



Original Article

Cue choice and spatial learning ability are affected by habitat complexity in intertidal gobies

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Variation in the structural complexity of a habitat is known to have significant effects on the evolution of different populations and can shape behavior, morphology, and life-history traits. Here, we investigated whether habitat complexity influences a species' capacity for spatial learning and cue choice by comparing the performance of 4 goby species from 2 contrasting habitats in a spatial task. Gobies were collected from dynamic, homogenous sandy shores and stable, spatially complex rock pool habitats. We trained fish to use a T-maze to find a hidden reward and asked whether they used local visual landmarks or body-centered methods for orientation (i.e. turn direction) to do so. It was expected that fish from rock pools would learn the spatial task much faster and use different orientation cues than fish from sandy shores. We found that rock pool species learnt the location of the reward arm much faster, made fewer errors and used both types of cues available (visual landmarks and turn direction) to locate the reward, whereas sand species relied on turn direction significantly more than plant landmarks to orientate. The results of this study provide support for the hypothesis that the spatial complexity of habitats in marine environments has a significant effect on the evolution of fish cognition.

Key words: cue use, ecology, gobies, habitat stability, spatial learning and memory.

INTRODUCTION

It is well understood that the environment that an animal inhabits has a major impact on the evolution and development of its morphology, life-history characteristics and behavior (Brown and Braithwaite 2005). However, it has only been within the last 20 years that the role the environment plays in shaping learning ability and memory has actually begun to receive the attention it deserves (Healy and Braithwaite 2000). Researchers are now interested in exploring the possibility that learning and memory are specialized to suit the specific needs of a species habitat and way of life (Balda et al. 1998; Brown and Braithwaite 2005). Enhanced spatial memories have been correlated with increased size in certain brain areas (i.e. telencephalon) in a number of species. For example, food storing birds and rodents possess a significantly larger hippocampus and a greater spatial memory capacity than do closely related species that do not cache food items (Shettleworth 2003; Vander Wall and Jenkins 2003; Lucas 2004; Healy et al. 2005). Likewise, larger telencephalons have been noted to occur in animals that live in spatially complex habitats such as coral reefs (Kotrschal et al. 1988; Marchetti and Nevitt 2003). Central to these comparative studies is the assumption that the maintenance, operation and production of the neural machinery required for spatial learning and memory is energetically costly and thus should only be invested in by animals

with lifestyles and ecologies that demand it (Odling-Smee et al. 2008).

Although the links between habitat complexity, brain development and spatial learning ability have been reasonably well studied in terrestrial taxa, far less is known about these relationships in aquatic systems, and especially in marine environments. Numerous fish species live in structurally complex and changing habitats, thus it is expected that they should also display reasonable spatial learning and memory capabilities. Spatial complexity and stability within a habitat affects the speed at which fishes learn, the duration of these memories, and the type of spatial cues they use to orientate (Mackney and Hughes 1995; Brown 2003; Odling-Smee and Braithwaite 2003; Odling-Smee et al. 2008). Furthermore, a species' tendency to focus on certain cues or to learn certain associations is also likely shaped by natural selection. In this way, animals are less likely to pay attention to untrustworthy or unhelpful sources of information, reducing the risk of wasting time processing unneeded information and making potentially costly mistakes. Brown and Braithwaite (2005) found that Panamanian bishops (*Brachyraphis episcopi*) from habitats of low-predation pressure made fewer errors, solved the spatial task much faster and navigated more actively within the maze, than fish from high-predation sites. Furthermore, they found that fish collected from more stable rivers relied on local landmarks to navigate, whereas fish from less stable rivers relied on extra-maze cues.

Further support for the theory that spatial learning abilities are ecologically driven comes from studies on 2 lake dwelling sympatric

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species of 3-spined stickleback (*Gasterosteus aculeatus*) by Odling-Smee et al. (2008). Reproductively isolated “benthic” and “limnetic” sticklebacks were trained to locate a goal in a T-maze using either turn direction or plant landmarks. Despite using similar types of spatial cues, benthics learned the task almost twice as quickly and made fewer errors than did limnetics (Odling-Smee et al. 2008). Benthic species live within the spatially structured littoral zone, where they feed mainly on invertebrates within the vegetation and sediments and thus may require better spatial memories; whereas limnetic species live in the comparatively homogeneous open water column where they feed predominately on plankton. Studies of river and pond dwelling sticklebacks revealed that fish from pond habitats relied more heavily on landmarks to orientate than did fish from rivers, which were more likely to use a body-centered algorithm (series of turns) to orientate (Girvan and Braithwaite 1998; Odling-Smee and Braithwaite 2003). Landmarks are likely to be unstable and thus unreliable as directional cues in turbulent fast flowing rivers but are likely to be highly stable in ponds. It may be that the reliability of cues is more important than their availability in determining the preferred cues to use in spatial learning. Mackney and Hughes (1995), showed that closely related stickleback (*Gasterosteidae*) species obtained from more changeable habitats (i.e. estuarine and marine), displayed a shorter memory for foraging skills, whereas freshwater populations obtained from stable landlocked ponds, displayed longer memory for foraging skills.

The family Gobiidae consists of thousands of phenotypically diverse species inhabiting a range of aquatic environments (Nelson 1994) making them ideal candidates for comparative analyses of spatial learning and memory processes. Gobies can be broadly broken up into a number of phylogenetic lineages which broadly correspond to the habitats that they occupy (Thacker and Roje 2011); 2 of which include species that occupy intertidal sandy shores and those that inhabit rock pools on rocky reefs (White 2014, Chapter 9). These groups show extensive behavioral, habitat, and life-history differences that likely have deep phylogenetic origins (Thacker and Roje 2011; White 2014, Chapter 9). Such differences are likely to be reflected in measures of cognitive performance and the cues they use for orientation.

The majority of fish species that inhabit sandy shores enter and leave the intertidal zone with each tide cycle (Gibson 1999). This sandy intertidal zone is mobile, constantly changing and relatively homogeneous in nature. Disturbance by wave action and tides render local visual landmarks unreliable indicators of location for use in orientating to resources. In this environment, fish ought to rely on egocentric information and/or global cues (e.g. wave direction, location of shore) for orientation and long-term memory formation of particular locations is likely to be less valuable. Intertidal rock pools on the other hand are stable, structurally complex habitats that retain water at low tide acting as refuges for intertidal fish (Gibson 1982; Silberschneider and Booth 2001). The fish occupying these pools tend to remain there for extended periods and likely become very familiar with their surroundings (White and Brown 2013). The large number of stable landmarks in this environment provides excellent opportunities to use them as orientation cues. These greatly contrasting habitats likely lead to divergent evolution of a huge number of physiological and behavioral traits. Here, we contrast the learning and memory capabilities of 2 rock pool-dwelling species and 2 sandy shore dwelling goby species, asking specifically whether habitat stability and spatial complexity influences the use of visual cues in orientation. By using multiple species from each habitat, we intend to demonstrate that there is a general

evolutionary response to living in each environment. A broader phylogenetic approach to comparative cognition such as this provides better insight into the selective pressures shaping cognition in the marine environment.

The ecological cognition hypothesis predicts that natural selection would favor the evolution of enhanced spatial memories in rock pool-dwelling species so they can avoid the risk of becoming stranded in unsuitable areas due to the retreating tides and orientate within their home pools. Sandy shore species, by contrast, would be subject to a different suite of selective pressures because their habitat is homogenous, mobile and constantly changing and thus a highly developed spatial memory is less likely to be useful in this context and may even be a hindrance. We predicted, therefore, that gobies found in rock pools will use landmark cues for orientation to a greater extent than sand-dwelling species, and that rock pool-dwelling species will perform better in the spatial learning task than gobies found on sandy beaches.

METHODS

Study animals

Four species of intertidal gobies of differing ecologies were tested for specific cue use during a spatial learning task. Two rocky intertidal species: Cocos frillgoby (*Bathygobius cocosensis*) and Kreff's goby (*B. krefftii*), which were collected from rock pools at a number of rock platforms. Additionally, 2 sand-dwelling species: Eastern long finned goby (*Favonigobius lentiginosus*) and Hoese's sandgoby (*Istigobius hoesei*), were collected from a number of sandy beaches in the Sydney region of New South Wales, Australia.

We tested a total of 65 fish for cue use (17 Cocos frillgoby, 16 Kreff's goby, 18 eastern longfin goby, and 14 Hoese's sandgoby). Fish were allowed to acclimate in the laboratory for a period of 1 month before testing began. They were housed in groups of 8–10 in 4 flow-through seawater aquaria (64 × 42 × 26 cm: 70 L) held in a seawater facility. All aquaria were maintained at the same seawater flow rate (1 L/1 min⁻¹) and temperature (18–22 °C), and were illuminated for 12 h each day with full spectrum UV lights. Fish were fed a combination of live brine shrimp (*Artemia franciscana*), live black worms (*Lumbriculus variegatus*) and the commercial dry food “tetra color bits” every second day during the settling period. Three weeks before testing began fish were lightly anesthetized using a solution of 50 mg/1 MS222 buffered with sodium bicarbonate (fish placed in a bucket containing 1.5 L solution until subdued), their total length was measured and they were tagged using Visible Implant Fluorescent Elastomer tags (VIE: Marine Technology, Inc. 2014) to aid individual identification. Recovery from tagging is almost immediate and has no long-term effects on their behavior (White and Brown 2013).

T-maze apparatus

The apparatus and procedures used in this experiment were based on those used by Odling-Smee and Braithwaite (2003) and Odling-Smee et al. (2008). Two 4-arm, cross mazes constructed from 3-mm polyvinyl chloride plastic (Figure 1) lined with shell grit were submerged into aerated seawater to a depth of 10 cm within a rectangular pool (1.3 m × 0.85 m and 0.2 m high) connected to a flow-through system.

A removable screen 10 cm wide and 16 cm high could be slid into runners, enabling any 1 of the 4 arms to be shut off to produce a T-maze. A trap door (10 × 16 cm) held by runners 8 cm from

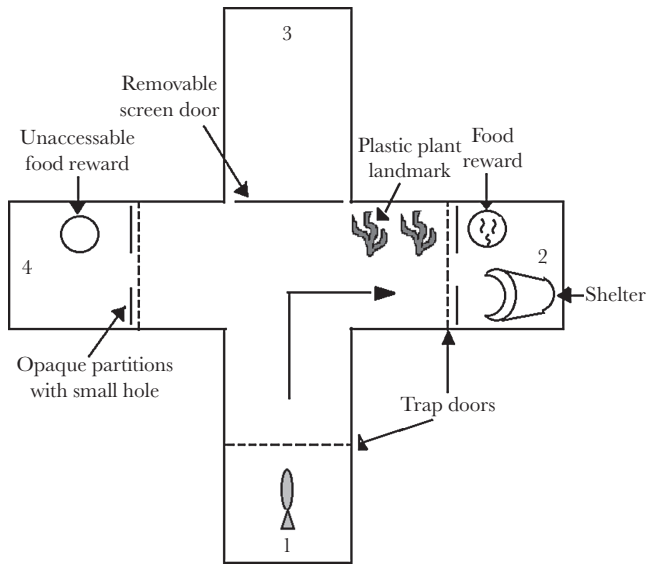


Figure 1 Diagram of the spatial learning task. Numbers indicate the sequence of start box positions for a run of 3 consecutive trials starting at position 1. The arrow indicates the correct route a right turn trained fish had to take in order to obtain food and shelter.

the central core of the T-maze was used to create the start box for each trial. Two opaque PVC partitions (10×16 cm) were positioned in the maze 10 cm from the ends of each arm. At the base of each partition was a small doorway (4 cm high and 2 cm wide) through which the fish could swim to reach the end of the arm. Food rewards were provided by placing blackworms into glass petri dishes (3 cm in diameter and 1.5 cm deep), which were only visible to the fish after it had passed through the doorway in the PVC partition. An artificial shelter, half a ceramic pot 5 cm in diameter, was also positioned behind the partition at the ends of each arm, to help reduce the stress caused by forcing fish that normally seek out cover into an open area. Therefore, fish received a double reward (food and shelter) when it entered the correct arm. If a fish swam into the wrong arm, trap doors (10×16 cm) held in grooves just behind the PVC partitions could be lowered in the opposite arm, preventing the fish from swimming back and reaching the food. Therefore, rewards were limited to fish that chose the correct arm first. Two landmarks (plastic plants) were placed in the maze, 1 in entrance to the correct arm just visible from the exit of the start box, and the other also in the correct arm just in front of the small doorway leading to the reward (Figure 1). Fish behavior was monitored remotely on a laptop computer connected to a web camera (Microsoft Lifecam VX-2000) positioned 1 m above the centre of each maze. Trials were recorded directly to an external hard drive connected to the laptop computer. Black curtains surrounded the maze to reduce the availability of external maze cues and prevent disruptions created by viewers.

Pretraining

The function of pretraining was to allow fish to become accustomed to the maze and the start box. During pre-training, fish were transferred from their holding tanks to the maze via small hand nets. Within the maze no shelters or landmarks were present, and petri dishes containing large amounts of blackworm were placed in both arms of the maze. From pretraining onwards fish

were only ever fed when performing trials inside the maze. Groups of 8 fish were given free access to the entire T-maze for ten 24 h periods alternated with 24 h in their home tanks, both mazes were used simultaneously. During each pretraining session, we randomly selected a start box with the restriction that no start box was used more than twice in a row. To minimize handling effects, an opaque cover was placed over the start box for 5 min before each session began to allow the fish to settle. After the 5-min calming period had elapsed, the trap door was removed and fish were allowed to swim freely throughout both arms of the T-maze.

Training

Fish were given 3 training trials every second day, and we randomly chose the order in which the fish were trained for each day. Fish were trained to find a reward (shelter and 3 blackworms in a petri dish) in 1 arm of the T-maze. A petri dish was also positioned in the opposite arm except that it was inverted so that fish could not access the reward. This ensured that fish were not using olfactory cues to orientate. To control for any directional bias, we trained half the fish from each species to turn left and half to turn right. At the beginning of each 3 trial session, we randomly selected the start box with the restriction that no start box was to be used twice in a row for the same fish. In this manner, fish could not rely on extra-maze cues for orientation. A single fish was placed into the start box and given 5 min to settle before the cover and trap door were removed and the fish was allowed access to the maze. We recorded the arm the fish started in, time taken for the fish to leave the start box and to enter an arm, and which arm the fish chose first. Arm entry was determined to have taken place when the base of the caudal fin had passed through the doorway in the PVC partition. To complete a trial correctly, fish had to swim through the PVC partition into the arm containing the reward (food and shelter) before it entered the unrewarded arm. Once fish had consumed the reward and/or entered the shelter they were left for a further 3 min and the trial terminated. If the fish chose the unrewarded arm it was still able to swim into the opposite arm to obtain the food and shelter reward, but this trial was scored as "incorrect". If the fish did not enter an arm after 10 min the trial was terminated. Once a trial had been terminated, we encouraged fish to swim into the rewarded arm (i.e. if they had left it or not entered it during the trial), lowered the trap door, and then manipulated the maze so that the previously rewarded arm became the start box for the next trial. This ensured that fish rotated around the arms of the maze: clockwise (for left turners) and anticlockwise (for right turners), allowing us to train individuals using blocks of 3 trials, with minimal handling between trials.

For the first 12 trials, trap doors in the arms of the T-maze were removed allowing fish access to both ends. However, for the remainder of the experiment, once a fish had entered the hole in the partition of 1 arm, the trap door was lowered over entrance to the opposite arm. Therefore each fish was only rewarded if they chose the "correct" arm first. To ensure that each individual had learned the task, fish had to perform 9 correct trials out of 10. Fish were trained until they had reached this criterion or for a total of 45 trials after which they were given a probe trial.

Probe trials

Once fish reached criteria, 3 probe trials were conducted interspersed by additional post criterion training trials. Between any 2 probe trials fish needed to perform a minimum of 4 correct trials

out of 5 consecutive trials in order to reach criterion. These probe trials tested individual preferences for spatial cues by placing the 2 possible orientation cues (turn direction and landmarks) in conflict with one another by moving the plant landmark. For example fish trained to turn left would now find landmarks positioned in the right arm of the maze (opposite side from that of training). By observing which arm the fish entered, it was possible to determine which cue the fish used to orientate. During probe trials, the food and shelter rewards were placed at both arms of the maze so that fish were not being punished for choosing one or other of the available cues. Fish were allowed to enter an arm and feed or hide in the shelter for a period of 3 min before they were removed from the maze.

Statistical analysis

In most cases, the data were normally distributed, but where necessary nonparametric statistics were used. To test if learning task performance was affected by species differences or habitat type, we used a general linear model with a binomial error structure design with number of trials to reach criteria (probe trial 1), percentage of errors to criteria (probe trial 1), or ability to learn (i.e. did the fish reach probe trial stage before the 45 trial cut off or not) as the dependent variable, and direction fish was trained to turn (left or right) and habitat or species as fixed factors. Size was not included in any of the above comparative analyses because it is confounded by species size differences. Thus we analysed size at a species level for each of the 4 performance measures using regressions split by the direction in which fish were trained to turn.

Results of all 3 probe trials were combined (i.e. total number of probe trials where fish chose to use landmarks) and we used a chi-square test to determine whether rock pool fishes and sand fishes differed in the types of spatial cues used (i.e. turn direction or landmarks) during the probe trials. This test questioned whether the proportion of rock pool fishes using landmarks was different to that of the sand fishes, we also performed the chi-square test for each species separately. All statistical analyses were performed using StatView Version 5.0.1 (SAS Institute Inc. 1998) and Excel version 12.2.3 (Microsoft, 2008).

RESULTS

Learning task performance

Analyses of the number of trials to reach criteria (the first probe trial) detected a strong effect of species and a strong species \times turn direction interaction, but no effect of turn direction (GLM: Species: $F_{3,58} = 5.04$, $P = 0.004$; Turn direction: $F_{1,58} = 1.30$, $P = 0.26$; Species \times Turn direction: $F_{3,58} = 3.59$, $P = 0.021$). Both rock pool species, Cocos frillgoby and Kreffit's goby, made it to criteria (probe trial stage) in significantly fewer trials than the sand species, eastern longfin goby ($P \leq 0.05$ in both cases). Much of the variability in learning between species was related to an interaction between size and underlying turn biases. Regression analyses of learning at the species level revealed a correlation between body size and turn direction for all species. In both rock pool species and one sand species larger fish (T_L : 4–6 cm) trained to turn left learnt the location of the reward arm faster than smaller (T_L : 2–3.5 cm) left turn trained fish (Regressions: Cocos frillgoby: $R^2 = 0.569$, $F_{1,8} = 9.244$, $P = 0.019$, Kreffit's goby: $R^2 = 0.517$, $F_{1,7} = 6.43$, $P = 0.044$, Hoese's sandgoby: $R^2 = 0.9$, $F_{1,4} = 27.27$, $P = 0.014$). Although the opposite was true for the sand species eastern longfin goby;

smaller fish trained to turn left learnt the location of the reward arm much faster than larger left trained fish (Regression: $R^2 = 0.65$, $F_{1,8} = 13.00$, $P = 0.008$). Although there were no significant differences in learning noted between small and large fish trained to turn right in the maze ($P > 0.05$ in all cases). Over all, rock pool species required marginally fewer trials to reach criteria than sand dwelling species (GLM: Habitat: $F_{1,60} = 3.602$, $P = 0.062$) (Figure 2), however, we found no effect of turn direction and no habitat \times turn direction interaction on the number of trials to fish required to reach the first probe trial ($P > 0.05$ in both cases).

Analyses of the percentage of errors fish made before reaching the first probe trial detected a strong effect of species, but no effect of turn direction or an interaction between the 2 variables (GLM: species: $F_{3,54} = 9.22$, $P < 0.001$; Turn direction: $F_{1,54} = 0.01$, $P = 0.91$; Species \times Turn direction: $F_{3,54} = 0.13$, $P = 0.94$). Eastern longfin gobies made the most errors followed by Hoese's sandgoby and the 2 rock pool species (Kreffit's gobies and Cocos frillgoby) made the fewest errors. Regression analyses of the percentage of errors made by fish revealed that there was no correlation between body size and turn direction for all species except the sand species eastern longfin goby where smaller fish (T_L : 3–4 cm) made fewer errors than larger fish (T_L : 4.5–6) (Regression: $R^2 = 0.612$, $F_{1,17} = 25.20$, $P < 0.001$). Over all sand dwelling species made significantly more errors before reaching the first probe trial than did rock pool species (GLM: Habitat: $F_{1,58} = 16.23$, $P < 0.001$) but once again there was no effect of turn direction and no effect of the interaction between habitat

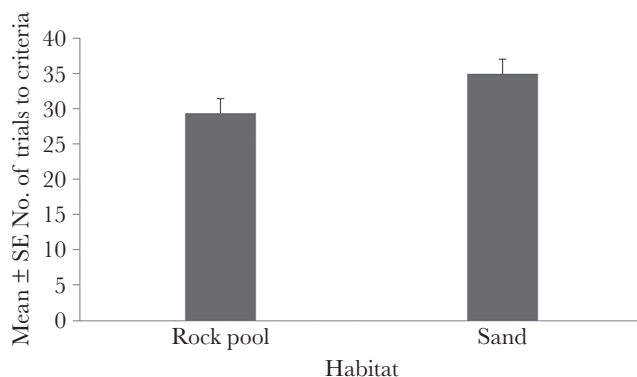


Figure 2
Mean (\pm SE) number of trials required by rock pool and sand-dwelling fish to reach criteria.

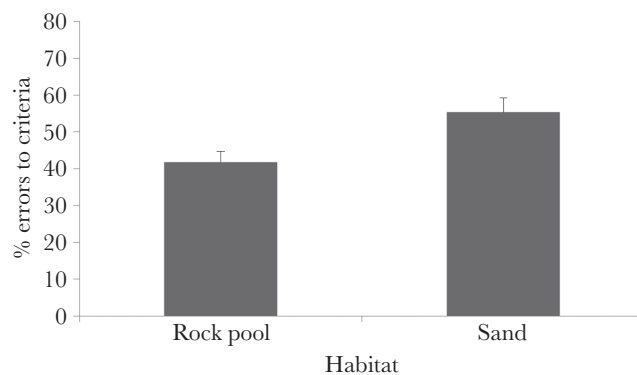


Figure 3
The percentage (\pm SE) of errors made by rock pool and sand-dwelling fish before reaching criteria.

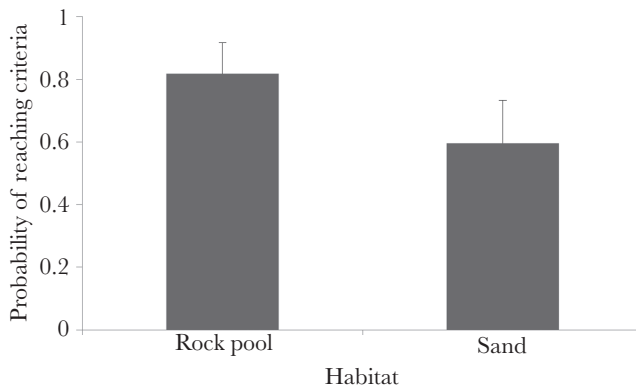


Figure 4

Mean (\pm SE) proportion of fish that made it to probe trial stage based on their own learning ability (i.e. before the 45 trial cut off) for rock pool and sand-dwelling species.

and turn direction (GLM: Turn direction: $F_{1,58} = 0.012$, $P = 0.91$; Species \times Turn direction: $F_{3,58} = 0.003$, $P = 0.96$) (Figure 3).

A total of 8 eastern longfin goby, 4 Hoese's sandgoby, 3 Cocos frillgoby, and 3 Krefft's goby failed to make it to criteria before the 45 trial cut off. The GLM found no effects of species or turn direction or habitat \times turn direction interaction on fishes ability to make it to probe trial stage before the 45 trial cut off ($P > 0.05$ in all cases). However, results of Fisher's PLSD analysis revealed a marginal difference between the sand species eastern longfin gobies and the rock pool species Cocos Frillgoby ($P = 0.089$). In total, 6 individuals from the rock pool species and 12 individuals from the sand species failed to make it to criteria before the 45 trial cut off. Habitat origins had significant effects on fishes learning abilities; sand species were significantly less likely to make it to probe trial stage before the 45 trial cut off than were rock pool species (GLM: Habitat: $F_{1,68} = 4.17$, $P = 0.045$) (Figure 4).

There was no effect of turn direction or a habitat \times turn direction interaction on fishes ability to make it to probe trial stage before the 45 trial cut off (GLM: Turn direction: $F_{1,68} = 1.3$, $P = 0.26$; Habitat \times Turn direction: $F_{1,68} = 1.1$, $P = 0.30$).

Spatial cue use

When analysed separately, all species were found to use a combination of both cues (plant landmark and turn direction) to orientate (Chi-square test χ^2_2 : Cocos frillgoby: $P = 0.67$, Krefft's: $P = 0.11$, eastern longfin goby: $P = 0.17$). However, there was a slight trend for the sand-dwelling species, Hoese's sandgoby, to use turn direction cues for orientation, but this was not statistically significant (Chi square test χ^2_2 : $P = 0.086$). When combined into habitat groups, it was found that rock pool dwelling species used a combination of the 2 available cues (plant landmark and turn direction) to orientate, whereas sand-dwelling species relied more readily on turn direction (Chi-square test χ^2_2 : rock pool $P = 0.132$; sand $P = 0.033$) (Figure 5).

DISCUSSION

Fish often live in a wide variety of complicated and changing habitats and are thus likely to have developed a range of learning and memory abilities to survive in them. The results presented here add to a growing body of evidence indicating that both learning capacity and navigation techniques vary considerably between

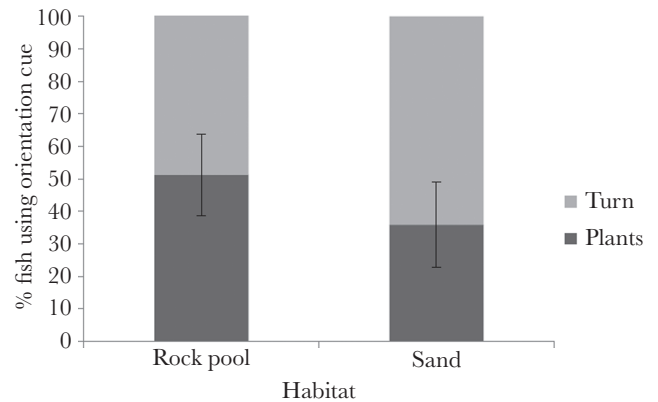


Figure 5

Percentage (\pm SE) of rock pool and sand-dwelling fish using plant landmarks and turn direction for orientation during probe trials.

species occupying contrasting environments. As we predicted, rock pool species learnt the location of the reward much faster and made fewer errors during training than did sand-dwelling species. Furthermore, rock pool species used a combination of the 2 available cues (plant landmarks and turn direction) to orientate which likely highlights the importance of locating the position of stable refuges. Sand species, in contrast, were more likely to use turn direction than plant landmarks to orientate. We would like to make it clear that we do not rule out the possibility that sand species can also use landmarks for orientation, it does appear, however, that their preference for body-centered methods of orientation (i.e. series of turns) overshadows the use of other cues. A partner study also found that rock pool species relied on both local and extra-maze (global) cues, whereas sand species showed a preference for extra-maze cues (White 2014, chapter 6). Studies exploring the spatial learning abilities of animals have often observed that when a navigational problem can be solved in more ways than one then multiple orientation strategies will be utilized in conjunction with one another (Etienne et al. 1990; Able 1993; Collett and Zeil 1998; Odling-Smee et al. 2008).

Within spatially complex rock pool habitats, fish avoid predators by learning and remembering the spatial position of safe refuges using the abundant, stable landmarks (Markel 1994; Burt de Perera and Guilford 2008). This type of information is likely to be of much less importance to sand species, which respond to danger by displaying quick zigzag escape trajectories and burying under the sand (Whitely 1932; Murdy and Hoese 1985). Although sand-dwelling species do use refuges if they are available, their presence and position are always transient, and therefore information concerning their whereabouts is unlikely to be retained. Moreover, in order to access rewards in the maze, fish were required to swim through a small door. The area behind this door was often slightly darker than the rest of the maze and as a result may have been more attractive to the rock pool dwelling species that are used to hiding in crevasses and under rocks (White and Brown 2013; White 2014, chapter 2). Dodd et al. (2000) demonstrated that shannies are highly attracted to dark areas as they may indicate the position of shelter such as a crevice or rocky overhang. In contrast, sand species observed in this study did not appear to notice the door until much later in the training period, instead they swam up and down the arms of the maze or remained in the start box hiding under the shell grit. Nevertheless, we expect that hunger should have equally motivated these species to locate the reward arm.

The differences in learning performance and spatial cue preferences exhibited by rock pool and sand species may have arisen due to genetics, differences in experience, or a combination of the 2. All 4 goby species are genetically distinct from one another, however rock pool species are more closely related to one another than they are to sand species and vice versa (Thacker and Roje 2011; White 2014, Chapter 9). Molecular evidence also suggests that a single species colonized each habitat type and then over time speciation occurred (Thacker and Roje 2011; White 2014, Chapter 9). Therefore, it is possible that learning, cue choice and habitat preference of the species studied here may have evolved in parallel with their phylogeny. That is rock pool and sandy bay environments may be genetically selecting for specific and divergent spatial learning abilities and cue preferences. Differences in prey capture tactics may also select for differing spatial learning abilities and cue use, e.g., rock pool species actively search for mobile prey items, such as amphipods, brachyurans, isopods, and polychaetes (Randall and Goren 1993), so they may rely on spatial learning to a greater extent (i.e. must remember complex environmental features) in order to return to key areas where these small invertebrates commonly hide. Whereas sand species feed by sifting sand through their gill rakers to filter out any infauna (Myers 1999), sitting stationary and sucking in mouthfuls of sand is unlikely to require much in the way of neural machinery.

Alternatively, learning ability and preference for a particular cue may be acquired during an early phase of development (Odling-Smee and Braithwaite 2003) or via continual reinforcement through use. For example, experiments on homing pigeons demonstrated that birds exposed to a full view of the natural landscape during development were more likely to use visual landmarks to orientate than their siblings reared in a loft with opaque windows (Braithwaite and Guilford 1995). In the case of this study, experience of a less stable environment during early ontogeny may cause individuals to rely on body-centered methods of orientation (i.e. series of turns) to a greater extent later in life and require constant and prolonged exposure to spatially complex tasks before they are able to learn to retain spatial information. Furthermore, rock pool species have more experience with spatially complex habitats, and in turn they may learn subsequent spatial tasks more readily. It may be that when fish recruit to their perspective habitats, they choose orientation cues and learning strategies that are appropriate to that location. Future experiments could test this by rearing fish in contrasting environments and observing the development of their orientation strategies.

Our results are consistent with the expectation that species experience a trade-off between the costs and benefits of investing in learning and memory abilities (Dukas 1999). The maintenance, operation, and production of the neural machinery required for spatial learning and memory is likely to involve quite high fitness and energetic costs (Mery and Kawecki 2003, 2004, 2005; Odling-Smee et al. 2008), which means that only animals with an ecological demand for spatial learning and memory should be actively investing in them. Although we did not test explicitly for the costs of learning here, we suggest that habitats that differ in spatial complexity are selecting for differing investments for spatial learning and memory abilities. We found that gobies living in more spatially complex habitats such as rock pools developed spatial memories of the T-maze arena much faster than species living in spatially simple sand habitats. Further support for the costs of learning can be found in a partner study that demonstrated

that gobies from rock pool habitats had a significantly larger telencephalon, an area of the brain that has been linked to spatial learning ability, than gobies from sandy shores (White 2014, chapter 6).

The results of this study indicate that turn preference and body size can also affect a species spatial learning. Practically, all vertebrates exhibit some form of turn biases or limb preference (Vallortigara and Rogers 2005; Bissaza and Brown 2011). In this context, strong turn biases interfered with the fish's ability to rapidly locate the reward, similar observations were made in poeciliids (Brown and Braithwaite 2005). Moreover, our data indicate that body size may influence turn bias. For all species, except eastern longfin goby, smaller fish appeared to have a preference for making right turns, however, no turn bias was present in larger individuals which learnt the location of the reward faster. Others studies have also noted shifts in turn biases or limb preference occurring during ontogeny. For example, both parrots and humans tend to be ambidextrous early in development before settling on a preferred hand (Corbetta and Thelen 2002; Brown and Magat 2011). Although we are not testing directly for laterality here (i.e. all our tests are cued and rewarded) our results do suggest that laterality may be influencing the gobies turning preference and hence making them appear slower to learn than they actually are. Future studies should focus on determining if these species do in fact exhibit some form of laterality by performing uncued and unrewarded turn preference tests in a T-maze.

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