

Social learning in a maze? Contrasting individual performance among wild zebrafish when associated with trained and naïve conspecifics



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ABSTRACT

Social learning facilitates informed decision making about foraging, mating and anti-predatory tactics among animals. We investigated the occurrence of social learning through performance in a spatial task among wild-caught zebrafish. Individual fish (demonstrators) were trained through a novel food finding task in a maze for 8 days. Demonstrators were paired with naïve individuals (observers) and subjected to trials through maze for 4 days followed by removal of the demonstrators and further training of observers for 4 more days. Paired naïve individuals were subjected to trials through the maze in similar fashion separately and the performance of observers were compared with theirs. Our results showed that observers associated with knowledgeable conspecifics did not perform the task better than naïve-paired individuals. Performances across trials improved for both sets while number of mistakes committed increased indicating no learning. The presence of a demonstrator could have increased the observer's activity, increasing the chances for the observer to come in contact with the stimulus. Performance of observers and naïve-paired fish were probably affected by social distraction. Sex and body-size of the dyads (demonstrator-observer pairs and naïve pairs) could also have interfered with information transfer among individuals.

1. Introduction

Learning that involves the use of socially provided information is termed as social learning (Brown and Laland, 2003). Social learning can be facilitated by a number of psychological mechanisms that may range from simple non-imitative reflexive contagion to complex forms like goal emulation and copying (Brown and Laland, 2003; Davis and Burghardt, 2011; Heyes and Galef, 1996; Laland, 2004; Pearce, 2008; Shettleworth, 2010; Zentall, 2006). Social facilitation, for example, is the increase in arousal or activity of a naïve individual (observer) by mere exposure to another animal (Davis and Burghardt, 2011; Zentall, 2003). Presence of conspecifics can have several added benefits such as enhanced object exploration and foraging mechanisms (Miller et al., 2014). On the other hand, following or being with a knowledgeable individual (demonstrator) exposes a naïve individual to similar features of environment, thereby facilitating 'guided learning' (Brown and Laland, 2003). Social learning enables naïve individuals to gain information crucial for survival such as foraging (Galef and Giraldeau, 2001), mating (White, 2004) and anti-predatory (Griffin, 2004) tactics. Indeed, studies on social learning strategies are increasing rapidly and emphasize the importance of flexible context-dependent use of subtle biases as a general feature of social learning (Rendell et al., 2011). The

use of information acquired through social learning is not always beneficial and it is still unclear as to how animals disentangle false and misleading information from that which is correct and appropriate (Giraldeau et al., 2002). Self-learning through personal interaction with the environment may implicate costs of risking predation or injury, as well as costs of opportunities missed due to loss of time or energy. It is expected therefore that the acquisition and utilization of knowledge through 'demonstrators' would necessarily bestow fitness benefits on naïve learners who will tend to economize on the costs of learning for themselves. This study investigates possibility of social learning among individuals of a natural population of zebrafish.

Experimental evidence across multiple fish species reveal involvement of social learning strategies in the context of foraging (Brown and Laland, 2002; Coolen et al., 2005; Duffy et al., 2009; Laland and Williams, 1997; Reader et al., 2003). Individuals may learn the location of feeding patches from observing foraging conspecifics (local enhancement: Giraldeau, 1997). In strongly gregarious species, a small group of informed leaders may be able to lead the shoal to food through social facilitation (Reebs, 2000). This study investigates the possibility of social learning about a food-finding task using zebrafish as a model. Zebrafish is a highly gregarious species with individuals aggregating in small to very large numbers to form shoals (Miller and Gerlai, 2011;

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Suriyampola et al., 2016). While several studies have investigated individual learning (spatial learning: Roy and Bhat, 2016; visual discrimination: Colwill et al., 2005; active-avoidance learning: Xu et al., 2007; associative learning: Cofiel and Mattioli, 2011; Sison and Gerlai, 2010; conditioning: Bilotta et al., 2005; Spence and Smith, 2008), few studies explore learning in social context among zebrafish. Information on predators can be socially transmitted in zebrafish through visual perception of alarmed conspecifics and sensing of chemical cues released due to skin injury (Hall and Suboski, 1995a,b). Further, information about location of an escape route in order to flee an artificial predator (moving trawl net) has been shown to be acquired through observation of knowledgeable conspecifics (Lindeyer and Reader, 2010). Abril-de-Abreu et al. (2015a,b) in two recent studies demonstrated the relevance of social eavesdropping for agonistic interactions in zebrafish and showed that eavesdroppers were able to integrate the gained eavesdropped (public) information with past social experiences (private social information).

Here, we examined the occurrence of social learning in zebrafish in the context of a spatial performance task. In accordance with the social-learning terminology, we termed trained knowledgeable conspecifics ‘demonstrators’ and the naïve subjects paired with them ‘observers’ (Brown and Laland, 2003; Davis and Burghardt, 2011). We first investigated if performance of observers in a spatial task improved when paired with demonstrators. Individual fish were trained in a maze to find a food reward associated with a color. Following this, the trained individuals (demonstrators) were paired with naïve conspecifics (observers) and subjected to trials in a similar fashion. As a separate set, paired naïve individuals were subjected to the same training regime. We compared the performances of ‘observers’ with the ‘naïve-paired’ individuals to understand the occurrence of social learning. We expected that performance of fish when paired with demonstrators would be better than performance of fish paired with naïve conspecifics.

2. Materials and methods

2.1. Population

A wild population of zebrafish, *Danio rerio*, was collected from a medium-slow moving stream Seripetkalwa situated in the Chittoor district of Andhra Pradesh, located in Southern India. Zebrafish were found in side-pools covered with emergent vegetation and along the vegetated areas of the stream. The total dissolved solids at the time of

collection was 227.5 ppm, indicating reasonably high water turbidity. The stream substrate consisted of mix of sand, silt and mud. The relative abundance of zebrafish (number of individuals of zebrafish/total number of individuals caught) was 0.32. The abundance of predatory fish was low while overall fish species diversity was high.

2.2. Maintenance of wild-caught population

Fish were brought back to the laboratory and transferred to bare housing aquaria of dimensions $45.7 \times 25.4 \times 25.4$ cm equipped with a standard corner filter with aeration. The average body-size (total length) of the fish was 2.8 cm ($n = 30$) at the time of sampling. Approximately 30 individuals were maintained per aquarium with mixed sex and similar density for all aquaria. Holding room temperature was maintained at 25 °C and lighting condition was set at 14:10 h light: dark cycle to mimic the natural conditions essential for courtship and spawning. The fish were fed freeze-dried blood-worms or *Artemia* alternately, feeding once daily in the morning. The fish were maintained in these stock aquaria for at least 8 months before the commencement of experiments to ensure full grown adults. The average body-size (total length) of the fish was 3.37 cm ($n = 30$) before the start of the trials.

2.3. Setup

A square shaped testing arena of dimensions $44.5 \times 44.5 \times 21.5$ cm was constructed for examining social learning in zebrafish through a spatial performance task (Fig. 1). The tank consisted of an inner square shaped layer with sides measuring 28.5 cm. The separation between the sides of the inner layer and corresponding sides of the outer wall was 7.5 cm. The diagonal ends of the tank-walls were connected to the edges of the inner square by 10 cm connections thereby forming four separate chambers. Each chamber was divided into three sections separated by two windows (Fig. 1). Each chamber had a main door leading to the center of the test arena. A removable colored window-pane (red, yellow, green or blue) identified each main door. The colors for doors were selected based on a previous study in *Amarillo* fish by Burt and Macias Garcia (2003). Previous studies have reported that zebrafish do possess color vision (Cameron, 2002) and are able to distinguish colors (Arthur and Levin, 2001; Colwill et al., 2005; Fettsko, 2002; Fettsko et al., 1996). A food reward associated with an artificial plant was always placed inside the right-hand section of the

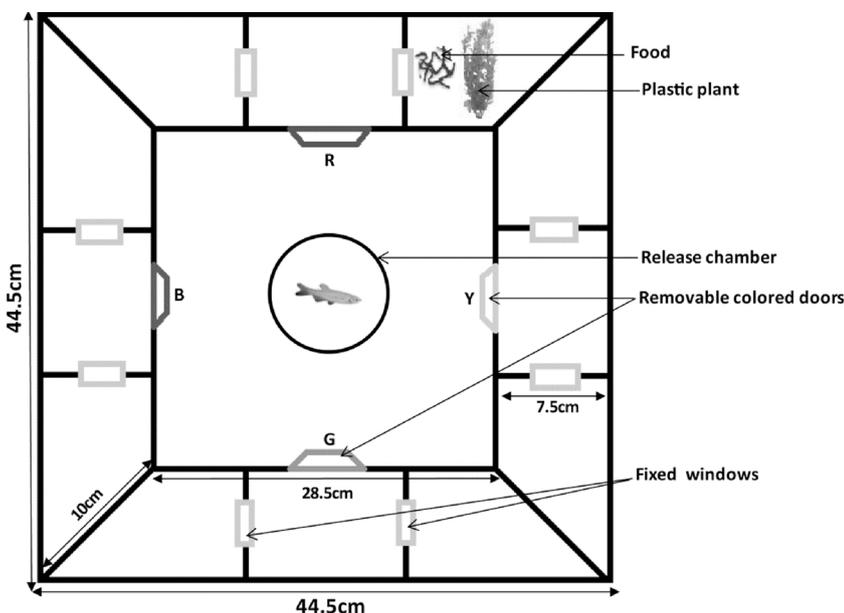


Fig. 1. Schematic diagram of the experimental setup. The square shaped arena has 4 chambers each with a coloured, removable main door. Each chamber is divided into 3 sections by two fixed windows. Codes R: Red, Y: Yellow, G: Green and B: Blue.

red-door chamber (Fig. 1). Pilot experiments conducted using the same testing arena without a reward had not revealed preferences for a particular colored door or door position by fish (Mann Whitney *U* test, $n = 10$, $p > 0.05$). Hence the choice of the red door chamber for food reward was random. Previous studies in zebrafish where the use of a red cue card predicted the presence of food (Sison and Gerlai, 2010; Williams et al., 2002) support our selection. The position of food-reward and plant within the red-door chamber was kept unchanged. An individual fish would have to explore the maze and locate the food inside the right-side section of the red-door chamber. The task in question therefore comprised a color-association component followed by a turn component. The outer wall of the testing tank was externally lined with brown cardboard paper to prevent any local external cues from affecting fish behavior. The walls of the inner layer and the diagonal connections were all opaque. The testing arena was positioned under a white ceiling and uniform lighting conditions.

2.4. Experiments

2.4.1. Experiment 1: test involving observers paired with demonstrators

The first set of experiments was conducted to test whether individuals performed a spatial task better when associated with trained conspecifics. Subjects (naïve individuals) are subsequently termed here as ‘observers’ and trained conspecifics ‘demonstrators’, in accordance with previous studies (Brown and Laland, 2003; Davis and Burghardt, 2011; Webster and Laland, 2008). We stick to this terminology throughout the rest of our study. 15 fish were selected from the wild-caught population maintained in the stock aquaria. Individual fish were kept in labeled transparent 1 l cylindrical plastic containers (filled with 520 ml of aerated water) throughout the period of experiments. This method of isolation was necessary to identify fish individually in order to facilitate the tracking of individual behavior over the course of experiments (Brown and Braithwaite, 2004; Roy and Bhat, 2015, 2016). For each day during the trials, fish were fed only after the test in order to control for hunger levels. Water in the holding containers was changed every day after the experiments in order to ensure welfare of the fish. Individual fish were trained across the maze setup described in the previous section, for 8 days to find the food reward. The tank was filled with well aerated water to a depth of 12 cm. A test fish was transferred into an opaque cylindrical release chamber in the middle of the maze and allowed 2 min to acclimatize (Fig. 1). Post acclimatization, the release chamber was slowly raised and the fish was allowed to explore the arena. Once the fish entered the red-door chamber and reached the right-hand section, a lump of freeze-dried blood worms was put near the plant using forceps. The behavior of the fish was video recorded for 20 min using a high definition camcorder Canon LEGRIA HF R306 placed at an optimum distance overlooking the maze. After the experiment, the fish was carefully netted out and transferred into its respective container for successive trials. The uneaten food was siphoned off and water in the tank was changed to avoid olfactory cues from affecting learning. An individual fish was subjected to a trial only once each day. In this way, the fish was trained through the maze to locate the food reward for 8 consecutive days. The position of red door, with the plant and the reward, was shuffled (not rotated) every day but kept constant (i.e. their relative positions were maintained) through all trials for other individuals tested during a day. No two consecutive days had the red door in the same position.

On the 9th day, 15 naïve fish (observers) were isolated from the population stock to be subjected to trials in presence of the previously trained individuals (demonstrators). The observers selected were from a different stock tank than that of the demonstrators used in the previous section. This ensured that there were no interfering effects of familiarity between demonstrators and observers while being tested for social learning. As described in the previous section, individual fish were kept in labeled transparent 1 l containers throughout the period of experiments. Each demonstrator was then paired with an observer. In a pair,

the observer contrasted the demonstrator in body size, i.e. a smaller observer fish was selected for a bigger demonstrator, and vice versa. Among the 15 pairs, the observer fish were smaller than the demonstrator fish in eight pairs. The pairs were subjected to trials across the maze setup as described in the previous section. At a time, a demonstrator and its observer counterpart were transferred into the release chamber in the testing arena, acclimatized for 2 min and allowed to explore the maze to find the food reward. Food was introduced whenever the demonstrator or the observer first entered the correct compartment. Excess food was carefully siphoned off immediately after the demonstrator or observer had stopped feeding in order to minimize the chances of olfactory cues affecting the other’s behavior. The behavior of the demonstrator-observer pair was recorded for 20 min. The trials involving paired individuals lasted for 4 days (9th – 12th day). On the 13th day, the demonstrators were removed from the experiments and the observers were tested alone across trials for four more days, i.e. 13th–16th day. The demonstrators were returned to the stock tanks. The observers therefore underwent 8 trials, 4 in presence and 4 in absence of demonstrators. Following the 16th day trial, an intermission of four days was given during which the observers were not subjected to trials. During this time, the fish were fed normally in their respective containers. Post-intermission, the observers were re-tested on the 21st day for their ability to remember the task. The video-recordings were analyzed for (1) time taken by a demonstrator to find the food reward on each day (performance time) from the time of release, from the 1st to the 12th day, (2) the number of wrong entries made by a demonstrator till the discovery of food reward on each day from 2nd trial to 12th trial, (3) time taken by an observer to find the food reward on each day (performance time) from the time of release, from the 9th to 21st (test) day and (4) the number of wrong entries made by an observer until the discovery of food reward on each day from 10th day trial to the test trial on 21st day. An upper-limit of 20 min was assigned for the performance time if an individual was inert or failed to find the food-reward within the duration.

2.4.2. Experiment 2: test involving paired naïve conspecifics

28 (naïve) fish were sorted out from the stock population, 14 each from 2 different stock tanks in order to maintain uniformity with selection of observer-demonstrator pairs. Individual fish were kept in labeled containers throughout the period of experiments as described previously. 14 pairs were then formed with paired individuals contrasting in body size. The behavior of the fish was video recorded for 20 min. The paired individuals were subjected to training in the maze setup for 4 consecutive days. On the 5th day, one individual from each pair was removed randomly. The single individuals then continued to be tested across trials for four more days, i.e. 5th–8th day. The individuals removed were returned to the stock tanks. Following the 8th day trial, an intermission of four days was given during which the fish were not subjected to trials. During this time, the fish were fed normally in their respective containers. Post-intermission, the individuals were re-tested on the 13th day for their ability to remember the learnt task. The video-recordings were analyzed for (1) time taken by any one random individual to find the food reward on each day (performance time) from the time of release and (2) the number of wrong entries made by any one individual until the discovery of food reward on each day from the 2nd trial to the test trial. This meant that the same individuals would not have been analyzed on consecutive days since the choice was random. This was based on a previous study by Spence et al. (2011) where the time until commencement of feeding by any single individual in a group of three zebrafish was recorded across trials. An upper-limit of 20 min was assigned for the performance time if the individual that was being scored for the behavior was inert or failed to find the food-reward within the duration. The tested individuals from this experiment are termed ‘naïve-paired’ henceforth.

2.5. Statistical analysis

Differences in performance over trials between observers (Experiment 1) and naïve-paired individuals (Experiment 2) were tested using ANCOVA with treatment (demonstrator-paired and naïve-paired) as factor and trials as covariate. Similarly, a repeated measures ANOVA was conducted with treatment as a factor to test the differences in number of mistakes over trials between the two groups. Performance time of first and last trials, between observers and naïve-paired individuals was compared using Mann Whitney *U* test. Further, a Mann Whitney test was conducted to compare performances during the 1st and 4th trials of the demonstrators and naïve-paired fish in order to test whether presence of a conspecific affected performance of individuals. In order to test for memory of observers and naïve-paired individuals, the performance time and number of mistakes committed during the last trial and test was compared using Wilcoxon paired-sample test.

All data analyses were conducted using Statistix v1.8 and R software (2013).

3. Results

Performances and number of mistakes made by the demonstrators and observers during the spatial task are graphically represented in Fig. 2a, b. No fish (demonstrator, observer or naïve-paired) was found to be inert in any of the trials. The ANCOVA results (Experiment 1 versus Experiment 2) showed a significant effect of trials ($F_{1,12} = 34.80, p < 0.001$) and treatment ($F_{1,12} = 63.62, p < 0.001$), but no significant interaction effect on performance time

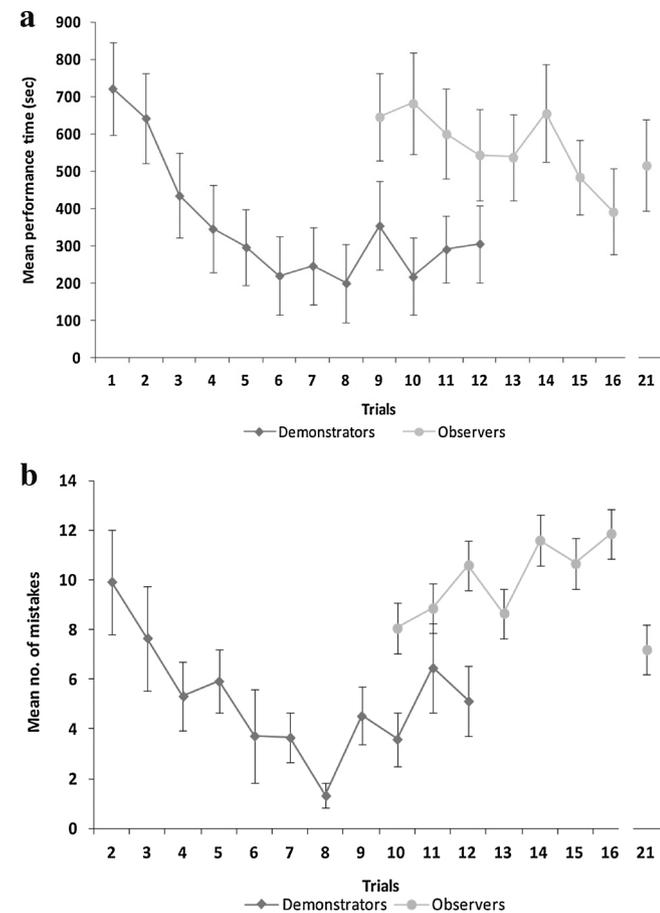


Fig. 2. Representation of performance in the maze among demonstrators and observers. (a) Mean performance time, and (b) mean no. of mistakes made by demonstrators over 12 trials (Day1 to 12), and observers over 8 trials (Day 9–16) and the test trial. Standard errors (\pm) are indicated by error bars.

Table 1 Results of (a) ANCOVA comparing performance time, and (b) repeated measures ANOVA comparing number of mistakes made by observers and naïve-paired individuals.

(a)		
Performance time	F (df)	p
Trial	34.80 (1,12)	< 0.001
Treatment (Experiment 1 and Experiment 2)	63.62 (1,12)	< 0.001
Trial X Treatment	1.97 (1,12)	0.19
(b)		
Number of mistakes made	F (df)	p
Trial	0.83 (6)	0.56
Treatment (Experiment 1 and Experiment 2)	0.02 (1)	0.89
Trial X Treatment	0.40 (6)	0.87

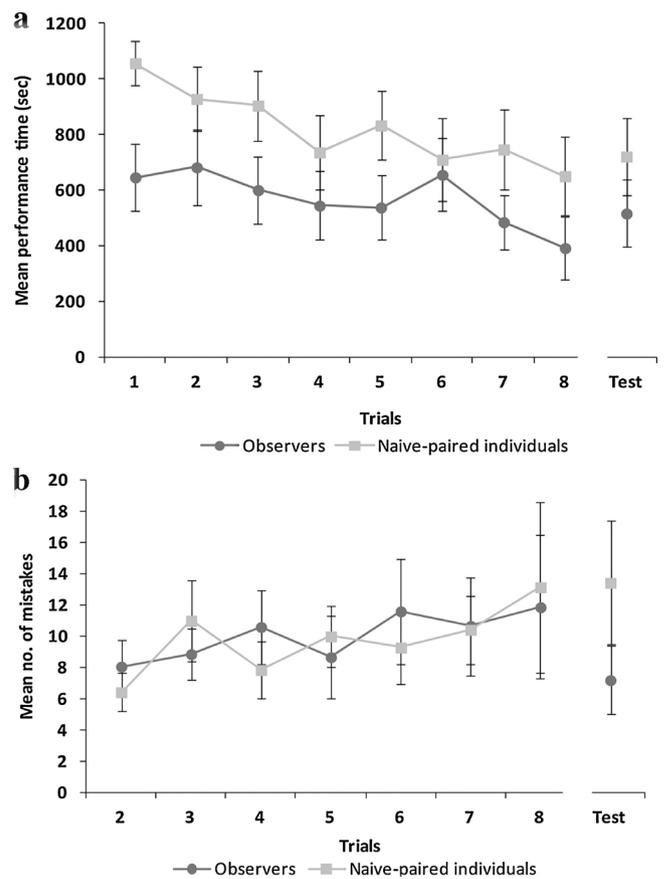


Fig. 3. Test for occurrence of social learning through comparison of Experiment 1 and 2. (a) Mean performance time of observers and naïve-paired individuals over 8 trials and test trial. (b) Mean no. of mistakes made by observers and naïve-paired individuals over trials 2–8 and test trial. Standard errors (\pm) are indicated by error bars.

of individuals (Table 1a, Fig. 3a) (therefore indicating no significant differences in slopes of these performance curves). There was no significant effect of trials, treatment or interaction trial X treatment on number of mistakes made by individuals in finding the food reward (Table 1b, Fig. 3b). A significant difference was found between performance time of observers and naïve-paired individuals during the 1st trial (Mann Whitney *U* test, $U = 156.5, p = 0.02$), while no significant difference was found between performance time of observers and naïve-paired individuals during the 8th trial (Mann Whitney *U* test, $U = 138.0, p = 0.16$). Furthermore, difference in performance time between single (mean = 722.1 s, S.E \pm 125.3), and naïve-paired (mean = 1057 s, S. E. \pm 78.4) individuals during the 1st trial was

significant (Mann Whitney U test, $U = 156.5$, $p = 0.02$), as was the performance time between single (mean = 346.5, sec S.E. \pm 116.8) and naïve-paired (mean = 735.9, sec S. E. \pm 134.8) individuals during the 4th trial (Mann Whitney U test, $U = 154.0$, $p = 0.03$).

In the test for memory, no significant difference was found in performance time (Wilcoxon paired-sample test, $T = 36$, $n = 15$, $p = 0.22$) (Fig. 3a) and number of mistakes made (Wilcoxon paired-sample test, $T = 48$, $n = 15$, $p = 0.53$) (Fig. 3b) by observers during 8th trial and test. Similarly, no significant difference was found in performance time (Wilcoxon paired-sample test, $T = 46$, $n = 14$, $p = 0.95$) (Fig. 3a) and number of mistakes made (Wilcoxon paired-sample test, $T = 52$, $n = 14$, $p = 0.99$) (Fig. 3b) by naïve-paired individuals during 8th trial and test.

4. Discussion

The results showed that the performance of all fish improved across trials, and the performances of observers were better than the performances of naïve-paired individuals (Fig. 3a). However, no significant interaction effect (i.e. treatment X trials) on performance indicated there was no difference in performance rates between the observers and naïve-paired fish. Individuals were considered to have learnt the task only when both the performance time as well as number of mistakes decreased significantly over time (trials). Though the performance time of all individuals decreased over trials (Fig. 3a), the number of mistakes made increased with time (Fig. 3b) which indicated that they had not learnt the task. The observers performed significantly better than the naïve-paired individuals. They also had a significantly lower initial performance time than naïve-paired individuals (Fig. 3a) implying that the presence of demonstrators aided the observers in finding the food reward when first exposed to the spatial maze. Performance time during the 8th trial was not significantly different for the observers and naïve-paired individuals. The demonstrator may have encouraged the observer to move more on its own, thus increasing its chance to enter the correct compartment and find the food reward (Social learning processes: Brown and Laland, 2003). The decrease in performance time of both observers and naïve-paired individuals across trials in presence of a conspecific could have been because of an increase in boldness and familiarity with the test tank. The fish became increasingly familiar with the test tank with subsequent trials. This observation is similar to a previous study where wild-population juveniles of zebrafish exhibited improvement in performance in a novel maze due to increasing familiarity but did not show high learning capacities (Roy and Bhat, 2016). While the number of mistakes increased with trials, their speed also could have been higher owing to increasing familiarity with their surroundings thus leading to shorter time in reaching the correct compartment (i.e. improved performance) along subsequent trials. Further, no differences between the rate of change of performance of observers and naïve-paired fish indicated that presence of a conspecific (naïve or trained) does not impact rate of learning in the maze. Finally, a significant difference between the 1st as well as 4th trial performances of demonstrators and the naïve-paired individuals was found, with the clear better performance demonstrated by individuals when they were solitary. This strengthened our observation that presence of a conspecific (naïve or trained) does not improve individual learning in maze. These results are in partial agreement with a previous study on Atlantic salmon where naïve fish paired with trained conspecifics learnt the task of associating a darting motion with food availability and the presence of an untrained conspecific decreased the learning rates due to 'social inhibition' (Brown and Laland, 2002). The paired naïve individuals also performed worse than the fish who learnt by themselves (Brown and Laland, 2002).

Group living facilitates transmission of novel information from knowledgeable individuals to naïve conspecifics (Rendell et al., 2011). However in our study, we did not observe transfer of information about the novel food-finding task between the demonstrators and the

observers. A previous study by Giraldeau and Lefebvre (1987) showed that pigeons that were allowed to scrounge from an informed partner failed to learn how to obtain food by themselves whereas pigeons that could not scrounge did easily learn from a conspecific tutor. Furthermore, the fact that the observers on an average took more time to enter the correct compartment than the demonstrators first did, on all trials, suggests that there was no transfer of information between demonstrators and observers. The performance of demonstrators improved rapidly during the first 8 days in the absence of observers (Fig. 2a, b). The greater learning capacities exhibited by the demonstrators, prior to the introduction of observers could be attributed to a more detailed exploration and information obtained through first-hand sampling of the maze environment as asocial learners.

Social learning can be adaptive only if individuals can use social learning selectively and can also collect personal information according to circumstances (Kendal et al., 2005; Laland, 2004). Organisms face a trade-off between acquisition of expensive but accurate information through individual sampling (asocial learning) and utilization of less reliable but cheap information obtained through observing or interacting with others (*Costly information hypothesis*: Boyd and Richerson, 1985). A study by Webster and Laland (2008) showed that minnows would copy a foraging patch choice under simulated predation risk only when using private information was costly. Therefore individuals would have to adopt flexible learning strategies (social or asocial) depending on the circumstances (Laland, 2004). The maze in this experiment necessitated individual fish to explore all the chambers before being able to locate the food reward. The single individuals like the demonstrators were able to successfully sample the maze and gain precise information about location of food reward and its association with red colour but this may not have happened for paired conspecifics (trained or naïve) owing to possible social distractions (see below). This implied that in a novel task in an artificial setup, gaining personal information is probably more beneficial than learning from experienced individuals. The fact that this observation has been made in a social species like zebrafish point to the need for further investigation of circumstances that lead to switching from social to asocial strategies.

The other interesting observation was that the mean performance time and number of mistakes made by the demonstrators increased significantly during the 9th day trial when the naïve observers were introduced into the experiment, compared to the 8th day trial (Fig. 2a, b). Zebrafish is a highly social species (Suriyampola et al., 2016) and isolation for a period of 8 days could have elevated the urge for interacting with conspecifics among demonstrator individuals. The demonstrators could have become inquisitive about their new tank-mate and consequently took more time to find the food on the 9th day. The presence of observers could have distracted the demonstrators through the next three days (10th to 12th day) as evident from the consistently greater time they took to get to the food (Fig. 2a). Therefore, the learning of colour-association with food reward by the observers during the first four trials was likely affected due to the distraction and could have resulted in a non-significant decrease in the performance time and increase in the number of mistakes made by observers across the first four trials. Perhaps for the observers, exploring a novel environment was less important when a conspecific was present. Though initially the performance of demonstrators worsened after introduction of observers into the experiment on 9th day, it improved during the subsequent trials (10th to 12th day). The observers could have been viewed as potential competitors.

One shortcoming of the study that could have affected our results of comparison made between the performances of observers (Experiment 1) and naïve individuals (Experiment 2) was the differential acclimatization of the naïve pairs to the experimental setup than the observer-demonstrator pairs. The naïve fish (observers) put in with demonstrators were joining a more acclimated and knowledgeable fish. On the other hand, the two naïve fish were both equally new to the test arena, as new as the observer fish, but without an acclimated partner.

Familiarity of one of the individuals of the pair may significantly reduce the stress associated with the learning task as in the case of demonstrator-observer pair. Therefore a naïve individual (of the two naïve pair in Experiment 2) that was familiar with the setup could have performed differently than when both individuals were naïve. The other potential confounding factor for our results could be the sex of the observers and the demonstrators used in the experiments. Individuals for the experiment and the control sets were randomly selected from the stock tanks and there might have been same sex (both male-male and female-female) and opposite sex dyads, which might have behaved differently. Size differences within the observer-demonstrator pair could also have possibly affected social learning. A previous study in sticklebacks by Duffy et al. (2009) showed that observers and demonstrators of two different size classes copied the patch choice of larger demonstrators significantly more than the patch choice of smaller demonstrators. In our case, the careful construction of pairs in which observers and demonstrators (or the two naïve fish) differed in size, but in both directions, facilitated tracking of individual behaviour. Nevertheless it could be that the smaller fish could have scored less well, or “learnt” less well when tested as demonstrators than when tested as observers. Further studies are essential to confirm the effects of body size on social facilitation between size-unmatched dyads.

Habitat complexity and relative fish abundance could determine the prevalence of social learning among fish populations in nature (Coussi-Korbel and Fragaszy, 1995). In complex habitats, locating food patches individually would be tedious and costly. Thus, more individuals would tend to depend on the fewer informed conspecifics. The test population had been maintained in the laboratory in bare aquaria. Hence, learning from conspecifics might not have been a preferred strategy. Again, competing individuals might not be inclined to transmitting information about productive patches. Therefore, social learning among fish occurring at high densities would be less common. The density at which fish were maintained in the laboratory could have been high which could have disfavored information transfer. Comparative studies between populations are however warranted to understand the effects of these factors on social learning.

In the test for memory, the observers showed similar performances during the probe trial compared to the last trial. The naïve-paired fish too performed similarly during the probe trial when compared to the last trial. Development of strong memory abilities is an energetically expensive process and would happen in organisms which inhabit environments that demand its requirement (Dukas, 1998, 1999; Odling-Smee et al., 2008). Spatially complex habitats would provide for an enriched rearing environment for development of increased neural plasticity and enhanced cognitive processes (Salvanes et al., 2013). Being native to structurally less complex habitats, the need for retention of memory of associative cues indicating location of food patches among individuals of Seripetkalwa population may not be adaptive. However, comparative studies across populations would help understand the role of habitat complexity in determining memory processes.

Overall, the study revealed that a naïve fish in association with a knowledgeable conspecific did not perform a spatial task better than when two naïve individuals were subjected to the same task. Further studies are needed that can disentangle the interaction components associated with social groups and the role of ecological factors (habitat complexity, predation pressure) among this species. Comparative studies among natural populations of zebrafish would help understand the influence of these factors in modulating social learning strategies and role of selective forces in shaping evolution of specific learning traits.

Compliance with ethical standards

The authors declare that they have no conflict of interest. The studies complied with the existing rules and guidelines outlined by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. The studies also adhered

to the Institutional Animal Ethics Committee's (IAEC) rules and guidelines of IISER Kolkata (Reg. No. 1385/ac/10/CPCSEA). Zebrafish (*Danio rerio*) is designated as a species of Least Concern by IUCN's Red List of threatened species. The zebrafish collections were not made inside any reserved forest or protected area. No animals were euthanized or sacrificed during any part of the study and behavioural observations were conducted without any chemical treatment on individuals. At the end of the experiments, no individuals were sacrificed and all zebrafish were returned to the stock tanks and continue to be maintained in the laboratory.

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