

# Episodic-like memory in zebrafish

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**Abstract** Episodic-like memory tests often aid in determining an animal's ability to recall the *what*, *where*, and *which* (context) of an event. To date, this type of memory has been demonstrated in humans, wild chacma baboons, corvids (Scrub jays), humming birds, mice, rats, Yucatan minipigs, and cuttlefish. The potential for this type of memory in zebrafish remains unexplored even though they are quickly becoming an essential model organism for the study of a variety of human cognitive and mental disorders. Here we explore the episodic-like capabilities of zebrafish (*Danio rerio*) in a previously established mammalian memory paradigm. We demonstrate that when zebrafish were presented with a familiar object in a familiar context but a novel location within that context, they spend more time in the novel quadrant. Thus, zebrafish display episodic-like memory as they remember *what* object they saw, *where* they saw it (quadrant location), and on *which occasion* (yellow or blue walls) it was presented.

**Keywords** Zebrafish · Episodic-like memory · Declarative memory · Object recognition · One-trial test

## Introduction

Episodic memory is generally defined as a retrieval of a memory trace of past experience that can be identified according to what happened, the context in which the event occurred, and when the event took place in subjective time (Tulving 1993). In other words, episodic memory is the *what*, *where*, and *when* of a personal experience. Tulving (1993) also referred to the subjective consciousness of episodic memory, which is difficult, if not impossible, to establish in animals. Because of this, some researchers have theorized that humans are the only species capable of true episodic memory (Suddendorf and Busby 2003; Suddendorf and Corballis 2007; Zentall 2005). However, several “episodic-like” memory tests have been developed for animals. For example, Clayton and Dickinson (1998) were able to demonstrate memory for *what*, *where*, and *when* in Scrub jays by utilizing the species' innate food-storing habits. This is considered the first evidence of episodic-like memory in a non-human animal. More recently, a species of cleaner fish, the wrasse, have been shown to keep track of the *what* and *when* in a foraging task (Salwiczek and Bshary 2011). In addition, cuttlefish, one of the few invertebrates tested outside of bees, have been found to recall what they have eaten, where they ate it, and when they ate various foods (Jozet-Alves et al. 2013).

Eacott and Norman (2004) developed a slightly different episodic memory paradigm that did not use food reinforcers and instead focused on a basic habituation task in order to evaluate the *what*, *where*, and *during* which occasion (what they referred to as context) an event occurred and subsequent memory formed. Their initial experiments involved exposing dark Agouti rats to two different objects (the *what* aspect of memory) that had been placed in a specific location (the *where* aspect of memory).

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Each rat first experienced two exposure phases followed by a trial phase. The exposure phases consisted of placing rats in two contexts, first a black floor and white walls with object A on the left and object B on the right and then a black/white floor and wood walls with object A on the right and object B on the left. In the test phase the object (A or B) was placed in both a familiar location and a novel location, which depended on the original context experienced. For example, in the test phase for context one (black floor, white walls) object A would be placed on both the left and right sides (Eacott and Norman 2004). As a result, the object on the right side was novel and rats, which have a tendency to investigate novel objects (Ennaceur and Delacour 1988), were found to spend more time exploring that object. This finding suggested that the rats were able to recollect the past location of the objects within a particular context (the *which occasion* aspect of memory).

Eacott and Norman's (2004) paradigm has since been modified for use with mice (Dere et al. 2005), Wistar rats (Kart-Teke et al. 2006), and Yucatan minipigs (Kouwenberg et al. 2009) with similar positive results. The presence of episodic-like memory in many complex organisms has led some researchers to suggest that it is evolutionarily advantageous and has increased fitness in many species (Allen and Fortin 2013) and therefore may be present in many species yet untested.

Zebrafish (*Danio rerio*) have become an increasingly popular species used in a variety of research settings. While there is ample research on zebrafish biology, physiology, and genetics (e.g. Rinkwitz et al. 2011; Panula et al. 2011), research on cognitive abilities and the behavioural repertoire of zebrafish is not as extensive. Some past studies of memory have adapted experimental designs used with other species to assess basic memory function in zebrafish. For example, Williams et al. (2006) used a modified T-maze alternation task designed for rodents to assess zebrafish learning and memory abilities. They found that zebrafish could learn to alternate from one side of a tank to the other following an auditory cue, in order to obtain a food reward. Additional research has shown that zebrafish can learn to discriminate between artificial and naturally occurring scents in a classical conditioning task (Braubach et al. 2008) and between visual stimuli when trained in a T-maze (Colwill et al. 2005).

The above experiments all relied upon food-based reinforcements, which are commonly used in classical or instrumental conditioning paradigms. Food-based reinforcements may be problematic when employing paradigms for the investigation of certain types of learning and memory, however, as there may be species differences with respect to the importance of memory in foraging behaviour [e.g. compare Salwiczek and Bshary (2011) and Spence

et al. (2008)]. Cleaner wrasses, for example, can recognize each "client" fish that they clean (Tebbich et al. 2002) which can consist of upwards of 100 different species (Grutter and Poulin 1988). Thus, they are constantly faced with foraging choices that require remembering which clients they have already cleaned (Salwiczek and Bshary 2011). For cleaner wrasses a foraging task may be an appropriate experimental measure of memory capabilities given their natural history. By contrast, zebrafish are an omnivorous fish with a natural diet consisting of zooplankton, insects, and on occasion phytoplankton found in their ambient environment (Engeszler et al. 2007); as a result, they have little need to learn or recall a specific location or type of food presented (Engeszler et al. 2007). Foraging behaviour may therefore be a less naturally relevant indicator of memory abilities in zebrafish than in cleaner wrasses. Since zebrafish have been shown to have the capacity to recognize objects in a novel object recognition test (Braida et al. 2014; Lucon-Xiccato and Dadda 2014; May et al. 2015), the present study used a more relevant object-based episodic-like memory test.

In the current experiment, a variant of the episodic-like memory test designed by Eacott and Norman (2004) was used to determine whether zebrafish are capable of displaying episodic-like memory in a *what*, *where*, and *which occasion* task in which no external reinforcements were used to motivate behaviour.

## Materials and methods

### Subjects

Wild-type short-fin zebrafish (*Danio rerio*;  $n = 107$ ) were obtained from Big Al's Aquarium and Fish Supply (Edmonton, AB). All fish were adults of minimally 8 months of age, experimentally naive, and mixed genders. Zebrafish were housed in a 3-shelf benchtop Aquatic Habitats (AHAB, Aquatic Ecosystems, Inc. Apopka, FL) housing system in polyurethane tanks at a density of 5–10 fish per 3-L tank or 10–50 fish per 10-L tank. All fish were fed once daily on a mixture of freeze-dried shrimp (Omega One Freeze Dried Mysis Shrimp nutri-treat, Omega Sea Ltd, Sitka, AK) or fish flakes (New Life Spectrum Optimum Fresh H<sub>2</sub>O Flakes, New Life International Inc., Homestead, FL). The temperature of the habitat was maintained at  $27 \pm 1.5$  °C, dissolved oxygen at 5–10 mg/L, and pH of 7.0–8.0. Lighting in the housing room was on a 12-h light/dark cycle with lights on at 8 AM. Habitat water was continuously circulated, filtered through a 50- $\mu$ m filter and an activated carbon canister, and UV irradiated (Holcombe et al. 2013). This research was approved by the MacEwan University Animal Research Ethics Board, protocol

number 05-12-13, and is in accordance with the Canadian Council for Animal Care (CCAC) guidelines.

### Naïve preference test

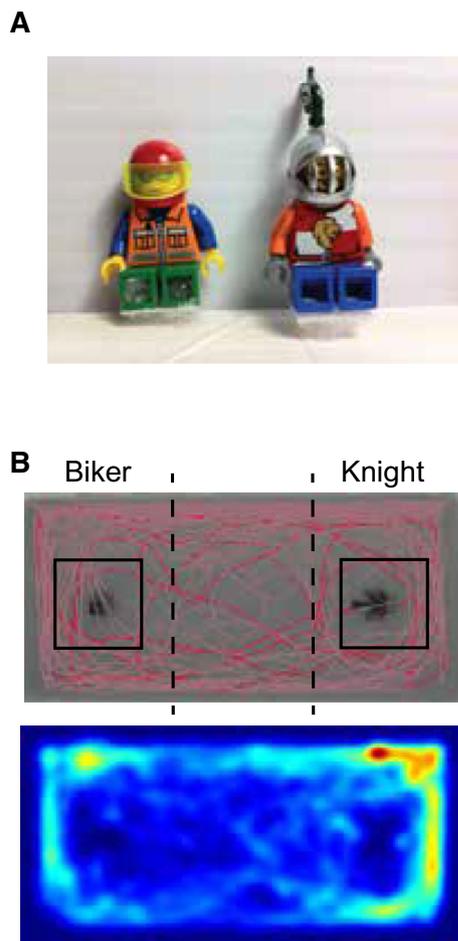
Animals exhibit natural preferences for some stimuli over others (Heyser and Chemero 2012; Ennaceur 2010). In order to ensure that approach behaviour towards certain stimuli used in testing was not due to such pre-existing preferences, it was necessary to choose objects for the experimental test that would be equally preferred by naïve fish (Bevins and Besheer 2006). The objects used were two pairs of colour-balanced LEGO® figures in seated positions (May et al. 2015) (Fig. 1a). The “Biker” figure had green legs, an orange body, blue arms, yellow hands, and a yellow face, and a red motorcycle helmet with a yellow visor. The “Knight” figure had blue legs, a red body, orange arms, grey hands, a yellow face, and a silver helmet with

green plume. Both objects were attached with Velcro to the floor of a 15-L rectangular fish tank (29 cm L; 14 cm W; 18 cm H). The walls and floor of the tank were lined with white corrugated plastic fixed in place with silicone in order to eliminate the influence of external visual stimuli. The experimental room was illuminated with diffused lighting in order to avoid light reflections on the water surface and to eliminate shadows. This ensured that software error in tracking of fish movement was minimized. An enclosure of white corrugated plastic sheets surrounding the test arena served as visual barriers for the fish.

Prior to testing, the tank was filled with water taken from the fish habitat to a height of approximately 6 cm, which was deep enough to cover the highest point on each figure, yet shallow enough to discourage fish from swimming above the figures. Biker and Knight figures were placed facing each other in the centres of the right and left thirds of the tank, and their locations were randomized for each fish. The tank was placed on a seedling heat mat (Hydrofarm Horticultural Products, Petaluma CA) to assist in maintaining the water temperature between 26 and 28 °C. For each session, an individual fish was netted from the naïve population in the aquatic habitat, moved into a transfer tank, and brought into the adjacent experimental testing room. Naïve fish ( $n = 30$ ) were individually exposed to the two objects for 10 min. Each trial began when the fish was netted from the transfer tank and placed into the middle of the tank and released perpendicular to the long wall of the tank at midpoint between the two objects in order to avoid biasing their approach behaviour. A video camera was suspended approximately one metre above the experimental arena to track and record the zebrafish in each trial. The camera was connected to a computer running Ethovision XT® (v7) motion-tracking software (Noldus, VA) and was used to measure the time the fish spent in direct proximity to the objects by calculating the time spent within a 9.6-cm<sup>2</sup> virtual box around each object (Fig. 1b), time in the left, middle, and right tank zones, average swimming velocity (total distance travelled/duration of trial), total distance moved, and time the fish was immobile during the 10-min test period.

### Episodic-like memory test

The test sessions for memory began with the transfer of the fish from the habitat to the testing room in a 3-L polyurethane transfer tank. Prior to each trial, the subjects were placed into a white 15-L holding tank (29 cm L; 14 cm W; 18 cm H) that was filled with habitat water to a depth of 6 cm and maintained at a temperature of 26–28 °C. Trials began when the fish were transferred into the experimental arena; a square aquarium made of non-toxic polyurethane (29 cm L; 29 cm W; 18 cm H, manufactured by Polyrama,

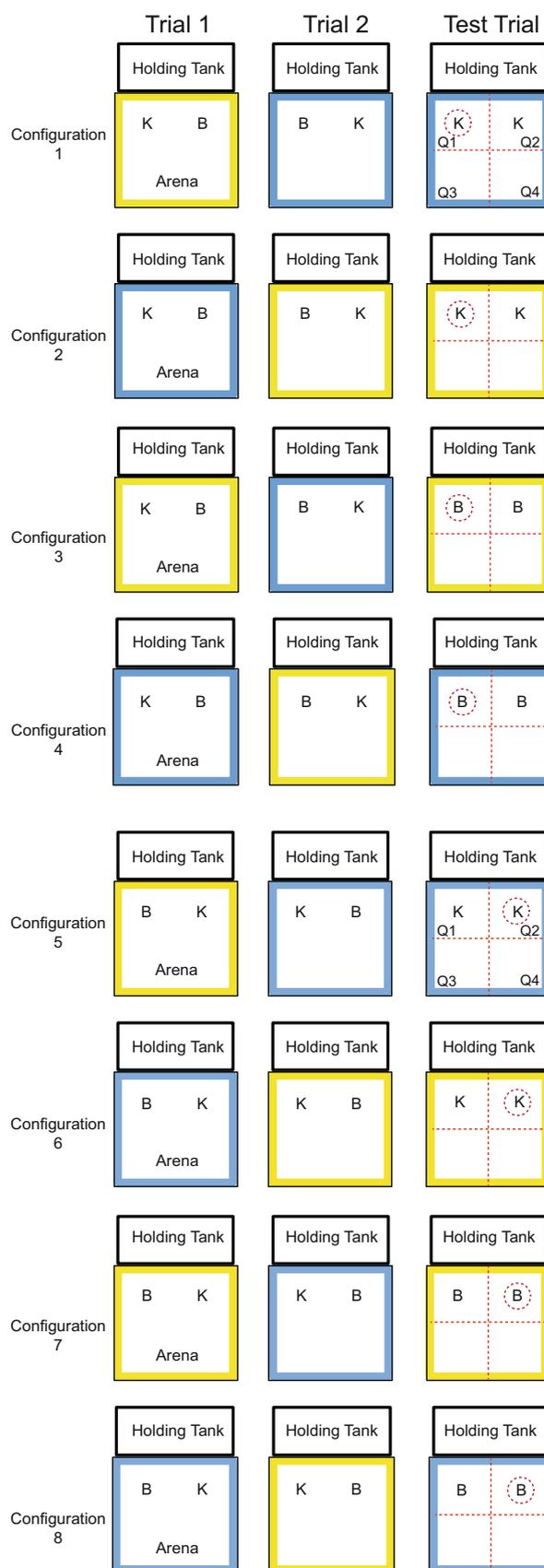


**Fig. 1** Naïve object preference testing. **a** Photograph of the Biker (left, 4 cm high) and the Knight (right, 5 cm high). **b** Representative trackplot from an individual zebrafish (above) and heatmap (pseudocolour representation of the location of the fish; below) over the 10-min naïve preference trial

Edmonton, AB). Non-reflective white corrugated plastic lined the bottom of the arena to ensure high contrast between the fish and the background. The holding tank and the arena were placed on a 53 cm × 53 cm heat mat (Hydrofarm Horticultural Products) to help stabilize water temperature during trials.

The arena was sectioned into four virtual quadrants measuring 14.5 × 14.5 cm (Q1–4) in the software program. To create distinctly coloured contexts, blue and yellow corrugated plastic sheets could be inserted to line individual walls of the arena. Previous studies have demonstrated that zebrafish exhibit colour preferences (Avdesh et al. 2012; Bault et al. 2015), indicating that zebrafish can reliably distinguish between these two colours. The sequence of blue and yellow context presentations was counterbalanced across groups of fish to offset any potential colour preference, as well as to balance familiarity of the contexts across the eight configurations (Fig. 2). To ensure consistent placement of objects across conditions, as well as to prevent the objects from floating, the objects were affixed to the centre of Q1 and Q2 of the testing arena and the bottom of the LEGO® stimuli.

Zebrafish were randomly assigned to one of eight experimental conditions with 9–10 fish per condition (Fig. 2). For each session, similar to the naïve preference test, an individual fish was netted from the naïve population in the aquatic habitat, moved into a transfer tank, and brought into the experimental testing room. The experiment started when the fish was placed into the holding tank for 2 min to habituate the fish. In the first learning trial, the fish was placed into the arena for 5 min. The fish was exposed in the arena to one of two contexts (blue or yellow walls) and the two objects (Biker and Knight), each seated in one of two quadrants (Q1 or Q2). The fish was then placed back into the holding tank for 2 min while the context and relative positions of the objects were reversed for the second learning trial. Next, the fish was placed back into the experimental arena for learning trial two for 5 min. The fish was then removed and placed into the holding tank for 2 min, while the arena configuration was modified for the test trial. The test trial involved placing the fish in one of the two coloured contexts that it had encountered in either trial one or trial two. In the test trial, however, the two objects were identical, with the object in Q1 or Q2 having never been previously encountered in that particular context-object-quadrant combination. Thus, in half of the trials, the context-object-quadrant combination in Q1 was novel to subjects, whereas the context-object-quadrant combination in Q2 was familiar. In the other half of the trials, the context-object-quadrant combination in Q2 was novel to subjects, whereas the context-object-quadrant



**Fig. 2** Episodic-like memory testing procedure. Fish were randomly assigned to one of eight configurations. Zebrafish were individually placed in the holding tank for 2 min, then into the arena for 5 min of each trial. After trials 1 and 2, they were placed back into the holding tank for 2 min. Context colour (*blue* or *yellow*) is presented in each configuration in the order shown by the diagram. “K” represents the position of the LEGO® Knight, and “B” represents the position of the LEGO® Biker. The target object that is in the location/context combination not previously presented to the fish is *circled in red* on the diagram. The arena was subdivided into four virtual quadrants (shown with *dotted lines*) in Ethovision for post hoc analysis (colour figure online)

combination in Q1 was familiar (Fig. 2). In our first block, we ran the first four configurations; then, a second block was run with a new batch of zebrafish in the last four configurations (Fig. 2). There were no statistically significant differences between these blocks in D1, D2, or location shift (see below), so we grouped these blocks together. There was a significant difference in velocity between block one trials ( $4.8 \pm 0.2$  cm/s) and block two trials ( $6.4 \pm 0.2$  cm/s;  $P < 0.0001$ , Mann–Whitney test); however, there was a similar significant decrease in velocity between trial 1 and trials 2 and 3 (and no difference between trials 2 and 3) in both blocks, so that these data have also been grouped.

We calculated the discrimination index “D1” (also called  $D_1$ ) (Antunes and Biala 2012) with the formula:  $D1 = T_N - T_F$ , where  $T_N$  was the time the fish spent in the quadrant with the novel object-context quadrant and  $T_F$  was the time the fish spent in the quadrant with the familiar object-context quadrant. A discrimination index “D2” [also called “DI” (Broadbent et al. 2010; Antunes and Biala 2012)] was calculated with the formula:  $D2 = D1 / E_{T2}$ , where  $E_{T2}$  was the total time spent in Q1 and Q2 (Antunes and Biala 2012). Location shift between learning trial two and the testing trial was calculated by subtracting the time spent in learning trial two from the testing trial. The average velocity and time spent in each quadrant were recorded with the motion-tracking software program. Statistical analysis was performed with GraphPad Prism 5 or 6 software (CA, USA), and all data were analysed for normality with the D’Agostino and Pearson’s omnibus normality test. Normally distributed data were analysed with paired  $t$  tests, one-sample  $t$  tests, and one-way ANOVAs with Tukey’s multiple comparison test, whereas nonparametric data were analysed with the Wilcoxon signed rank tests or Mann–Whitney tests. An alpha level of  $P < 0.05$  and 95 % confidence intervals were used for assessing statistical significance in all tests. Elimination criteria included freezing for more than 2 min with freezing behaviour defined as an average velocity of  $< 2$  cm/s ( $n = 1$ ) and less than ten per cent of the total trial (30 s) in the quadrants containing objects ( $n = 6$ ).

## Results

### Naïve object preference task

To assess naïve preference of zebrafish for the two objects, we divided the rectangular tank into thirds and calculated the time spent in each zone containing the objects. There was no significant difference in the time spent in each zone (Biker:  $227 \pm 11$  s, Knight:  $208 \pm 11$  s,  $n = 30$ , paired two-tailed  $t$  test,  $P = 0.311$ ). Second, we quantified the time spent in close proximity to each object by calculating the time within a virtual box around the object, which is similar to how exploration has been assessed in other object recognition studies (Ennaceur and Delacour 1988). There was no significant difference in time spent near the objects (Biker:  $14 \pm 1$  s, Knight:  $17 \pm 2$  s, Wilcoxon signed rank test,  $P = 0.173$ ). We therefore used these objects for the remainder of the study.

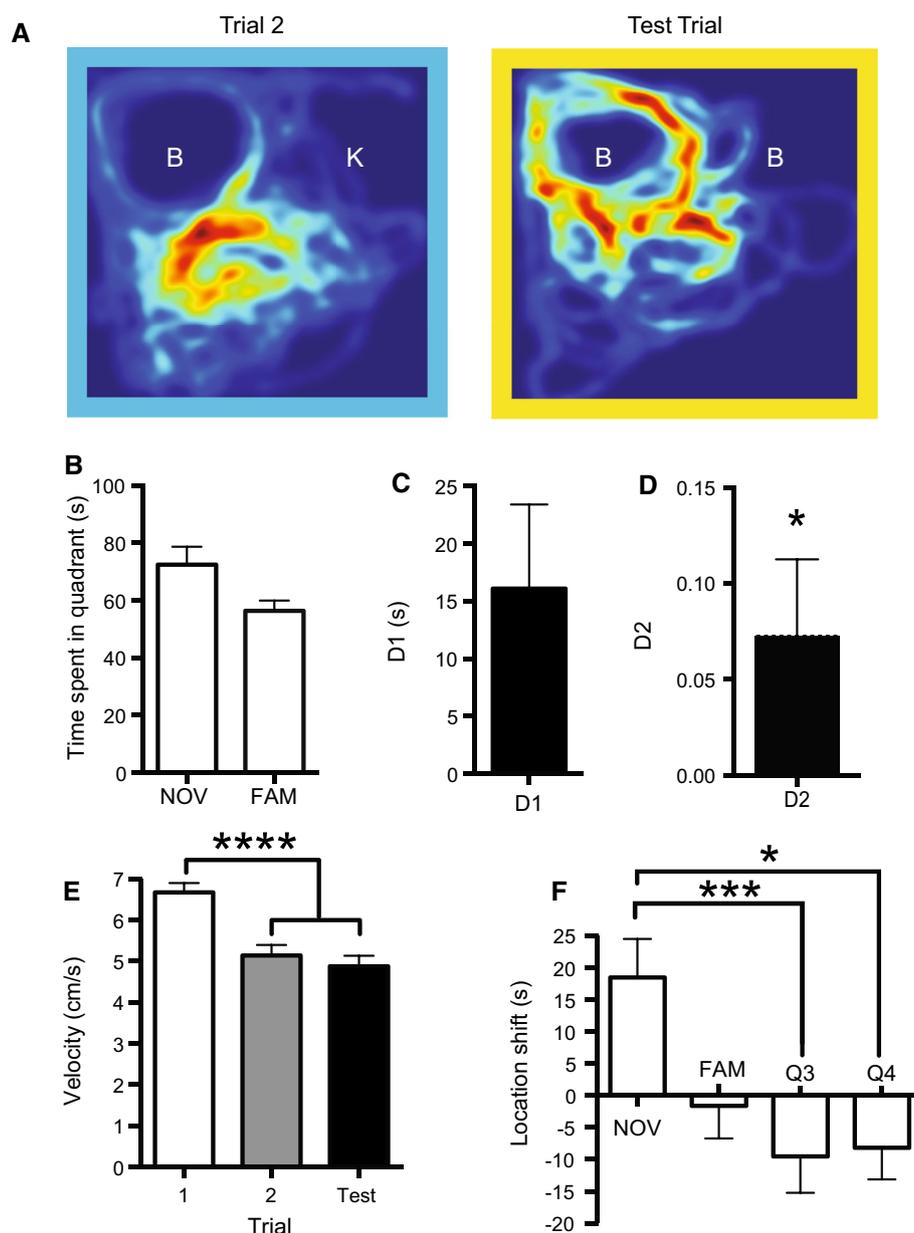
### Episodic-like memory task

To assess performance in the episodic-like memory task, we compared the relative time the zebrafish spent exploring the objects in Q1 and Q2 of the arena. During the test trial, in half of the trials, Q1 represented a novel situation to each fish (they had not encountered the object in Q1 with the same context in either learning trial 1 or 2), whereas the object in Q2 had been experienced within the presented context during one of the learning trials. In the other half of the trials the opposite occurred with Q2 containing the novel situation for each fish. When all results were combined, there was a trend towards fish spending more time in the novel object-context quadrant compared to the familiar object-context quadrant (novel:  $72.5 \pm 6.2$  s, familiar:  $56.4 \pm 3.6$  s) (Fig. 3b). This difference, however, was not statistically significant ( $P = 0.1069$ , Mann–Whitney test,  $n = 70$ ). There was also a trend towards a difference in exploration time between the novel and familiar object-context quadrants that was just shy of significance ( $D1 = 16.1 \pm 7.3$  s, Wilcoxon signed rank test,  $P = 0.069$ ,  $n = 70$ ) (Fig. 3c). To take into account the discrimination between the novel and familiar object-context quadrants, a very common measure of examining memory in object recognition tasks (May et al. 2015), we calculated D2 and found a significant preference for the novel object-context quadrant ( $D2 = 0.07 \pm 0.04$ ,  $P = 0.035$ ,  $n = 70$ ) (Fig. 3d).

To further investigate location preference in the test trial, we attempted to compare the preference of location between all three trials; however, we found a significant difference in velocity between group means as assessed by a Kruskal–Wallis test ( $H = 28.21$ ,  $P < 0.0001$ ). Post hoc

**Fig. 3** Episodic-like memory testing: 5-min trial.

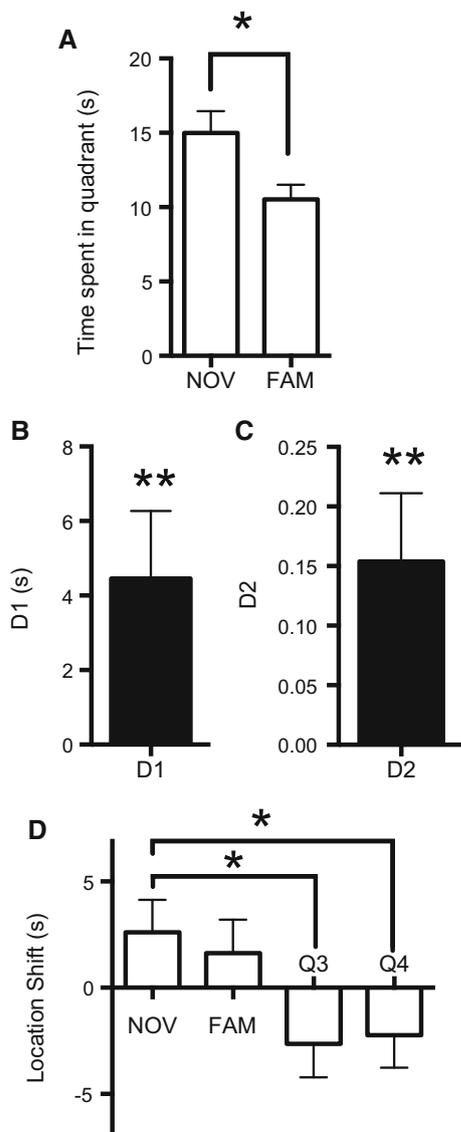
**a** Representative heatmaps from a single zebrafish in learning trial 2 and the test trial. The novel object-context was in Q1 (the “B” on the *left*) in the test trial. **b** There was no significant difference in time spent in the novel object-context quadrant (NOV) compared to the familiar object-context quadrant (FAM) in the test trial. **c** D1 was not significantly different from zero in the test trial. **d** D2 was significantly different from zero in the test trial.  $*P < 0.05$ . **e** There was a significant difference in velocity between trial 1 versus trial 2 and trial 1 versus the test trial.  $****P < 0.0001$ . **f** There was a significant difference in location shift between trial 2 and the test trial in the novel object-context quadrant compared to Q3 and Q4.  $*P < 0.05$ ,  $****P < 0.0001$



comparisons using the Dunn’s multiple comparison test found a significant difference in velocity between learning trial 1 ( $6.7 \pm 0.2$  cm/s) and learning trial 2 ( $5.1 \pm 0.3$  cm/s,  $P < 0.0001$ ), and a significant difference between learning trial 1 and the test trial ( $4.9 \pm 0.3$  cm/s,  $P < 0.0001$ ; Fig. 3e), but no significant difference between learning trial 2 and the test trial. Since elevated swimming velocity can be related to stress levels, such as increased anxiety (Maximino et al. 2010), only comparisons between learning trial 2 and the test trial, which did not differ in velocity, were compared. The fish spent significantly more time in the novel object-context quadrant during the test trial compared to the same quadrant in learning trial 2 ( $18.49 \pm 6.05$  s, Wilcoxon signed rank test,  $P = 0.004$ ,

Fig. 3f). There was no significant shift in time spent in the familiar object-context quadrant during the test trial compared to the same quadrant in learning trial 2 ( $-1.62 \pm 5.10$  s, Wilcoxon signed rank test,  $P = 0.419$ ). Furthermore, there was a decrease in time spent in Q3 ( $-9.53 \pm 5.70$  s, Wilcoxon signed rank test,  $P = 0.016$ ) and no significant change in Q4 ( $-8.17 \pm 4.93$  s, Wilcoxon signed rank test,  $P = 0.117$ , Fig. 3f). Comparison of time shifts indicates a significant difference between the novel object-context quadrant and Q3 and Q4 (Kruskal–Wallis test;  $H = 14.32$ ,  $P = 0.0025$ ) (Fig. 3f). This suggests that in the test trial the fish prefer the novel object-context combination and spent proportionally less time in the quadrants without objects.

To determine whether the fish immediately preferred the novel object-context combination in the test trial, we analysed performance in the first minute of the trial (compared to the full 5-min trial above). In this initial time epoch zebrafish spent significantly more time in the novel object-context quadrant compared to the familiar object-context quadrant (novel:  $15.0 \pm 1.5$  s, familiar:  $10.5 \pm 1.0$  s;  $P = 0.0364$ ,  $n = 70$ , Mann–Whitney test) (Fig. 4a). There was also a significant difference in exploration time between the novel and familiar object-



**Fig. 4** Episodic-like memory testing: first minute of trial. **a** In the first minute of the test trial there was a significant difference in time spent in the novel object-context quadrant (NOV) compared to the familiar object-context quadrant (FAM).  $*P < 0.05$ . **b** D1 and **c** D2 were significantly different from zero in the test trial.  $**P < 0.01$ . **d** There was a significant difference in location shift between trial 2 and the test trial in the novel object-context quadrant compared to Q3 and Q4.  $*P < 0.05$

context quadrants (D1 =  $4.5 \pm 1.8$  s,  $P = 0.003$ ,  $n = 70$ ) (Fig. 4b). We also calculated D2 and found a significant preference for the novel object-context quadrant (D2 =  $0.15 \pm 0.06$ ,  $P = 0.005$ ,  $n = 70$ ) (Fig. 4c).

We also calculated the location shift from learning trial 2 to the test trial in the first minute of each trial. The fish spent significantly more time in the novel object-context quadrant during the test trial compared to the same quadrant in learning trial 2 ( $2.61 \pm 1.53$  s, Wilcoxon signed rank test, difference from 0,  $P = 0.012$ , Fig. 4d). There was no significant shift in time spent in the familiar object-context quadrant during the test trial compared to the same quadrant in learning trial 2 ( $1.63 \pm 1.57$  s; Wilcoxon signed rank test, difference from 0,  $P = 0.267$ , Fig. 3f). Furthermore, there was no decrease in time spent in Q3 ( $-2.64 \pm 1.58$  s; Wilcoxon signed rank test, difference from 0,  $P = 0.099$ ) and a significant decrease in Q4 ( $-2.23 \pm 1.53$  s; Wilcoxon signed rank test,  $P = 0.046$ ; Fig. 3f). Comparison of time shifts indicates a significant difference between the novel object-context quadrant and Q3 and Q4 (Kruskal–Wallis test;  $H = 9.963$ ,  $P = 0.0189$ ) (Fig. 4d).

## Discussion

Our research demonstrates that zebrafish have the capacity for episodic-like memory in a *what*, *where*, and *which occasion* learning task. When presented with a familiar object in a familiar context, *but a different location within that context*, fish spend more time in the quadrant that this object is located in. These results indicate that the fish can remember *what* object they previously saw (Biker vs. Knight), *where* they saw that object (Q1 vs. Q2), and on *which occasion* (in which context: yellow or blue walls) the object was presented.

Episodic-like memory has been demonstrated in animals using related paradigms by quantifying an increased preference for the object in a test trial with similar context but different location (Eacott and Norman 2004; Dere et al. 2005) or at a different time (Kart-Teke et al. 2006; Kouwenberg et al. 2009). For example, Dere et al. (2005) used the *what*, *where*, and *when* paradigm to investigate object recognition in mice. In this study, the context was kept consistent but the location of a previously presented object was altered in the test trial. The time that the mice spent exploring the object in the new location was used to quantify episodic-like memory. In this task, mice could recall, in a similar context, *what* object was different, *where* an object had been previously located, and how recently it had been seen in that place (Dere et al. 2005). In the present study, zebrafish similarly spend more time in the quadrant with the object that had not been encountered

in the same combination of context and location—thus demonstrating episodic-like memory.

Other studies of episodic-like memory have used a similar method of defining object exploration and episodic-like memory (Eacott and Norman 2004; Kouwenberg et al. 2009). Kouwenberg et al. (2009) used minipigs and defined exploration of the object as time spent not only in the quadrant but also in sniffing and investigating the object itself. In order to verify that zebrafish in the current experiment were indeed exploring the object itself in the new location (quadrant), we examined time spent in the quadrants containing the object. This is slightly different than the sniffing and examining behaviour of the minipigs; however, given that zebrafish generally explore visually in their environments and thus must move close to the object in order to inspect or explore it, time spent in quadrants is comparable to the sniffing and investigating behaviour that minipigs and rats engage in.

In this study, zebrafish were able to demonstrate object learning and memory without any type of reinforcement. This is consistent with recent studies on object recognition in zebrafish (Braida et al. 2014; Lucon-Xiccato and Dadda 2014; May et al. 2015). As in the avian and mammalian literature, previous research on memory in other fish has focused mainly on foraging-based memory tasks. For example, Salwiczek and Bshary (2011) explored the *when* and *what* of episodic-like memory of cleaner wrasses (*Labroides dimidiatus*) in a foraging task. Their results indicated that in order to maximize food intake, the wrasses had to, and did, recall *when* and *where* they interacted with a particular food source. Individual wrasses were first given the opportunity to choose between two high-quality food plates that were presented at different time intervals (5 vs. 10 min, 5 vs. 15 min, and 10 vs. 15 min). Wrasses were found to choose the plate that allowed them to forage, indicating that they could recall the time at which the food last appeared and choose appropriately (Salwiczek and Bshary 2011). These results suggested that the fish had a concept of *when* to forage. The fish were then given the choice between a low-quality food plate that was consistently available and a high-quality food plate that was only sometimes available. Again the fish chose the high-quality food more often when it was accessible, demonstrating memory for *what* (Salwiczek and Bshary 2011). Although this example demonstrates episodic-like memory in a foraging task, it highlights that fish species are able to perform well in memory and learning tasks.

The results of the present study with zebrafish illustrate episodic-like memory in a non-foraging, object recognition/habituation task. As previously indicated, the life history and behavioural repertoire of zebrafish make a food-based memory paradigm problematic. Zebrafish,

however, have evolved the ability to discriminate between colours (Hughes et al. 1998) and can recognize and remember colours in their environment (Avdesh et al. 2012; Bault et al. 2015; Colwill et al. 2005; Yu et al. 2006). Therefore, the present memory task, which involves distinguishing both environmental contexts and object stimuli on the basis of colour, may be appropriate for this species. Furthermore, discrimination indices such as D1 and D2 are used as valuable equations to quantify novel object-context preference in this test. The use of location shift also indicates where the fish prefers to explore from trial to trial. Unfortunately, in our task we were unable to compare learning trial 1 to the test trial because of significantly higher velocity in learning trial 1 (which was likely a result of stress). In future studies a longer habituation interval could be used to alleviate this stress. During this habituation interval a series of different objects could also be placed into the habituation arena to minimize the potential stress of a novel object.

Overall, our research demonstrates that zebrafish display episodic-like memory in a non-food reinforcement based test. Zebrafish are becoming an increasingly important animal model in a wide variety of subject areas, and the use of memory tests will hopefully aid in the development of nootropic drugs and in the treatment of memory disorders like Alzheimer's disease. Moreover, fish are generally underrepresented in both memory and learning research due to their relatively small brains and supposed inability to recall events or objects. Our research, however, suggests that zebrafish encode specific aspects of their environment and have a capable episodic-like memory system.

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