chicks (Chiandetti and Vallortigara, 2008; Chiandetti et al., 2015). The fries choose significantly more often the only corner that, after the affine transformation, maintained the correct arrangement of the long and short wall with respect to the left and right side rather than the corner that had the same featural disposition of the colour experienced during training with the incorrect geometry, or the inverted features but the correct geometry.

Our results also show that the reliance on the pure geometry of the environment is stable at all ages and is evident irrespectively of the rearing environment (whether circular or rectangular) and the living condition (either individually or in group). Despite the relevance of the featural information at test, which is augmented in our affine transformation because the colour blue is displaced from the shorter wall at training to the longer wall at test, the performance of all fish, in the preferential use of pure geometry, is not affected by the change in size of the feature. The only observed difference pertains to animals reared in groups, which make overall fewer choices for the pure geometric corner but choose it still significantly more than all the other alternatives.

A modulatory effect of exposure to both geometry and other individuals has been detected at one month of age during learning. Concerning the effect of the exposure to geometry, rectangular-reared fish needed a lower number of trials to reach the criterion, pointing toward a specific advantage of being previously exposed to the geometric cues of a rectangular rearing tank in dealing with geometric cues while learning. However, this holds true only for fish trained at one month of age, hence it seems a modulatory effect that is specific to a certain stage of maturation, when the fish are no longer fries but at the same time not yet mature adults. At this age, indeed, the size of the body makes the fish less susceptible to intra- and in-

ter-specific predation, which in turns gives the fish more time to learn the environmental features. Concerning the effect of the social rearing, our results show that one-month-old fish reared in groups of conspecifics, but independently from the shape of the rearing environment, tend to be slower in learning as compared to fish grown individually. One possibility to explain the negative effect of the group rearing is that the prolonged experience of living with conspecifics could have made fish more cautious when alone in a new enclosure while learning to get their bearings. Note that fear or stress potentially experienced by group-reared fish when trained alone are factors that unlikely can explain the observed difference *per se*, because we should expect it consistently at all ages when not increasing with age, given the gregarious nature of this species. Once fish were close to the maturity, starting from 5 months onwards, the prolonged experience has no longer detectable effects and there is rather a general decrease in the number of trials needed to learn the task. Fries, on the contrary, have the essential need to make the safer choice possible since they have to readily escape, in order to reduce the high risk of predation and intra-specific cannibalism. They could have been predisposed to quickly attend the shape of the environment in order to swim toward a safer environment and hide.

Convict fish (*Archocentrus nigrofasciatus*), when tested around maturity after social rearing with or without exposure to geometry, learnt the task in a comparable fashion (Brown et al., 2007). However, in convict fish, a negative effect of exposure to geometry emerged when fish grown in rectangular tanks had to learn to reorient about the features. At test, rectangular reared fish chose between three alternatives (the two geometrically correct corners and the corner that retains the sense “blue wall to the right”) while circular reared fish reoriented almost exclusively to the corner that maintained the same characteristics of sense (Brown et al., 2007). In our work, we did not find a comparable influence of the rearing experience: all the animals preferred to get their bearings toward the metrically correct corner that had no featural cue. Moreover, the blue wall was systematically disregarded at test; we speculate that the size increment resulted in a response of avoidance of novelty, probably showing a species-specific cautious attitude with subsequent avoidance for the feature, so strongly changed. Hence, an explanation based on species-specific differences is the most likely to account for the differences in the observed behaviour between convict and redbtail splitfin fish. However, a crucial factor never systematically addressed before is the age at which the chosen animal model is tested. As shown in our work, an assessment conducted only at one month of age would have partially masked the innate propensity of *X. eiseni* to deal spontaneously with geometry.

We conclude that the encoding of the geometric shape of the environment appears early in *X. eiseni* as

in other precocial species of vertebrates like the chick and does not require previous experience relative to the geometry of space, as angular cues, directional relationships or distances between metrically distinct surfaces (Spelke and Lee, 2012). Moreover, the presence of a conspicuous landmark does not detract priority to the encoding of geometry, regardless of exposure to geometric information, age and living conditions.

The modulatory effect of experience is limited to the learning phase of a specific age during the ontogenetic development in *X. eiseni*, i.e., one month. Most importantly, there are no differences depending on the shape of the rearing environment when different alternatives to the use of geometry are available at test. Hence, the use of the geometry of the extended surface layout appears to be robust, and consistently and successfully mastered in all conditions, despite the fact that in some conditions the number of tested fish is limited to few individuals.

Another species of fish, the zebrafish (*Danio rerio*), spontaneously reorients by the environmental layout of a rectangular tank, likewise the redbtail splitfin (Lee et al., 2012). The two species of fish differ instead in the use of landmarks for reorientation. Again, the encoding and use of the geometric information seem a shared and basic mechanism upon which natural selection has modeled species-specific differences due to particular pressures and living niches for further learning processes to take place. The zebrafish, indeed, was also shown to form an articulated memory of the spatial organization of a variety of mazes (Sison and Gerlai, 2010); animals exploring complex labyrinths remembered the mutual arrangement of corridors, even when no differences in colours or other characteristics were available (Grossman et al., 2011), a map-like higher level representation of the environment that likely sets its root in the use of the environmental geometry.

These facts seem to support the idea of an innate core system to deal with layout geometry that arises with no prior experience navigating in geometrically structured environments and that is in some way essential to the survival of organisms in their natural habitat, where the spatial elements on a large scale, such as the presence of a mountain or a river bed, do not change for the duration of life of a biological organism, while the local cues, such as, for example, the presence of flowers, snow or mud, are constantly changing, in relation to atmospheric events and the alternation of the seasons. There is a computational advantage in relying on invariant properties of the environment rather than on its changeable features. Moreover, in real natural scenes, it is unlikely that surfaces present equivalent symmetries as those created in artificial enclosures. Still, such environments help to clarify how animals navigate and to what extent the mechanisms they use are predisposed or can be moulded by experience.

Ethical statements

The present research was carried out in the laboratory of the CIMeC (Center for Mind/Brain Sciences) of the 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 the Italian law. The experimental protocol has been authorized by the University of Trento Ethic Committee for the Experiments on Living Organisms and the Italian Ministry of Health (authorization protocol: 2012–021A).

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