Dusky damselfish *Stegastes fuscus* relational learning: evidences from associative and spatial tasks

M. M. Silveira, J. J. Oliveira and A. C. Luchiari*

Departamento de Fisiologia, Centro de Biociências, Universidade Federal do Rio Grande do Norte, P.O. Box 1511, 59078-970 Natal, Rio Grande do Norte, Brazil

This study investigated the ability of the dusky damselfish *Stegastes fuscus* to associate conditioned and unconditioned stimuli (single CS–US) and to find a specific place in a clueless ambiece (spatial learning). After tested for colour preference and showing no specific colour attractively, the fish were trained to associate a colour cue with a stimulus fish (conspecific). Fish were then challenged to locate the exact place where the stimulus fish was presented. *Stegastes fuscus* spent most time close to the zone where stimulus was presented, even without obvious marks for orientation. The results confirm that *S. fuscus* show single CS–US learning and suggest the fish ability for spatial orientation. *Stegastes fuscus* appears to use multiple senses (sight and lateral line) for cues association and recall, and appear to perform relational learning similar to mammals. These data suggest the importance of cognitive skill for reef fishes that may have contributed to their establishment and evolutionary success in such complex environment.

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Key words: associative learning; cognition; colour preference; memory; spatial learning; visual cues.

INTRODUCTION

Animals often exploit several strategies to locate food sources, find mates, obtain safe shelters or return to familiar territory. The natural environment offers an ample variety of cues for navigation, including colour, shape and smells (Siebeck *et al.*, 2009), that are commonly used to find specific spots (*i.e.* conditioned stimulus, CS). Studies have shown that fishes learn and remember specific routes and locations by the use of cues from different sources such as landmarks, sun position and local enhancement (Odling-Smee & Braithwaite, 2003). The current knowledge about the role of learning and memory on fish orientation comes from experimental tasks involving conditioned association between environmental cues and reward location (Rodríguez *et al.*, 1994; Portavella *et al.*, 2002; Broglio *et al.*, 2003; Vargas *et al.*, 2004; Salas *et al.*, 2006; Sovrano *et al.*, 2007; Karnik & Gerlai, 2012). Because of the aquatic environment variability in information, individuals must rely on learning and memory to respond appropriately (Mazeroll & Montgomery, 1998).

Alternatively, orientating in the environment without any explicit marks requires navigation based on spatial orientation. Spatial memory is a cognitive process that allows

*Author to whom correspondence should be addressed. Tel.: +55 84 32153409; email: analuchiari @yahoo.com.br

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animals to remember different places and the dynamic relationship among items in each place (Johnson, 2010). The spatial learning ability provides flexibility, allowing animals to associate behavioural responses with complex environments based on previous experiences (Dodson, 1988). The construction of a mental map mainly requires some representative items and their location in the environment (O'Keefe & Nadel, 1978; Newman *et al.*, 2007). Hence, equivalent relations can be learned through multiple components relationship (internal or external stimuli and responses), previously and arbitrarily related by reinforcement contingencies, which is called relational learning (Karnik & Gerlai, 2012). According to this definition, both associative and spatial learning are among the relational learning processes (Shapiro & Eichenbaum, 1999).

Protocols using mazes and open fields have become a standard tool to investigate such strategies, because animals are allowed to explore allocentric cues (external marks) and are tested for their ability to form egocentric cues (single CS–US, spatial learning and memory). Here, the importance of associative cues acquisition and recall for a marine territorial species, the dusky damselfish *Stegastes fuscus* (Cuvier 1830) was investigated, and how fish orientate in the absence of obvious cues, *i.e.* cognitive mapping facilitation was addressed.

Stegastes fuscus is ideal for studying spatial learning because of its natural ability to establish territory around a rock den and chase intruders far away, returning after several metres distance (Ferreira *et al.*, 1998; Osório *et al.*, 2006). The fish navigate in shallow and deep waters of natural reef areas in order to find food, but keep the geographical area guarded as its home site (Ferreira *et al.*, 1998). As the species is colourful and inhabit clear water, vision appears to play a key role for territorial recognition, mate and juvenile recognition and chasing intruders. Vision is an important feature in reef areas, where hydrodynamics is intense, because of its straight signal source while olfactory and auditory signals show scattered routes of dispersion (Siebeck *et al.*, 2009). Therefore, the aims of this study were to investigate (1) whether *S. fuscus* prefer or avoid certain colour in a multiple choice preference test, (2) whether fish associate colour cues to a stimulus [single conditioned and unconditioned stimuli (CS–US) conditioning] and (3) how fish orientate without cues, *i.e.* cognitive mapping facilitation.

MATERIALS AND METHODS

A total of 31 adult *S. fuscus* (mean ± S.D. = 8.42 ± 0.75 cm, standard length, L_S), were collected from tide pools at Pirambúzios beach (6° 03′ 25″ S; 35° 05′ 53″ W), Nísia Floresta, RN-Brazil, using a cast net (3 m diameter; 10 mm mesh size). Sampling was authorized by the Brazilian Institute of Environment and Natural Resources (Licence Number IBAMA 41869-2/2013; SISBIO number 5413747). Fish were stored in 30 l tubs of aerated seawater prior to being transported to the Laboratory of Ornamental Fish at Departamento de Fisiologia at Universidade Federal do Rio Grande do Norte, where fish were stored in three 70 l tubs (10 fish per tank), each fish in a separate compartment (20 cm × 20 cm) delimited by glass walls. Fish were socially isolated in order to avoid aggressive behaviour and contest mortality. Fish, however, could maintain visual, acoustic and olfactory contact through holes on the glass walls. The glass tanks formed a closed recirculating water system, in which water was kept aerated and filtered (mechanical, chemical, biological and UV light filters), maintained at 28° C, range ±1° C, salinity of 35 and a 12L:12D cycle for 1 month prior to the experiments. Fish were fed *ad libitum* twice daily with adult *Artemia salina*, shrimp paste and dry commercial food (38% protein, 4% lipid, Nutricon Pet; www.nutriconpet.com.br). All animal procedures were

performed with the permission of the Ethical Committee for Animal Use of the Universidade Federal do Rio Grande do Norte (CEUA 037/2013).

COLOUR PREFERENCE

For the colour preference test, the same methodology previously applied for fish colour discrimination (Luchiari *et al.*, 2007; Luchiari & Pirhonen, 2008; Luchiari *et al.*, 2009) was used. Hence, six 50 cm diameter transparent plastic tanks were divided into four lateral compartments of similar size with a 7 cm hole in the central region to allow the fish to move between compartments. Water depth was 15 cm. The laboratory was illuminated with fluorescent tubes set at 12L:12D. Different environmental colours were randomly chosen for each compartment, by covering the chosen compartment with layers of gelatin filter (LeeFilters; www.leefilters.com; blue: λ_{max} 435 nm, green: λ_{max} 534 nm, yellow: λ_{max} 546 nm, red: λ_{max} 610 nm). Light intensity was set around 60 lx for each compartment by adding layers of the same gelatin filter. The test apparatus is shown in Fig. 1(a).

Colour preference was observed individually (one fish per tank) for a period of 5 days ($n = 16$). Each fish was placed in the experimental tank 1 day before observations started. On the day after, during the first and fifth days of each 5 day period, the compartments were not covered and light intensity was around 230 lx. During these days, the frequency of visits in each compartment was observed to check possible preference between non-coloured compartments. On the second, third and fourth days the observations were made with compartments set with different colours. Colours (gelatin filter layers) were added on the first day and were removed on the fourth day after lights were turned off in the experimental room. Visiting frequency was observed throughout 5 days and data were collected every 2 min for a 20 min period at 0800, 1100, 1400 and 1700 hours, making a total of 40 observations per day. Food was not offered during the experimental days in order to prevent any stimulus that could propel the fish to choose one specific compartment because of any other driving force than colour. It is well documented that fishes are able to withstand longer periods of starvations (Simpkins *et al.*, 2003; Montserrat *et al.*, 2007) and 5 days of fasting is not long enough to induce energy losses based on lipid levels (Miglavs & Jobling, 1989).

For statistical analysis, the non-parametric procedure of Friedman ANOVA was used for multiple group analyses of visit frequency. Friedman test was used because fish preference for one compartment instead of the others provides dependent data. In cases where the Friedman test was significant ($\alpha < 0.05$), the non-parametric Dunn's *post hoc* test was used to determine significant differences among compartments. To compare the visiting frequency across the days and among the compartments, a two-way ANOVA was performed.

LEARNING

The tank used was a squared (100 cm × 100 cm × 20 cm; length × width × depth) open-field glass tank with all lateral wall covered by white sheet to prevent the fish from seeing
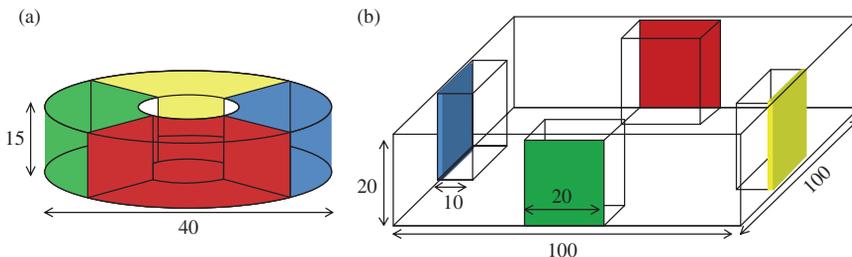

FIG. 1. Schematic views of the tanks used for the (a) colour preference test and (b) learning test of *Stegastes fuscus*. The numbers show the dimensions of the tanks in cm. The learning tank (b) had all walls covered with white sheet and a start box (10 cm diameter) placed in the centre to serve as a release apparatus.

outside. Four small tanks ($20\,cm \times 10\,cm \times 20\,cm$; length $\times$ width $\times$ depth) were placed lying at the centre of each wall and equidistant from the corners. The test apparatus was adapted from Karnik & Gerlai (2012). Each small tank had a different colour paper (cues for the fish orientation): a blue, a green, a yellow and a red card ($20\,cm \times 10\,cm$) attached to the back wall [Fig. 1(b)]. A start box (10 cm diameter) was placed in the middle of the tank and served as a release apparatus. The start box was removed from the tank by a hook connected to a 2 m grip. The tanks were filled with seawater (same temperature and quality of the system water) to a 15 cm depth, and changed everyday to ensure water quality. The experimental procedure was divided in four phases: (1) habituation, (2) training, (3) associative learning test and (4) spatial learning test. A total of 14 fish were used for this procedure.

On the habituation phase (1), each fish was individually allowed to explore the testing tank for 5 days. On the first and second days, fish were placed in the start box for 1 min. The box was then lifted and fish could swim for 1 h. On the third and fourth days, the same procedure was performed but fish were allowed to explore the tank for 20 min. On the fifth day, fish were placed to explore the tank for 5 min. After each trial, fish were moved back to their holding tanks.

Following the habituation, the experimental fish underwent a training phase (2). In this phase, the blue background tank (inside the testing tank) received one fish to be the unconditioned stimulus. The stimulus fish attracted the attention of the experimental fish because *S. fuscus* is highly aggressive and the opponent presence induces agonistic behaviour display in order to preserve territorial ownership. The other small tanks remained empty but had different colours (green, yellow and red). On the training phase, each experimental fish was left for 1 min in the start box and 5 min free to explore the open field. After 5 min exploring the tank, fish returned to the start box for the next trial. This procedure was sequentially repeated four times totalling 24 min training section per day. The training phase lasted 5 days. All fish training was recorded using a digital video camera recorder (SONY DCR-SX45; www.sony.com) placed 2 m above the tank.

The associative learning test (3) took place 24 h after the last training section. All procedures and conditions were the same as in the training phase, except that no stimulus fish was presented and the experimental fish were allowed to explore the tank for only 5 min after released from the start box. The exploring period (5 min) was recorded for learning analysis.

On the day after the associative learning test, the spatial learning test (4) was applied. The same procedures were kept but no colour cards were available as cues. Hence, after being released from the start box, the experimental fish had no cues to locate the stimulus fish's place. Fish were allowed to explore the open field for 5 min during which recording was performed for posterior analysis.

The behaviour of the experimental fish was analysed using the Any-Maze video tracking software (www.anymaze.com). The open-field tank was divided in areas: four equal areas located around each small aquarium ($1050\,cm^2$ each) plus the central and corner areas ($5000\,cm^2$). Random choice (the chance of the fish being in any area of the tank randomly; y) was considered at $y = 11\%$ [$5000 + (4 \times 1050)\,cm^2$]. The time the fish spent in each area was calculated for the training phase, for the associative learning phase and for the spatial learning phase. The time spent in the central and corners areas were calculated as a unique area because these areas could not be associated to any stimuli or cues. The percentage of time spent in the tank areas was compared by repeated measure Friedman ANOVA test, because data showed dependence and non-normal and homoscedastic distribution (according to Shapiro–Wilk and Levene tests, respectively). The time spent at the blue and target zone (where stimulus fish was presented) during the training, the associative learning test and the spatial learning test were compared by the Kruskal–Wallis ANOVA test. The *post hoc* test used was Student–Newman–Keuls. A mixed model test using non-linear mixed-effect (NLME) modelling was performed because it allows the inclusion of fixed effects (days and colours), random effects (fish) and an auto-regressive process in behaviour to describe the time spent in each colour area by the fish (Pinheiro *et al.*, 2009); it is a stronger test to compare the fish behaviour during the training and testing days. The NLME was analysed using R software version 3.0.2 (R Development Core Team; www.r-project.org). For all tests, a probability level of $P < 0.05$ was used as an index of statistical significance.

RESULTS

COLOUR PREFERENCE

During the first observation day of the experiment (compartments without colours) the experimental *S. fuscus* showed equal distribution among the compartments (Friedman ANOVA, $\chi^2 = 6\cdot69$, d.f. $= 3$, $P > 0\cdot05$). When the coloured gelatine filters were placed over the compartments (days 2, 3 and 4) the average visit frequency in each compartment did not differ (Friedman ANOVA day 2: $\chi^2 = 3\cdot19$, d.f. $= 3$, $P > 0\cdot05$; day 3: $\chi^2 = 2\cdot40$, d.f. $= 3$ $P > 0\cdot05$; day 4: $\chi^2 = 2\cdot18$, d.f. $= 3$, $P > 0\cdot05$; Fig. 2). On the fifth day (coloured gelatine filters were removed the previous evening), the fish showed no preference for any of the compartments (Friedman ANOVA: $\chi^2 = 2\cdot77$, d.f. $= 3$, $P > 0\cdot05$). Two-way ANOVA showed a non-significant effect of the testing days ($F_{3,60} = 0\cdot00$, $P > 0\cdot05$), or compartment colours ($F_{3,60} = 0\cdot81$, $P > 0\cdot05$), and the days $\times$ colour compartments interaction term was also found to be non-significant ($F_{2,45} = 0\cdot68$, $P > 0\cdot05$).

LEARNING

The experimental *S. fuscus* were first examined on the training phase: blue colour cue represented the conditioned stimulus, and a conspecific fish represented the
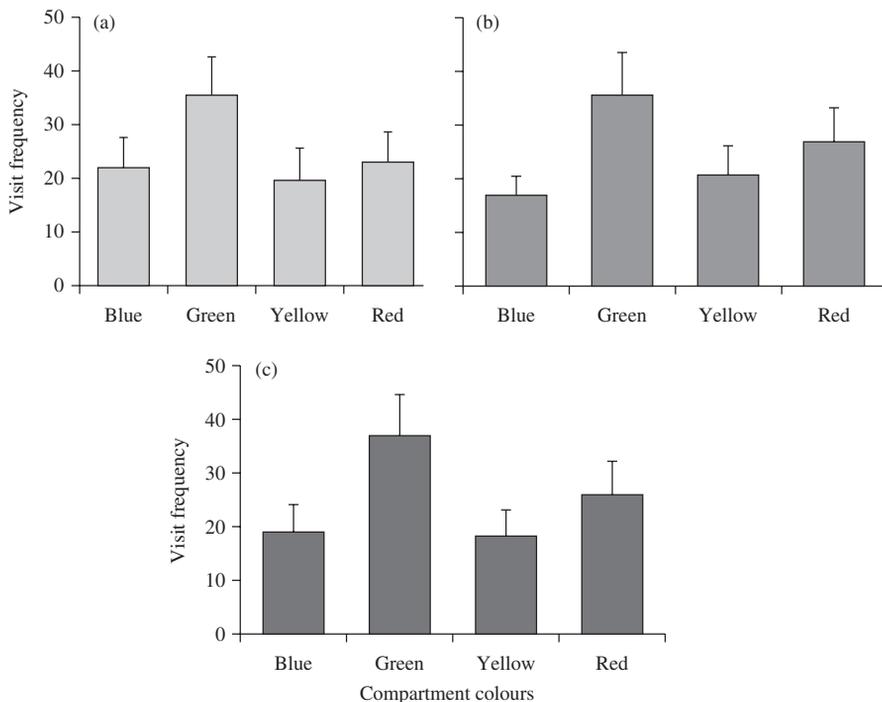

FIG. 2. The mean $+$ S.E. frequency of visits (20 min observation periods at 0800, 1100, 1400 and 1700 hours over a 3 day period; in total 120 observations per individual) of *Stegastes fuscus* to blue, yellow, green and red ambient colours on (a) day 2, (b) day 3 and (c) day 4. Values for days 1 and 5 are not presented in the as the compartments were without colours and are not shown. There was no statistical difference of fish visit frequency in each compartment (Friedman ANOVA, $P > 0\cdot05$).

unconditioned stimulus. The percentage of time fish spent in each zone of the tank, determined by the colour it showed (paired training) is shown in Fig. 3. Fish spent a lower percentage of time in the zones where no stimulus fish was presented (mean $\pm$ s.e. green: $6 \cdot 6 \pm 9 \cdot 3$ on day 1, $5 \cdot 7 \pm 7 \cdot 4$ on day 2, $4 \cdot 3 \pm 6 \cdot 7$ on day 3, $2 \cdot 4 \pm 4 \cdot 6$ on day 4 and $4 \cdot 3 \pm 4 \cdot 1$ on day 5; yellow: $3 \cdot 0 \pm 2 \cdot 7$ on day 1, $6 \cdot 4 \pm 10 \cdot 7$ on day 2, $5 \cdot 1 \pm 7 \cdot 3$ on day 3, $5 \cdot 6 \pm 7 \cdot 9$ on day 4 and $13 \cdot 0 \pm 10 \cdot 3$ on day 5; red: $4 \cdot 5 \pm 8 \cdot 3$ on day 1, $0 \cdot 4 \pm 0 \cdot 6$ on day 2, $0 \cdot 9 \pm 0 \cdot 8$ on day 3, $1 \cdot 0 \pm 2 \cdot 6$ on day 4 and $1 \cdot 5 \pm 2 \cdot 5$ on day 5). The mean $\pm$ s.e. time spent in the blue zone ($65 \cdot 8 \pm 10 \cdot 8\%$ on day 1, $68 \cdot 2 \pm 10 \cdot 3\%$ on day 2, $63 \cdot 5 \pm 10 \cdot 3\%$ on day 3, $67 \cdot 7 \pm 10 \cdot 5\%$ on day 4 and $64 \cdot 0 \pm 10 \cdot 6\%$ on day 5), where the stimulus fish was present, was above any random chance (Friedman ANOVA day 1: $\chi^2 = 26 \cdot 73$, d.f. $= 3$, $P < 0 \cdot 001$; day 2: $\chi^2 = 25 \cdot 54$, d.f. $= 3$, $P < 0 \cdot 001$; day 3: $\chi^2 = 22 \cdot 88$, d.f. $= 3$, $P < 0 \cdot 001$; day 4: $\chi^2 = 29 \cdot 93$, d.f. $= 3$, $P < 0 \cdot 001$; day 5: $\chi^2 = 36 \cdot 67$, d.f. $= 3$, $P < 0 \cdot 001$; Fig. 3). The experimental fish showed agonistic behaviour against the stimulus all time spent in the tank.

The percentage of time fish remained close to each colour cue after the stimulus fish removal is shown in Fig. 4(a). The fish spent the greatest amount of time close to the blue zone, where the stimulus was previously presented (Friedman ANOVA: $\chi^2 = 22 \cdot 63$, d.f. $= 3$, $P < 0 \cdot 001$). It is also notable that time spent in the yellow and red zones were below a random distribution. The spatial distribution of experimental *S. fuscus* when the open field was presented with no cues is illustrated in Fig. 4(b). It appears that fish exhibited strong preference for the place where the stimulus fish was previously presented. Friedman ANOVA test confirmed that fish spent most of the time close to zone 1 ($\chi^2 = 17 \cdot 45$, d.f. $= 3$, $P < 0 \cdot 001$), which showed the blue cue and the stimulus fish during the training phase. Only the blue and target zone data were plotted in Fig. 5, and the percentage of time spent at that area on the training phase (with colour and stimulus fish) compared on the associative test phase (only cue card) and on the
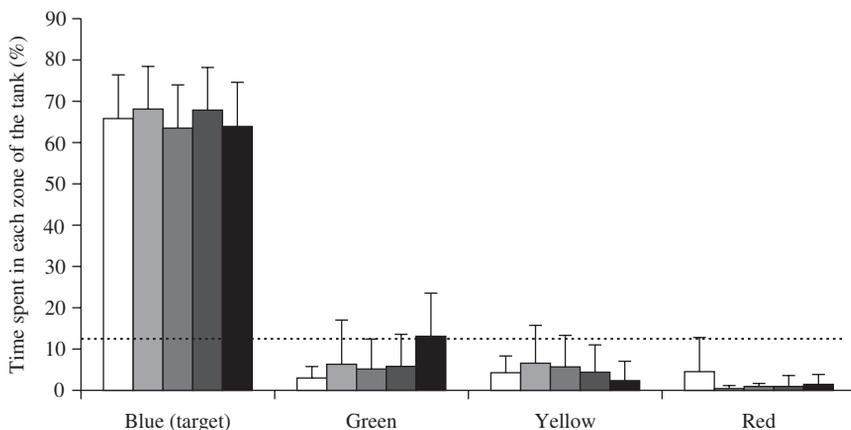

FIG. 3. *Stegastes fuscus* time close to each zone of the tank throughout training days. The data (mean + s.d.) show the percentage of time spent in each unit zone of the tank ($n = 14$). The dashed horizontal line indicates random choice (y) at $y = 11\%$ [$5000 + (4 \times 1050) \text{cm}^2$]. Each zone of the tank was presented in a different colour (blue, yellow, green and red) and stimulus fish was always presented at the blue zone. Fish was allowed to explore the tank for 20 min per day. The training days are represented by different colour bars: from white $=$ day 1 to black $=$ day 5. *Stegastes fuscus* showed strong preference for the target zone that contained the stimulus fish during all the five training days (Friedman ANOVA, $P < 0 \cdot 001$).
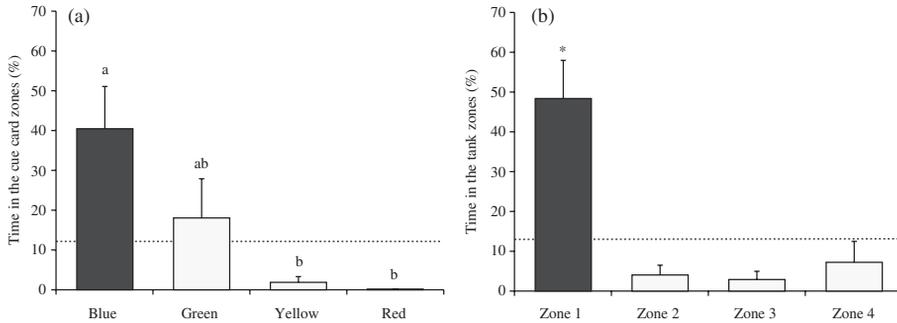
FIG. 4. Mean + S.D. ($n = 14$ fish) time *Stegastes fuscus* spent per zone of the tank when (a) only cue coloured cards were presented (no stimulus fish) and (b) neither stimulus fish nor cue cards were presented. The data are expressed as percentage of time in each zone marked by a different colour card (blue: zone 1, green: zone 2, yellow: zone 3 and red: zone 4). Fish were allowed to explore the tank for 5 min. Random chance is represented by the dashed horizontal line (11%). (a) Fish spent most of the time at the blue card zone, where stimulus fish was presented during the training phase; different lower case letters indicates significantly different values (Friedman ANOVA, $P < 0.001$). (b) Fish spent significantly more time at zone 1, where stimulus fish was presented in association with a blue card during the training phase; *significant value (Friedman ANOVA, $P < 0.001$).

spatial test phase. The results indicate that fish spent most of the time at the target zone, regardless of the presence of the cue or without any visual cue (Kruskal–Wallis ANOVA: $H = 5.58$, d.f. $= 2$, $P > 0.05$).

The NLME modelling showed that neither days (fixed effect) nor fish (random effect) affect the results, but time spent near each colour cue statistically differs (Table I). The most visited colour was blue (shown in Figs 3 and 4; Student–Newman–Keuls $P < 0.05$).

DISCUSSION

Stegastes fuscus learn to associate the presence of a conspecific with a visual colour cue in subsequent trials. Fish spent most time close to the colour cue alone on the testing day. Moreover, *S. fuscus* were also able to localize the place of the stimulus even without the colour cues. This is an important finding as it means that *S. fuscus* are not only able to perform associative learning but also suggests some spatial cognition.

TABLE I. Non-linear mixed-effect (NLME) modelling results obtained for the time *Stegastes fuscus* spent in each zone of the tank

Source	Effect	d.f.	MS	MSE	F	P
Colour	Fixed	3	10·51	0·020	519·26	<0·001
Day	Fixed	4	0·005	0·020	0·27	>0·05
Fish	Random	13	0·005	0·020	0·24	>0·05

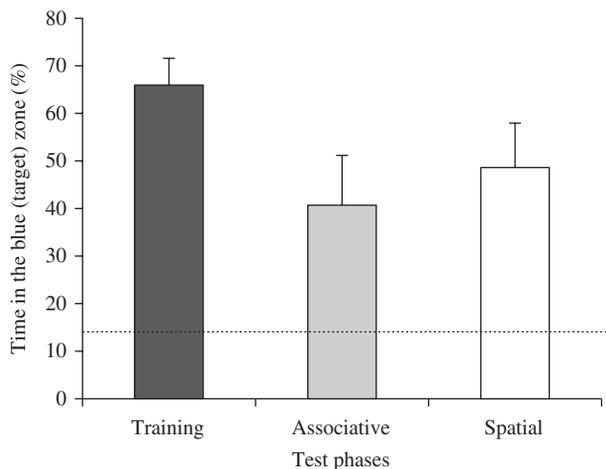MS, mean square; MSE, mean squared error.

FIG. 5. The mean + S.D. ($n = 14$ fish) percentage of time spent by *Stegastes fuscus* close to the specific zone of the tank where a stimulus fish–cue card was presented in three conditions test: during the training phase (stimulus fish and cue colour card presented), the associative learning phase (only cue colour card present) and the spatial learning test phase (no cues or stimulus). Random chance is represented by the dashed horizontal line (11%). Fish localized and showed preference for zone shown when the stimulus was presented and kept that pattern when only a cue was presented (associative) and also when no cues indicate the place (spatial). There was no statistical difference (Kruskal–Wallis ANOVA, $P > 0.05$).

Colour vision has been investigated for many coral-reef fishes. Although many marine fishes have very well-developed visual sense (Loew & Lythgoe, 1978; Levine & MacNichol, 1982), only a few studies approach the ability of *S. fuscus* to detect and behaviourally respond to colours. In this study, four different colours were used as cues for the associative learning task. A behavioural colour preference test was first performed in order to avoid using a colour cue that would attract the fish (if it showed any preference) on the associative learning test. Although fish failed to show preference for a particular colour (Fig. 2), they still may be able to distinguish colours. Fish, however, show no favouritism for a specific wavelength. Indeed, Siebeck *et al.* (2008) demonstrated that a close relative damselfish, *Pomacentrus amboinensis* Bleeker 1868, show surprisingly accurate colour vision. Meanwhile, the present result allowed the associative learning protocol using the US fish always combined to the same colour cue (*i.e.* blue; CS) to be performed.

During the training sessions of the associative learning task, in which a stimulus fish was presented together with the colour cue, the experimental fish spent most of the time close to the stimulus (Fig. 3) and displayed many agonistic signals against it. The aggressive behaviour, characteristic of damselfish species (Siebeck *et al.*, 2008), could be used as motivation for the animal to seek out the stimulus fish when inside the open-field tank. The results suggest that *S. fuscus* was motivated to stay close to the stimulus and that agonistic display may play an important role in the fish welfare, as previously reported for highly aggressive species by Huntingford *et al.* (2012). According to Rasa & Anne (1971), damselfishes show high appetence for aggression, and several studies indicate benefits of the aggressive display for the animal emitter; for instance, the most aggressive fishes acquire the most food (Adams *et al.*, 1998), obtain greater mating success (Berglund & Rosenqvist, 2003; Weir *et al.*, 2004), and

increase dopamine release in brain areas related to motivation (Kania *et al.*, 2012); although some costs can also be suggested for the aggressive species, as energy expenditure, risk of injury and stress response (Karino & Someya, 2007; Lehtonen, *et al.* 2010). The study succeeds in using a conspecific as US, and *S. fuscus* showed CS–US conditioning and place learning based on the seeking behaviour for the stimulus fish.

Many studies address conditioned learning in fishes, such as zebrafish *Danio rerio* (Hamilton 1822) (Pradel *et al.*, 1999, 2000; Al-Imari & Gerlai, 2008; Braubach *et al.*, 2009; Goméz-Laplaza & Gerlai, 2010; Karnik & Gerlai, 2012), goldfish *Carrasius auratus* (L. 1758) (Salas *et al.*, 1996; Broglio *et al.*, 2010), redtail splitfin *Xenotoca eiseni* (Rutter 1896) (Sovrano & Bisazza, 2008) and also *P. amboinensis* (Siebeck *et al.*, 2008, 2009; Holmes & McCormick, 2010). Although associative learning experiments with fishes commonly use food as US, a conspecific fish was used; the result corroborates the fact that fish show CS–US learning. The learning processes allow animals to obtain knowledge to respond, adapt and survive in unpredictable environments (Staddon, 1983). In fact, it appears that learning plays an important role in many aspects of the fish's life. In particular for those living in clear waters around coral reefs, where visual signals may be visible over many metres and indicate several different stimuli (*e.g*. food, predators and intruders).

Learning and memory have been investigated from many angles. The ability to associate stimuli (conditioned learning) brings numerous advantages and has been intensively studied in animal cognition. An important mnemonic characteristic of animals is the spatial memory, which is the ability to record information about the environment and its spatial orientation (Broglio *et al.*, 2003). In addition to the use of environmental landmarks as direct reference for orientation, fishes appear to be able to learn more complex spatial relations (Pitcher & Magurran, 1983; Nieuwenhuys & Meek, 1990; Braford, 1995; Northcutt, 1995; López *et al.*, 2000; Broglio *et al.*, 2003).

In the place learning test, the removal of all environmental cues [colour cards; Fig. 4(b)] did not prevent the fish from returning to the area where the stimulus was earlier presented. This is an important finding because it implies that the fish is able to learn not only association between US and CS, but also more than just a single associative task. While there are numerous studies on damselfishes (Thresher, 1978; Cleveland *et al.*, 2003; McDougall & Kramer, 2007; Ben-Tzvi *et al.*, 2009; Souza *et al.*, 2011; Manassa & McCormick, 2012), they have focused attention on the territorial behaviour or predator avoidance and neglected factors as their ability to recognize and navigate on their complex territory.

These results confirm the ability of conditioned learning (single CS–US) in *S. fuscus* and suggest their good performance in spatial learning task. The current state of the art regarding spatial cognition, however, lacks information on whether it in fact means spatial map knowledge or whether non-spatial solutions are used. For instance, it cannot be determined which visual stimuli are actually perceived and which are ignored by the fish. More than that, practically nothing is known about the behavioural responses involved in lateral line signals and magnetic orientation in fishes, what makes it difficult to discuss which signals were actually used for finding the stimulus place.

Therefore, these results show that *S. fuscus* perform visual discrimination tasks, identifies the reward-target site showing association and presents remarkable ability to recognize the environment. This species lives in coral-reef areas, where the environment is highly complex in terms of routes (courses and circuits) and patches (areas), have many species sharing the same space and food sources, and receives a

variety of incoming water currents and substrata that make ambient conditions very unpredictable. Therefore, it appears reasonable that *S. fuscus* form spatial maps of their environment in order to locate their shelter and territorial borders. Hence, the results are compatible with the idea that fishes present some kind of relational learning capacity. Whether this ability was solely used or used in combination to other aspects of ambient conditions is not known at this point. Nevertheless, some spatial orientation probably occurs in *S. fuscus*, because it spent most of the time close to the place where the stimulus was previously given. Hence, in order to understand how these fish orientate in complex coral-reef environment, studies approaching other relational learning tasks (for instance the animal's detection of the ambient geometry) may contribute to animal behaviour research.

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