

Spatial Learning and Memory Retention in Intertidal Gobies

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Total word count: 10, 172

Submitted in partial fulfilment of the requirements for the completion of a Masters in Philosophy (Masters in Research) degree at Macquarie University (2014).

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Abstract

It is well understood that an individual's learning capabilities and memory retention are strongly influenced by their environment. In a stable environment with little physical change, a longer memory span would be favoured. In contrast, a shorter memory span would be better suited in a constantly changing environment. The physical characteristics of an environment are also believed to mould what learning strategies an individual will use, such as learning the location of certain landmarks. This study investigates the learning and memory capabilities of four marine goby species found along the east coast of New South Wales. Two of these species are rock pool specialists while the other two occur in the flat sandy areas of the intertidal zone.

After a brief rest period following tagging, the gobies were introduced into a t-maze with no landmarks and trained to choose the right hand end for a food reward. Each individual was then randomly assigned to either a one month, two week or one week retrieval group to test memory retention. The results suggest rock pool species learned the task faster than sand specialists, while turn choice appears to return to random within one month in all species.

Introduction

Cognitive ecology

Cognition encompasses the ways animals receive, process, retain and act on the information acquired from their environment, forming the processes of perception, learning, memory and decision making (Shettleworth 2001). Formally coined over two decades ago (Real 1993), the field of cognitive ecology has seen an increase in studies that have helped explain the role of environmental influence on the evolution and development of an individual's brain and behaviour (Real 1993; Shettleworth 1998). In essence, an animal's cognitive ability is fine-tuned to match the niche it occupies. This occurs because there is heavy selective pressure acting on the outcomes of cognitive processes. Learning is a key part of life for all animals as they will experience change in their habitat either rarely or on a regular basis, and adapt their responses to suit temporary environmental conditions (White & Brown 2014a). Storing information from previous experiences in their environment allows an individual to respond accordingly to present challenges and provides a level of phenotypic plasticity (Giraldeau 1997; Braithwaite & Girvan 2003; Dall et al. 2005).

The ecological cognition hypothesis suggests that animal brain and behaviour are moulded by the experiences and challenges they encounter in their environment (Healy & Braithwaite, 2000). Selective pressures on cognitive ability favour animals that hone their behaviour to suit their environment, while behaviourally fixed animals will be at a disadvantage (Brydges et al. 2008). Foraging strategy is a common behaviour that animals will alter in accordance with environmental change and previous experience (Pitcher et al. 1982; Suboski 1989; Sackett et al. 1999; Braithwaite & Salvanes 2005; Laland & Janik 2006; Strand et al. 2010). For example, separate *Drosophila* larvae reared in resource rich and resource poor environments showed distinctly different foraging strategies (Vijendravarma et al. 2012). Different feeding strategies have also been studied in a population of green sea turtles which occupy two spatially dissimilar habitats (Cheng et al. 2013). These turtles have adopted either an intense foraging strategy to gain energy or a slower strategy to conserve energy. Also, Bakaloudis (2010) reported that short-toed eagles change their flight strategy during foraging trips based on wind velocity. Thus, by altering foraging strategy to suit temporary environmental changes, an individual can maximise resource intake efficiently.

Spatial learning

Learning can be defined as a change in the brain caused by information from the external environment, resulting in modified behaviour for a brief period (Broom 2010). Spatial learning and memory are two linked areas that have been intensely studied to help explain how animals are able to navigate around their environment when searching for important resources such as foraging sites, mates and shelter (Braithwaite & Girvan 2003). Up until recently, however, the majority of spatial learning studies have only investigated terrestrial animals, particularly small mammals and birds (Healy 1998; Shettleworth 2010). Caching behaviour has been of particular interest, as storing food is one of the benefits of maintaining a steady source of energy, but this is obviously only useful if the individual can recall the location of the cache. Animals will create caches when food is at its most abundant and then return to those caches in time of low food supply, sometimes even months later. Caching also seems to be heavily selected for in parenting. For example, when rearing dependent offspring, birds will travel to and from foraging areas, a behaviour that apparently preconditions the individuals to cache food (Smith & Reichman 1984). Studies have also shown birds have used adaptive strategies to hide their caches from conspecifics by moving their caches secretly (Clayton et al. 2007). Thus, caching as a foraging strategy is one that an individual would invest in not only for their own fitness, but also for reproductive success.

However in many cognition studies, only one species is investigated and the behavioural strategies reported were those adopted by individuals differing in age or experience. Kamil (1987) argued that studies focussing on one species fail to explain how environmental pressures shape the behavioural strategies exhibited by animals, arguing that a comparative approach should be used instead. In comparative studies in birds, authors explore the spatial learning capabilities in closely related species occupying different environments to elucidate the influence of environmental pressures. For example Krebs et al. (1990) reported a difference in spatial memory capabilities between two bird species with different caching behaviour. Recalling the location of food caches has obvious fitness benefits especially during the winter when food availability is low, and is believed to be the alternative strategy to storing energy as fat stores (Smith & Reichman 1984). Thus, it is a strategy seen in many

species including rats (e.g. Olton & Samuelson 1976), gerbils (e.g. Collett et al. 1986) and squirrels (e.g. Cahalane 1942; McQuade et al. 1986; Jacobs & Liman 1991).

Despite an array of comparative studies in birds and mammals, there is a notable gap in this information when it comes to fish. This trend, in part, is likely due to the once popular misconception that fish are merely vessels driven to behave in a way predisposed only by instinct, and are incapable of behavioural change (Laland et al. 2003). Cognitive studies in the past decade have now all but eradicated this stigma, and fish have quickly become a common taxa used in cognitive studies. Apart from sharing ancestral lineages with tetrapods which can reveal insight into cognitive ability across vertebrates, fish have more species than any other vertebrate group. Further, they occupy almost all aquatic niches, making them perfect candidates for comparative studies (Laland et al. 2003). The last two decades has seen the perception of fish morph from primitive and unchanging to that of a group capable of cognition likened to other vertebrates. For example, it is now widely known that they are able to form reliable cognitive maps (Reese 1989; Rodriguez et al. 1994; Burt de Perera 2004) and navigate around foraging sites with the aid of landmarks (Braithwaite et al. 1996; Salas et al. 1996; Lopez et al. 1999; 2000; Hughes & Blight 2000). Studies have also shown that fish can learn complex cues (Odling-Smee et al. 2008; Burt de Perera 2004a), locate a shelter quickly when threatened with predation (Aronson 1951, Markel 1994) and use geometric features in their surroundings to regain their bearings following disorientation (Sovrano et al. 2003). Interestingly, some authors have even likened their cognitive ability to mammals (Broglia et al. 2003) and even non-human primates (Bshary et al. 2002, Laland & Hopitt 2003, Odling-Smee & Braithwaite 2003a).

Habitat stability

Arguably one of the most important factors to an individual's survival is their ability to navigate in their environment. Navigating efficiently in their habitat allows an individual to reach essential resources by using the safest and most efficient pathways, rather than random movements that could expose them to predation or waste time and energy (Odling-Smee & Braithwaite 2003a). Selective pressures, such as environmental spatial characteristics, have moulded various strategies used to navigate successfully. For example, physically complex environments provide cues, aiding fish in forming cognitive maps to guide them. In this type

of environment, individuals are more likely to use landmarks to move between resources. On the other hand, spatially simple habitats that are homogenous in nature would offer little or no physical way to discern pathways. Also, these simple environments are often exposed to tough physical changes, such as currents, which would render any potential landmarks useless (Gibson 1999; Odling-Smee & Braithwaite 2003b). Under these conditions, individuals would have to rely on egocentric information; that is by keeping track of the turns and movements they make. For example, in freshwater habitats, ponds would represent stable environments whereas rivers, prone to flooding and changes in flow, would be considered less stable. This was illustrated in studies on sympatric three-spine sticklebacks (*Gasterosteus aculeatus*) residing in dissimilar habitats, which were trained to solve a maze. Those individuals that resided in ponds relied on physical landmarks, while those from the river habitat preferred to use egocentric cues (Girvan & Braithwaite 1998, Braithwaite & Girvan 2003). Along with spatial characteristics, habitat stability also influences what navigation strategy individuals will use.

Although all animals will cope with temporary changes in their environment through learning and memory, not all habitats exhibit the same variability. Some will be more prone to frequent physical changes, which would result in unreliable cues and would likely select for individuals to rely on egocentric information. In contrast, stable environments would show little spatial change over longer temporal scales which would allow animals to form cognitive maps that can be securely retained for long periods of time. As a result, individuals from spatially dissimilar habitats will demonstrate different learning capabilities and, in fact, animals with minimal cognitive skills are often associated with homogenous, mundane habitats (Potting et al. 1997). Thus, environmental stability plays an important role in the shaping of animals' behaviour (Biegler & Morris 1996; Brydges et al. 2008).

Odling-Smee et al. (2008) also investigated spatial learning capabilities in three-spine sticklebacks, which are especially useful in comparative studies as any difference between spatial learning capabilities can be inferred as being environmentally influenced. The pelagic individuals are found in the homogenous open water zone, while the benthic fish occupy the spatially complex lake floor. The fish were trained to solve a t-maze task relying on either egocentric information or landmarks. The authors reported that both species successfully

utilised both forms of information, though the pelagic species took double the time to learn the task. Thus, two similar species that have only recently diverged are demonstrating variation in learning capabilities that likely relates to the physical outlay and complexity of their habitats.

Learning is an essential skill for the survival and reproductive success of any animal, and memory capability also encompasses how well an individual learns. In basic terms, learning is a two-stage process; first an organism encounters a problem and sets out to find a temporary solution. The second part of the process forms a memory of this solution. Thus, when the problem is encountered again, reliable knowledge from previous exposures will enable the organism to perform the solution better than the previous time (Staddon 1981, Giraldeau 1997, Braithwaite & Girvan 2003, Dall *et al.* 2005). Without forming some memory of an interaction, an animal cannot learn, thus, any experience would be pointless. Memory in fish has already been shown across a wide variety of species occurring in different environments. For example, fish have been shown to avoid places where they had previously encountered dangerous situations (Czanyi & Doka 1993), approach alternative sides of an apparatus for a food reward (Williams *et al.* 2002) and successfully solve a maze in absence of continual training (Schluessel & Bleckmann 2012). The array of literature supporting the role of environmental stability on learning also extends to memory, and there is increasing evidence that predictability moulds memory retention rates (Warburton 2003). For example, homogenous habitats prone to physical change would require individuals to update spatial information often, and so would likely select for animals with shorter memory retention. In contrast, animals occupying a stable habitat where changes are infrequent would benefit from retaining spatial information for longer.

Ware (1971) reported rainbow trout (*Oncorhynchus mykiss Walbaum*) retain a conditioned reaction to prey for at least 14 days after training to approximately 3 months, after which reactions wane back to naivety. On the other hand, 15 spined sticklebacks (*Spinachi spinachia*) will show naïve levels after just 8 days (Croy & Hughes 1991). Mackney & Hughes (1995) investigated the memory retention in the context of food manipulation in three stickleback species found in marine, estuarine and freshwater habitats. The test fish were kept on frozen food for two months to decrease stimulation exposure then

exposed to live food again for ten consecutive days. During this time they learned to manipulate soft oligochaetes, which are soft and easier to handle and amphipods which are faster and have a tough exoskeleton. After fish were exposed to live food, they were deprived of stimulation once more for varying times and fed frozen mysid. When given amphipods after the deprivation period, the authors reported that both marine and estuarine species foraging efficiency diminished to naïve levels after 8 and 10 days, respectively. In contrast, the freshwater species showed consistent foraging efficiency even after 25 days. Thus, the difference between the foraging efficiency and memory retention appears to be an adaptive response to prey type, availability and also to their respective habitats.

Recently, Yoshida et al. (2013) investigated anticipatory behaviour in an intertidal goby species *Tridentiger trigoncephalus* in a tide-simulated task for a food reward. The gobies were confined to small tanks with a raised platform. When a simulated high tide was run, the platform would become submerged and the gobies would be able to access a food reward. After a few trials the gobies would approach the platform as the tide was rising. Once this behaviour was exhibited, the gobies were kept in separate tanks with no further exposure to the platform apparatus. The authors then reported this behavioural display even one month after the training period. Thus, these gobies appear to react to tidal changes as cues for investigative behaviour, as well as retain information about reliable foraging sites for long periods.

Learning and memory is especially important in the lives of animals occupying highly variable environments, as they will need to gather new information and alter their response frequently (White, 2014). An example of such a variable environment is the intertidal zone; a habitat lining the continental shelf which undergoes two tide cycles daily. It is also an environment with varying spatial complexity, from the bare sandy beaches to the more complex rock pools. Such contrasting habitats offers a unique opportunity to study the cognitive abilities of fish living there. Species in the family Gobiidae are frequently found across all habitats in the intertidal zone. Further, spatial learning is particularly important for rock pool specialists which exhibit homing behaviour, returning to home rock pools before low tide (White & Brown 2013). Thus, these individuals will require a thorough and reliable way of recalling the location of a pool suitably deep enough to avoid desiccation (Yoshiyama

et al. 1992). This family provides excellent candidates for comparative spatial learning studies, as species share some common morphological traits whilst occupying an array of different habitats. Despite this, there are only few cognitive studies on this family, and only a fraction of them have been comparative in nature.

The frillfin goby (*Bathygobius soporator*) is a species that is reported to occur in both spatially complex and simple habitats. In fact, both observational and genetic studies have suggested that the species may be going through the first stages of speciation (Tavolga 1950; Lima et al. 2005; de Lima-Filho 2012). Aronson (1951; 1971) studied the jumping ability of this species, reporting that agitating the water in rock pools sometimes encouraged the gobies to leap into neighbouring pools. Sometimes the gobies would even jump between pools in sequence until they reached open water. As this was at low tide, when adjacent pools were not connected by water, the gobies had no way of seeing out of their home pool. Thus, they must have been relying on a cognitive map to keep track of the location of surrounding pools. In the second study one half of the test gobies were permitted an overnight stay in the test pools, while the other half were kept inexperienced. The author reported incredible levels of accuracy in jumps, 95% in experienced individuals compared to 15% in naïve individuals.

Markel (1994) investigated the spatial learning capabilities in Black eye gobies (*Coryphopterus nicholsi*) by controlling the amount of time test fish were allowed to explore a test arena. Treatment individuals were given an extra 5 hours to become accustomed to the area, which included a single burrow for shelter. When threatened with predation, the treatment fish found the shelter significantly faster than naïve individuals. However when the burrow was changed, the treatment fish performed as they had in original tests. On the other hand, naïve fish showed little change across all tests. These results suggest that the treatment fish successfully learned the location of the burrow within the test arena.

White & Brown (2014b) were first to investigate spatial learning capabilities in intertidal goby species using the comparative methods. Two of the species (*Bathygobius cocosensis* and *Bathygobius krefftii*) commonly occupy rock pools, actively foraging during high tide and returning to a home rock pool at low tide (White & Brown 2013). In contrast,

the two sand specialists (*Favonigobius lentiginosus* and *Istigobius hoesei*) are commonly found along the relatively bare sandy shores. These sand gobies generally move towards the shore at high tide then retreat to deeper water at low tide. The gobies were kept in captivity and, when tested to locate a deep rock pool during a simulated low tide cycle, rock pool specialists exhibited strong spatial learning and high levels of return rates. Sand specialists, on the other hand, exhibited minimal or no homing behaviour and were stranded repeatedly. Thus, different capabilities observed in similar species from spatially dissimilar habitats are deduced as being environmentally influenced.

White & Brown (2014a) also showed that rock pool fish tend to rely on landmarks for navigating in comparison to sand-dwelling species that tend to rely on a mix of cues. The authors trained the same goby species from different habitats to solve a t-maze using either landmarks or egocentric cues. As expected, the rock pool specialists solved the maze faster and with fewer errors compared to the sand specialists. Interestingly, they were able to use both landmarks and egocentric information while the sand specialists preferred egocentric cues. These results support that behaviour, including cue choice, is heavily influenced by environmental stability and spatial complexity.

Here, I replicated this experiment using the same study species and apparatus but in the absence of landmarks in the hope that by doing so it would give the sand-dwelling species an advantage, since the gobies were required to use egocentric information to solve the task. Specifically, I addressed the following questions

- 1) Can intertidal gobies from rock pools solve a t-maze without the aid of landmarks?
- 2) Do sand and rock pool gobies differ in the time taken to solve the maze?
- 3) For how long do intertidal gobies retain egocentric navigational information?
- 4) Do the species differ in task memory retention?
- 5) Is there a correlation between body condition and learning/memory?

I hypothesised that all species would learn to solve the maze but in the absence of landmarks, sand-dwelling gobies should solve the task significantly faster and with fewer errors. I also hypothesised that all species would retain the information for some time, but the

rock pool species would show greater memory capabilities compared to the sand specialists owing to the stability of their rock pool environment. I also predicted that smaller individuals would learn to solve the maze faster.

Methods

Study animals

Despite the different environments they inhabit, the test gobies used in this study do share some common morphological traits well adapted to life in the intertidal zone. They are small in size (mean length = 4.2cm), have eyes placed high on the head, display cryptic colouring well suited to sand and rock habitats and have a well-developed circular pelvic fin which behaves like a suction cup (White & Brown 2013). This adaptation stabilises the gobies to the substrate and decreases the likelihood of them being washed away by waves (Kuitert 1996). They also display quite cryptic behaviour, seeking shelter under rocks and boulders. Four species of intertidal gobies were collected from two distinct environment types in the Sydney region of NSW, Australia, between April and May 2014. Two sandy shore specialists: Eastern longfin goby *Favonigobius lentiginosus* (Richardson, 1844) and Hoese's sandgoby *Istigobius hoesei* (Murphy & McEachran, 1982) were collected using a 30mx1.5m seine net (3mm mesh) and hand held nets in Chowder Bay. The other two species: Cocos frillgoby *Bathygobius cocosensis* (Bleeker, 1854) and Krefft's frillgoby *Bathygobius krefftii* (Steindachner, 1866) were collected using hand held nets at low tide along the rocky reefs of Dee Why beach and Chowder Bay. The number of fish captured at any one time varied between groups of 3 to 10, after which they were transported in a large, aerated bucket (15 litres) to the laboratory based at Sydney Institute of Marine Science (SIMS) in Chowder Bay. In all instances the transportation time was less than 30 minutes. A total of 12 individuals per species were collected (n = 48).

Once in the lab, the gobies were separated into species type and placed into one of four 52L black plastic tubs (64.5 x 41.3 x 27.6cm) kept in the seawater facility at SIMS. Fresh seawater kept at ambient temperature was run from Chowder Bay through a 200um filter into large tubs in the lab. From here, the water was distributed to the tubs, each running on a drip filter (2L/minute) and an outlet fitted with 25mm PVC piping for the water to escape. The

outlet was covered with a piece of 3mm mesh to ensure no gobies escaped, while still allowing adequate water flow. This mesh was rinsed twice weekly to avoid blockages and potential overflow in the tubs. As these goby species are from surrounding areas, no additional heating was provided. Water temperature were recorded daily (Serenity digital Thermometer) for the duration of the project and lighting was kept to 8 hours daily under full UV spectrum lights. Maximum water level was approximately 1.2cm from the lip of the tub. Due to the jumping nature of the gobies, a large (65 x 42cm) plastic grid (1cm x 1cm) was placed on each of the tubs to keep them from escaping.

The tubs housing the two sandy shore species had a fine sand substrate, while the tubs with the rock pool species had substrate of larger shell grit pieces. To encourage the gobies to utilise artificial shelters, four 12cm halves of 25mm white, non-reflective PVC pipes were also placed into each tub. The gobies were fed every second day a mixed diet of live copepods and amphipods as well as frozen brine shrimp *Artemia sp.* Water changes were also carried out once a week to maintain water quality. Once introduced into the lab, the gobies were allowed a one month rest period to settle, during which time I monitored their eating habits and their overall behaviour. These gobies were caught in compliance with NSW Fisheries (permit no. P08/0010-3.0) and kept under conditions approved by the Animal Research Authority (ARA ref. no. 2014/003) (Appendix A, page 40).

Tagging

One month after introduction into the tubs, the gobies underwent light anaesthesia (50mg/L MS222) for between 30 – 60 seconds and tagging using Visible Implant Fluorescent 113 Elastomer tags (VIE: Marine Technology, Inc. 2008) for unique identification. This tagging procedure took approximately 2 minutes per goby and all fish recovered within 2 minutes. All individuals were measured using calipers and weighed, and the results recorded. This was to investigate whether learning performance and memory retention were correlated to body condition. Once tagged, each individual was again placed into their holding tub and given another full week of recovery. During the recovery period overall behaviour and feeding habits were monitored closely.

Test apparatus

The test apparatus and procedures used in this experiment were based on those used previously by White & Brown (2014a). Two replicate cross form mazes with four arms were each submerged into a rectangular tub (100x50x17.8cm) of aerated seawater. These mazes were made from 3mm PVC plastic and were lined with a coarse shell grit substrate. A small (10x16cm) plastic screen could be removed and slid into any of the arms in the maze, producing a t-maze (Figure 1). This screen was moved at the beginning of each trial to establish a different outlay within the maze. The middle bar of the t-maze acted as the starting point to each trial. Plastic screens were also placed 10cm from the end of the other two arms to the left and right of the starting arm. These screens had a small (2x4cm) opening at the base for the gobies to swim through to reach the junction point. The end of both left and right arms had a small, glass dish (3cm in diameter x 1.5cm deep) and shelter constructed from a piece of halved 25mm white, non-reflective PVC (10cm). The shelters were the same as those in the holding tubs and were used to help reduce stress to the gobies as a result of exposure. A video camera was mounted above each of the mazes and the behaviour of the fish recorded onto a hard drive.

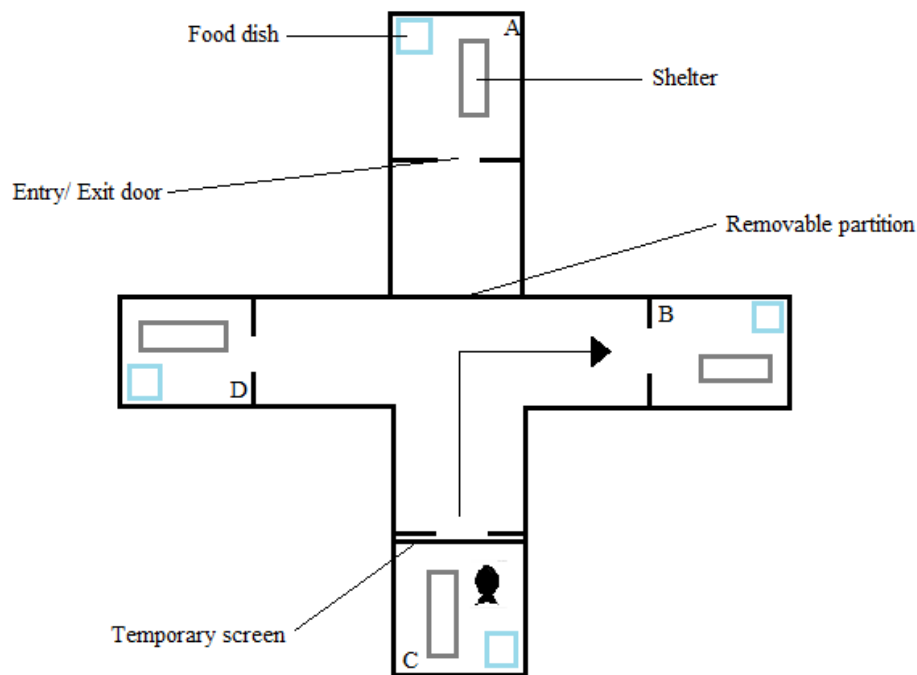


Figure 1: Layout of the spatial learning task. The letters indicate how three trials would begin in sequential start boxes starting from C. The arrow indicates the correct path a fish would need to follow in order to obtain a food and shelter reward.

Procedure

Groups of fish were given access to the maze for 24 hr to familiarise themselves with the layout, during which there were shelters in the maze but no food. After the familiarity session they were returned to their housing tanks, where they were fed live amphipods and allowed to rest for a further 24 hrs until testing began. Four individuals of each species were randomly selected and allocated to a test group. From these groups, a single goby was placed in the starting arm of the t-maze. After a 3 minute interval the start box was opened by sliding the solid partition upwards and the fish was free to explore the maze, the objective being to locate the reward arm. If the test gobies chose the correct arm, they were rewarded with the shelter as well as 2 individual brine shrimp delivered from a clear 3ml pipette into the clear food dish. Food was supplied only after completing the task in order to minimise chemical cues in the water. In the event a goby chose the wrong arm, a removable partition (10x16cm) was used to close off the correct arm to stop the goby from going back to get the reward. Apart from no food reward, the shelter in the wrong arm was fitted with clear plastic on either end, making it inaccessible. The goby would be kept in the wrong arm for 3 minutes, then gently encouraged to the correct arm where they would receive the food reward and available shelter.

Both arms, regardless of their food reward or lack thereof, were made to look identical so the gobies could not discern them from physical appearance. Apart from the dishes and shelters which were hidden behind the doorways, the maze was otherwise devoid of any physical objects that could be used as a cue. After a 15 min period the maze was reset: the reward arm became the start box and the opposite arm blocked off. The fish was then retested. In this manner the fish could only rely on turn direction to solve the maze. Each individual goby was trialled three times daily for 15 consecutive days (i.e. a total of 45 trials). In some cases, an individual would require encouragement to leave the start box to complete a trial. If this was necessary, the goby would be gently ushered out of the start box, which would then be partitioned to deny access back in. The gobies were given the first 6 days of trials to move on their own accord, after which they would be allowed 10 minutes before encouragement. All individuals were given a maximum of 30 minutes to choose a side, after which if no choice was made, they were given a fail score. If no choice was made, or if an individual

returned to the start box, they were then gently ushered into the correct box and given a food reward. To maintain water quality and reduce the effect of chemical cue residue from the food rewards, two thirds of the water in test tanks was changed before trials began each day. The substrate was also disturbed each day.

After the training period, all gobies were randomly assigned to one of three groups for memory retention trials. Some fatalities occurred between the training and memory trials; 3 *I. hoesei* and 3 *F. lentiginosus* perished before they could undergo memory trials. These treatment groups comprised 4 individuals of the rock pool specialists and 3 of the sandy shore specialists. Each species was broken down into three test groups were tested at one, two or four week intervals. Due to time constraints, the group of fish that underwent the learning trials first were assigned to the one month re-test group while groups tested later were assigned to the one and two week re-trials. During this period all the individuals were held in the housing tubs, fed every day but had no reinforcement training in the t-mazes. Thus, 14 individuals (ca 3 and 4 per species) were tested after one week, another 15 at two weeks and the final 15 at four weeks. During these tests, the original 30 minute time allowance was eliminated, thus the trial would only end when the individual chose a side. After these final trials, all test individuals were released at the original capture sites.

Statistical Analysis

All data were tested for normality prior to analysis. Any data displaying heterogeneity were log transformed to achieve a normal distribution. A one-way analysis of variance (ANOVA) was used to determine significant differences between performance times both between species over the 15 days of trials. This was followed by a post-hoc analysis to test for significance in time improvement within species throughout the training period. During the learning task in the experiment, the gobies were given one out of three possible scores depending on how they performed: 1 if they chose the correct side, 0.5 if they made no choice or 0 if they chose the incorrect box. Each goby performed 3 trials per day, from which an average was given for each of the 15 days they were tested. The gobies were also timed in every trial from the time they left the start box to the time they chose either the left or right box. The average of three daily trials were calculated and an ANOVA used to determine significance between turn choices over the 15 day training period. Average decision times

were also calculated over the 15 day trial period and analysed using a repeated measure ANOVA.

The second part of the experiment involved trialling each goby once, timing the trial from start to end and noting what direction the goby chose. The treatments were the periods of time allocated to each test group; one week, two weeks and one month. An ANOVA was carried out to test for significant differences in turn choice between treatments and between species. The relationship between each goby's weight and length was also graphed and the residuals used to calculate body condition. That is, whether an individual was under, normal or overweight for their length. Body condition was then used as a factor within and between species when compared to the success rates in both the learning and memory trials. An analysis of covariance (ANCOVA) was also carried out with species as the main effect and length of the individuals as the covariate. The memory data were analysed using a binomial generalised linear model. All analyses were carried out using Excel 2010 and StatView 5.0.1 (SAS Institute Inc.).

Results

Spatial learning task

During the trials, gobies showed varying degrees of boldness with some individuals leaving the start box immediately whilst others would approach the exit only to retreat back to the shelter. Many individuals, while exhibiting fast, deliberate motion, kept to the edges of the maze where there was some shadow caused from the lighting. Upon choosing the correct arm, many individuals swam immediately to the shelter and only investigated the food reward several minutes after the trial ended. During the trials, the gobies presented significantly different times to complete the task (ANOVA: $F_{3, 41} = 2.833$, $P = <0.002$) (Figure 2), with both rock pool species solving the maze significantly faster than the sand-dwelling species (ANOVA: $F_{1,43} = 4.067$, $P = <0.0009$). Between these groups, *B. cocosensis* demonstrated significantly faster times than *B. krefftii* (ANOVA: $F_{1,23} = 4.279$, $P = 0.0012$) while *I. hoesei* solved the maze significantly faster than *F. lentiginosus* (ANOVA: $F_{1,18} = 4.414$, $P = <0.0001$).

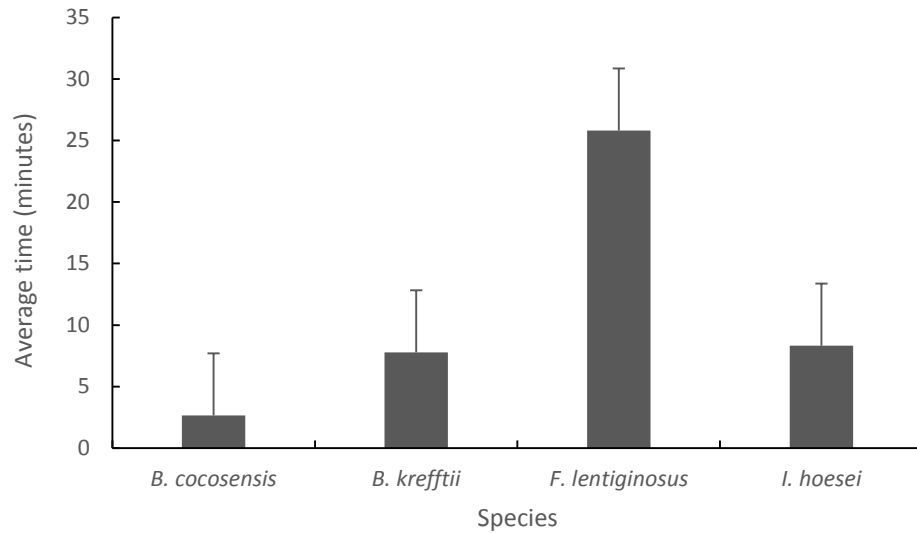


Figure 2: Mean (\pm SE) performance time taken to solve the maze per species.

There was a significant difference between the number of correct turns on average made between species (ANOVA: $F_{3,42} = 2.827$, $P = 0.0003$) (Figure 3). In contrast to the hypothesis, the rock pool species performed more correct turns than the sand species (ANOVA: $F_{1,20} = 4.351$, $P = 0.0004$) (Figure 4). Both rock pool species performed similarly (ANOVA: $F_{1,23} = 4.279$, $P = 0.067$) but there was a significant difference between correct turns performed by sand species, with *F. lentiginosus* choosing the correct arm more often than *I. hoesei* (ANOVA: $F_{1,19} = 4.381$, $P = 0.0001$). A post-hoc test for average scores across the 45 trials showed *B. cocosensis*, *B. krefftii* and *F. lentiginosus* all performed significantly better than *I. hoesei* ($P = 0.0002$, $P = 0.0001$, $P = 0.0181$, respectively), however, there was no significant difference between *F. lentiginosus* and rock pool specialists (*B. cocosensis* $P = 0.0822$, *B. krefftii* $P = 0.061$).

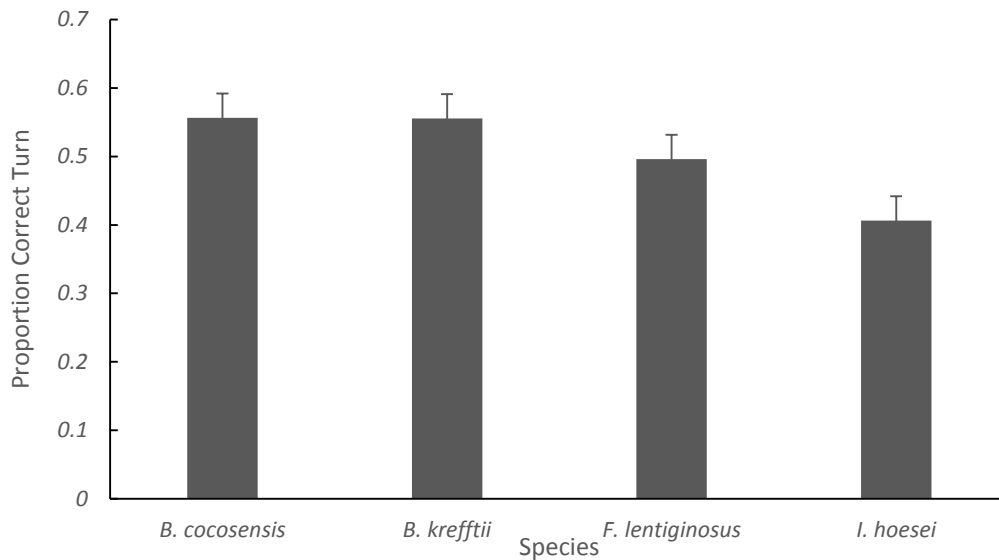


Figure 3: Mean (\pm SE) turn score achieved across 45 trials per species.

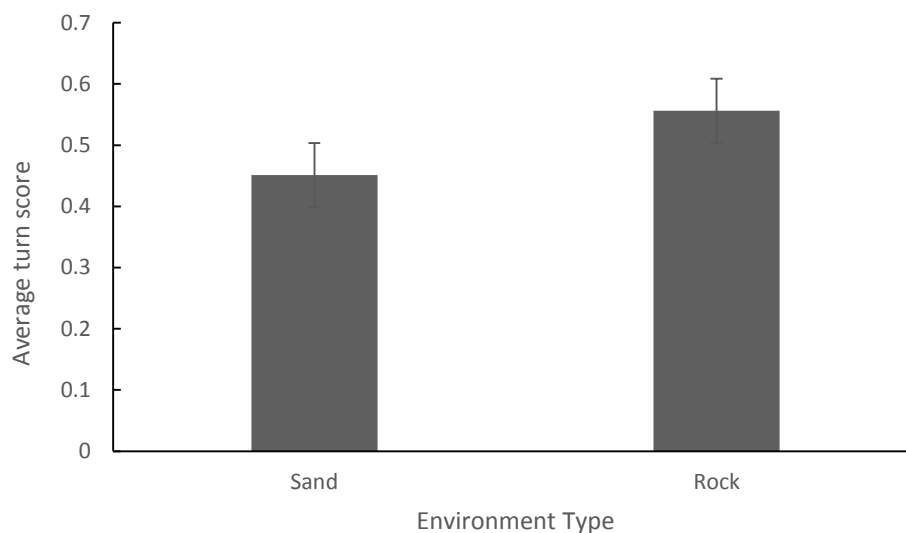


Figure 4: Comparison of mean turn score (\pm SE) between groups based on habitat types. Turn score is based on the average results accumulated in 3 trials over 45 days from the scoring system of 0, 0.5 and 1. Rock pool specialists and sand-dwelling species were grouped separately.

All species performed significantly more correct turns as time progressed (rmANOVA: $F_{3,42} = 5.719$, $P = 0.0022$) (Figure 5). A post-hoc analysis showed all species performance in turn choice improved significantly between day 1 and days 10 to 15 ($P = <0.02$ across all species). Further, there was a significant difference between performance in

both rock pool species and *I. hoesei* (*B. cocosensis* $P = 0.0013$, *B. krefftii* $P = 0.0006$). Rock pool species showed similar performance trends ($P = 0.8253$) as did the sand-dwelling species, though marginally ($P = 0.0596$). There was also a significant increase in correct turns within species over the 15 day period (ANOVA: $F_{14,42} = 6.280$, $P = <0.0001$). Furthermore, tests showed significant difference between average decision times between species (ANOVA: $F_{3,41} = 2.833$, $P = <0.0001$). *B. cocosensis* showed significantly faster decision times than all other species (*B. krefftii* $P = 0.0028$, *F. lentiginosus* $P = 0.0001$ and *I. hoesei* $P = 0.0027$) while *F. lentiginosus* showed significantly slower times (*B. krefftii* $P = <0.0001$, *I. hoesei* $P = <0.0001$).

Memory task

A comparison of turn choices was carried out between species using a Chi squared test, which showed no significant difference between species and chosen side ($P = 0.996$) or between species and treatments ($P = 0.940$) (Figure 6). A binomial generalised linear model showed no significant difference between species choice in the one month treatment. However, in the two week treatment, *F. lentiginosus* chose the correct side significantly more often than *B. cocosensis*, *B. krefftii* and *I. hoesei* ($P = 0.046$, $P = 0.001$, $P = 0.0014$, respectively). During the one week treatment there was a significant difference between the correct turns made by rock pool dwelling species, with *B. krefftii* choosing the correct side more often than *B. cocosensis* ($P = 0.046$). However, there was no significant difference between sand species and side chosen during the one week treatment ($P = 0.221$).

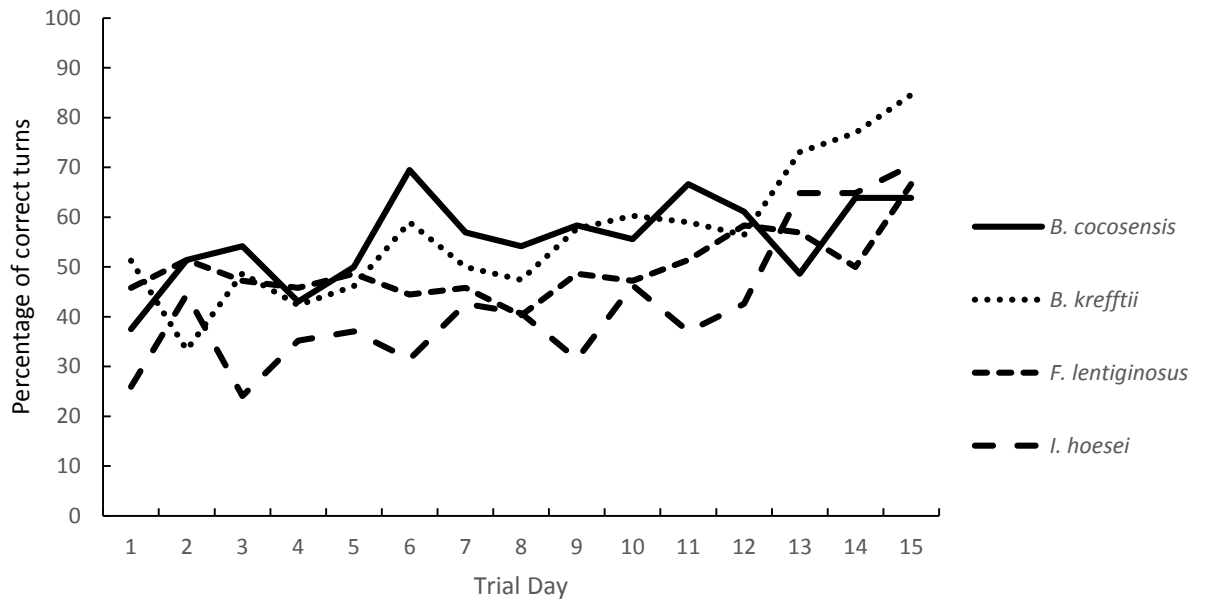


Figure 5: Percentage of correct turns performed per day on average per species, across the 15 trial days.

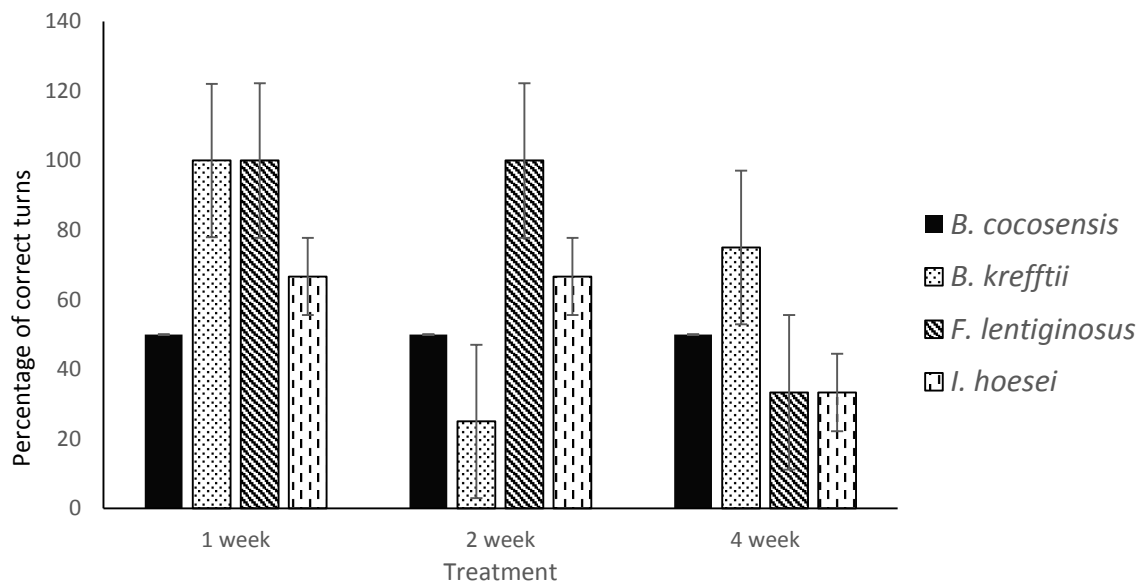


Figure 6: Percentage (\pm SE) of individuals turning in the correct direction (i.e. the same as training) during the memory retention trials.

Body condition

In the learning trial data, individuals that were lighter in weight for their length performed significantly better during the learning trials (ANCOVA: $F_{1,38} = 10.51$, $P = 0.0025$) (Figure 7), though this was only a significant trend in three of the species, with *I. hoesei* being the exception (Figures 8a – d). In contrast, individuals that were heavier for their size performed slightly better in memory trials, but not significantly so (ANOVA: $F_{3,34} = 2.502$, $P = 0.074$).

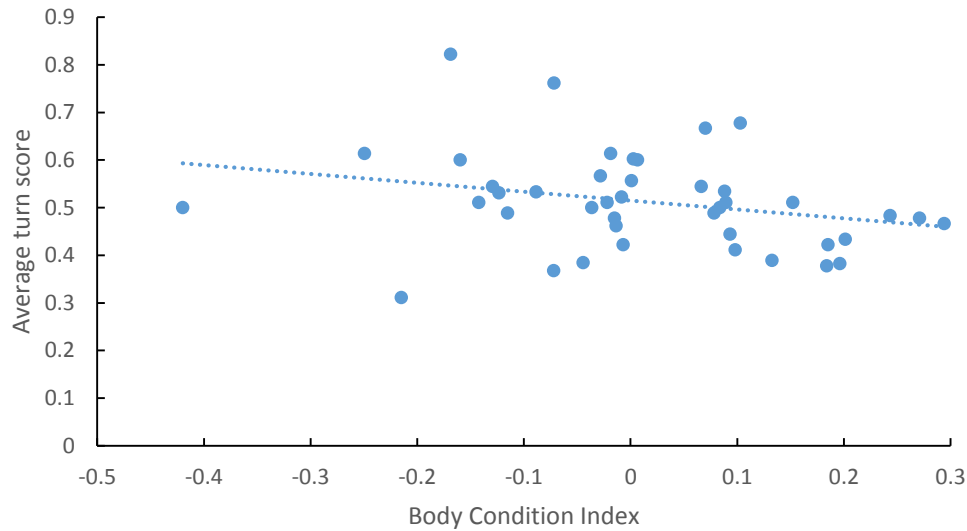


Figure 7: Body condition index and average turn score of each individual.

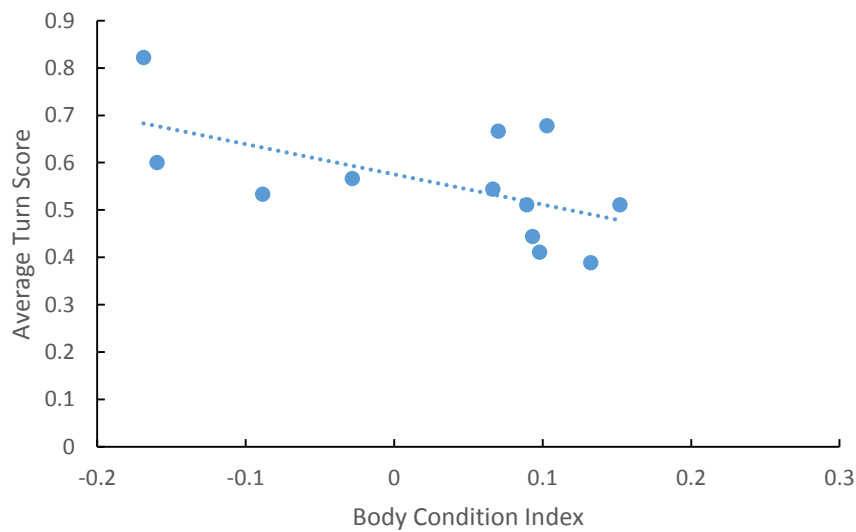


Figure 8a: Body condition index and average score turn for rock pool species *B. cocosensis*.

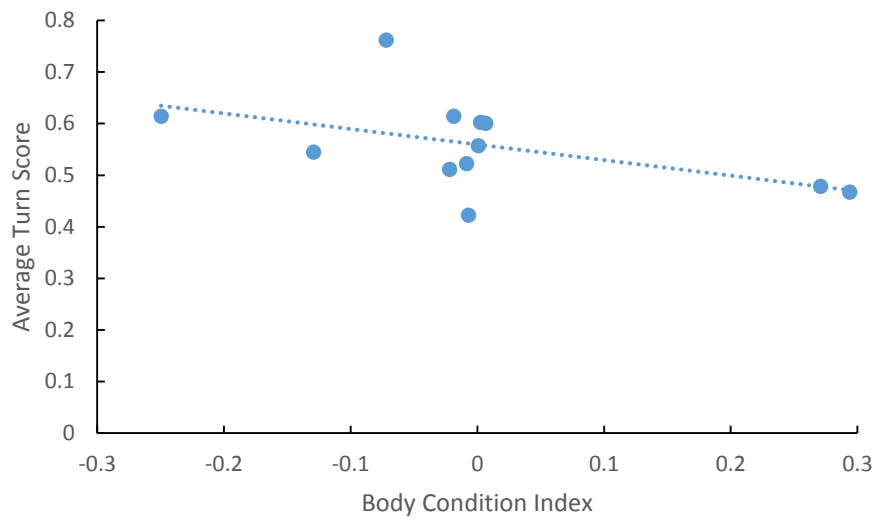


Figure 8b: Body condition index and average score turn for rock pool species *B. krefftii*

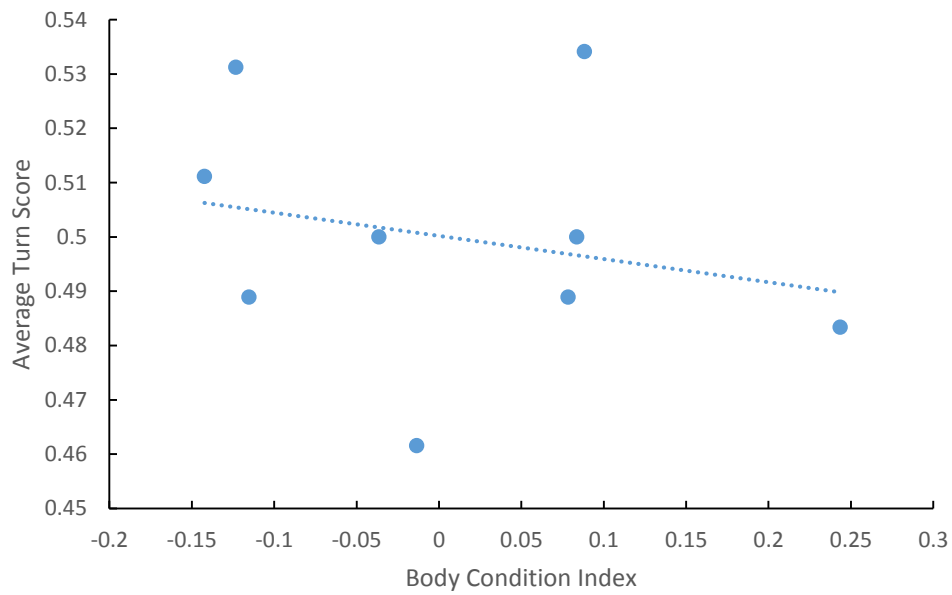


Figure 8c: Body condition index and average score turn for sand-dwelling species *F. lentiginosus*.

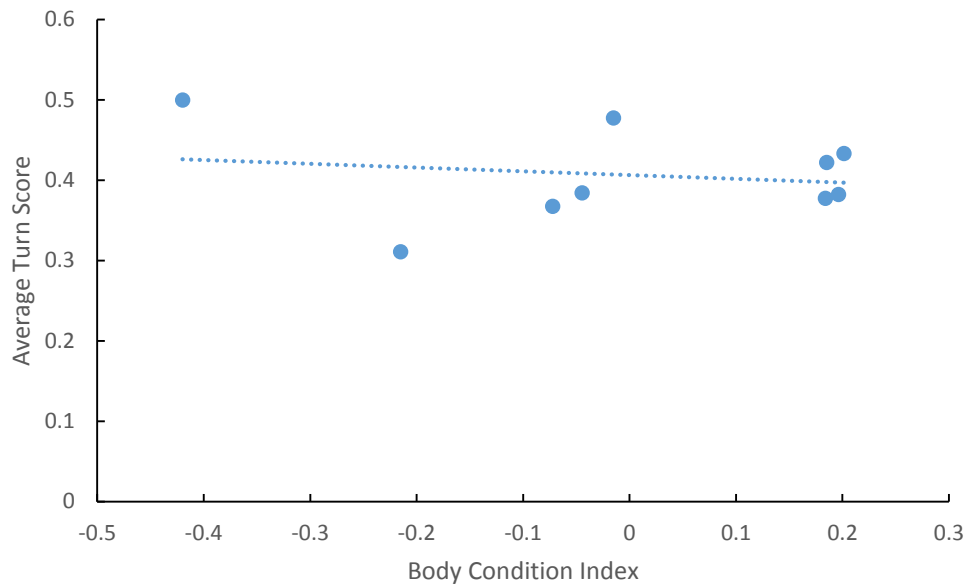


Figure 8d: Body condition index and average score turn for sand-dwelling species *I. hoesei*.

Discussion

Spatial learning

There is increasing evidence to support environmental pressures play an important role in developmental learning across taxa (Odling-Smee *et al.* 2008). For example, individuals inhabiting spatially complex environments would probably have greater spatial learning abilities compared to individuals living in more spatially simple environments. This is already a trend seen in birds, particularly those that demonstrate caching behaviour. It is also a trend seen in fish which have become a common taxon used in cognitive studies in the last decade. Before this, fish were commonly viewed as vessels surviving purely on instinct with no learning capacity (Laland *et al.* 2003). This archaic view is now recognised and an increasing number of studies agree that fish are capable of complex cognitive processes and are not low level vertebrates as previously suggested. For example, Warburton (2003) argues that the learning processes across all vertebrate taxa have far more similarities than differences. Further, some authors have compared problem solving skills in fish to those found in non-human primates (Bshary *et al.*, 2002, Laland & Hoppitt 2003). Fish are the most ancient of the extant vertebrate lineages, are more numerous than any other vertebrate taxa and occupy almost all aquatic environments. There are also many examples of sympatric

species, which occur when a single species diverges into two without the aid of geographical isolation (Dieckmann & Doebeli 1999). Thus, fish are excellent candidates for comparative studies investigating cognitive ecology.

The ecological cognition hypothesis suggests that animal brains and their behaviour are moulded by the experiences and challenges that they encounter while living in their environment (Healy & Braithwaite, 2000). Within the spatially complex rock platforms of the intertidal zone, gobies rely heavily on recalling the pathways to and from home rock pools to avoid becoming stranded at low tide (White & Brown 2013). Even within their home pools, rock pool specialists likely recall features and hiding spots accurately, particularly to hide from predators (Markel 1994; Burt de Perera & Guilford 2008) and return to guard nest sites (White & Brown 2014a). In contrast, sandy shore habitats usually lack visual cues and are subject to physical change which would make landmarks too unstable to supply reliable information. Thus, sandy shore species would likely use another form of navigation strategy to access foraging sites and shelter. Further, stable environments with little physical change would not require constant updates of spatial information, so it is likely that fish living here would exhibit longer memory retention than sand species.

Throughout the last decade, the relatively new field of cognitive ecology has seen an enormous increase in studies explaining cognition in the lives of vertebrates. It is now widely understood an individual's developmental behaviour and memory retention capabilities are strongly influenced by the environment they occupy. Environments that are spatially homogenous and prone to large scale physical changes will help form different behaviours compared to animals residing in complex, stable habitats. In contrast to the expected outcomes, the rock pool species made fewer errors than the sand dwelling species, despite the maze lacking any landmarks. Though the use of cues and preference in turn choice may be dependent on individual experience, the trend suggests that natural selection in context of their dissimilar habitats is in play. Thus, their performance shows a divergence in cognitive ability; rock pool and sandy shore environments are selecting for different learning abilities.

Though the sand-dwelling species made significantly more errors than the rock pool species, this could be as a result of their transient behaviour towards particular areas, with no

obvious fidelity to a particular locations. Though they will readily use shelters if made available to them, the time spent there is always brief and their positions are always changing. Murdy & Hoesei (1985) described the movement of these sand specialists when fleeing danger, most of which involve erratic movements to the nearest shelter or burying themselves under the sand. This apparently opportunistic escape behaviour is in contrast to the rock pool specialists, who can recall exact locations of shelters (Markel 1994; Burt de Perera & Guilford 2008) and exhibit strong homing behaviour to a particular pool (White & Brown 2013). Further, while the majority of the apparatus used here was usually well lit, some spaces caused slight shadows, particularly behind the partitions and between the maze wall and the shelters. In fact, several *B. cocosensis* individuals in this study approached the partitions and perched themselves vertically with their tails pointing down to the substrate. Also, it was common to see *B. krefftii* individuals move towards the reward arm by staying close to the path edges at the base of the maze walls. These darker areas may have been more noticeable to the rock pool species, given that under natural conditions they will actively search for shadowed areas to hide in (White & Brown 2013). Dodd (2000) reported shannies actively seek out these darker areas, possibly mistaking them for crevices or overhanging rock.

The formation and maintenance of spatial learning abilities and memory retention have survival value but are costly for neural mechanisms, thus we expect that only animals that depend on them for survival would invest in them (Mery & Kawecki 2003, 2004, 2005; Odling-Smee et. al. 2008). Rock pool species would have especially reliable memories as demonstrated by their homing behaviour, because losing track of their home pool may lead to them wrongfully choosing a shallow pool and risking desiccation (Yoshiyama et al. 1992; White & Brown 2013). Thus, rock pool species should invest more heavily in spatial learning and the maintenance of their cognitive map than sand dwelling species, which do not display homing behaviour. This would certainly explain why both rock pool species performed well in the learning trials and also why in previous studies have shown they will use both landmarks and egocentric cues (White & Brown 2013).

Homing to the correct pool twice per day obviously has great survival value to the rock pool specialists, thus it would appear that by using multiple forms of information to navigate, these species would decrease the likelihood of possibly fatal errors. Also, *B.*

cocosensis individuals caught for this study were found in closely spaced rock pools which are connected during high tide only. They were also found in larger pools with a shellgrit substrate and large boulders scattered throughout. This habitat is also joined to the smaller rock pools at high tide but much of the substrate joining the two becomes exposed at low tide. Thus, it may be that during foraging trips, *B. cocosensis* will leave the smaller rock pools towards the larger, more exposed pools. By using a combination of strategies to orient themselves, these individuals might navigate in the larger pools using egocentric information then revert to landmarks when closer to their home rock pool. This would be supported by previous which studies have shown that when a navigational challenge with multiple solutions is encountered, an animal may use multiple orientation strategies together to solve it (Etienne et al. 1990; Able 1993; Collett and Zeil 1998; Odling-Smee et.al. 2008).

On the other hand, sand dwelling species do not show homing behaviour but merely move forward towards the shore at high tide, and away from the shore to deeper water at low tide. Thus, they do not risk desiccation so the survival value of spatial learning and memory retention is lower than in rock pool specialists. This was certainly the trend observed in this study, with sand species making significantly more errors in their turn scores compared to their rock pool counterparts. During this study, *F. lentiginosus* individuals were the only species to demonstrate cryptic behaviour by burying themselves in the sand, while *I. hoesei* individuals darted quickly in a zig-zag motion toward the reward arm. Further *I. hoesei* individuals would use the shelter provided more often than *F. lentiginosus*, suggesting that they may rely on hiding spots more than burying behaviour.

During the learning trials of this study, a food reward was one of the incentives for the test gobies to choose the reward arm of the maze. Although it is assumed that all individuals were equally motivated by food, many individuals appeared to show greater interest in shelter instead. During the preliminary trials when the gobies were introduced to the layout of the maze, many individuals sought the first shelter they found and stayed there for some time. Thus, rather than simply giving them one reward, the shelter in the left arm of the maze was blocked off on both ends with clear, plastic partitions. When they approached this shelter, many of the test fish would swim vigorously against the plastic in attempts to get inside. Most of the gobies, particularly in the early days of the training period, would then turn their

bodies, leave the incorrect box and seek out accessible shelter in the correct arm. This was not always the case for individuals of the rock pool species *B. cocosensis*, many of which would attempt to access the blocked shelter then simply swim over the top of it and rest on the food dish. Thus, it appeared that some gobies found the shelter to be more of an incentive than the food, though it seems likely this is individual personality rather than species specific.

Variations in diet are also likely to affect the spatial learning abilities of these gobies which exhibit different foraging strategies and prey capture tactics. Rock pool gobies actively forage for mobile prey including amphipods, isopods and polychaetes (Randall & Goren 1993) which may require greater spatial learning abilities to recall where these invertebrates hide. Furthermore, it is vital that the paths these gobies follow are the most advantageous in terms of predator avoidance and high coverage. On the other hand, sand species feed by sifting mouthfuls of sand through their gill rakers and filter out any infauna (Myers 1999). Though the sand gobies may have a system to avoid depleted patches, feeding while stationary is unlikely to cost much in terms of neural machinery. Also, as *F. lentiginosus* in particular will commonly hide under sand, they probably won't need to invest as much time learning the pathways to shelters in order to escape predators. Furthermore, *F. lentiginosus* individuals took longer to solve the maze over the 15 day trial period than all other species and also took the longest time per trial even in the final days of the training period when their times had improved. Though this may be explained by the difference in complexities of their respective habitats, the difference in foraging and motivation as related to their diet may have also been in play.

Reproductive behaviour in gobies has some similarities but also obvious differences, particularly in their nesting preferences. Males will prepare and guard nests on the substratum (Thacker 2011) but rock pool specialists will seek out sites in protected areas in small caves, beneath rocks and between crevices (Tavolga 1954; Taru et al. 2002). In contrast, sand species will nest on open sediments and sometimes among shell fragments (Hernaman & Munday 2007; Rowe et al. 2008). Thus, we expect that sand species won't require spatial learning abilities to return to their nest sites as much as rock pool species, which likely navigate to nests in the same way they navigate to foraging sites.

Fish will navigate in their environment by the processes and cues made available to them, and there is increasing evidence that habitat type has strong selective pressure on the cues that fish will use for maximum navigation efficiency. Sensory systems make up an organism's arsenal of survival tactics, and this along with spatial learning assists with the formation of cognitive maps. For example, the blind Mexican cave fish (*Astyanax fasciatus*) cannot follow visual cues to navigate, instead they encode information into their spatial map through their lateral line by the wave perturbations caused as the fish moves closer to objects (Burt de Perera 2004b). Further, Braithwaite & Girvan (2003) investigated spatial learning in three-spine sticklebacks which reside in ponds and rivers, with only water flow direction as the only reliable orientation cue. Though all test individuals were able to solve the task, those from the river habitat learned the task twice as fast compared to pond individuals. Further, when the test fish were given a choice between landmarks or flow cues, the river population preferred flow direction while the pond population showed a preference for landmarks.

The results of this study support previous investigations reporting that rock pool species will readily use a mixture of cues to solve spatial tasks (White & Brown 2014a). While this is not to say that the sand gobies will not use landmarks, it appears they rely mostly on egocentric information. However, the expected outcome was that the sand species would learn the maze faster than the rock pool species, due to the nature of bare, sandy shore habitats. However, it should be noted that *I. hoesei* chose the correct side significantly more often than *F. lentiginosus*, but significantly less often than both rock pool species. Further, *I. hoesei* demonstrated significantly faster times than *F. lentiginosus*, however both rock pool species still solved the maze faster than both sand-dwelling species. This may be a representation of shelter preference in *F. lentiginosus*, which will often bury themselves in the sand. In contrast, *I. hoesei* generally move in quick, successive movements towards a large boulder or rock crevice. Thus, though rock pool species demonstrate enhanced spatial learning abilities, differences between species occupying the same areas should not be discounted.

The preference of spatial cues between rock pool and sand species may be from genetic predisposition or varied experiences in their respective environments, but the likelihood is a combination of both. Even though all these species are genetically distinct, the

rock species are more closely related to each other than they are to the sand specialists, and vice versa (Thacker & Roje 2011; White & Brown 2014a). Thus, it is expected that cue choice in these species evolved alongside their phylogeny, especially as selective pressures for cue choice would vary across environments. Alternatively, it may be phases during early development that determine preferred cue choice (Odling-Smee & Braithwaite 2003b). For example, spatial learning studies on homing pigeons found different rearing environments influenced cue preference (Braithwaite & Guilford 1995). Those pigeons raised with visual access to the surrounding environment were more likely to use landmarks for navigation than their siblings raised in an environment with opaque windows. Further studies could test this by raising sand dwelling gobies in a spatially complex habitat, or rock pool species in a simple habitat, and observing their preference of cues for navigation as adults.

Studies on sympatric populations of three-spined sticklebacks (*Gasterosteus aculeatus*) show support for habitat stability influencing cue preference. These occur together in several lakes but reside in dissimilar microhabitats. The pelagic population reside in the homogenous open water zone while the benthic population are found on the spatially complex benthos. Interestingly, when given the task of solving a t-maze, both populations did so successfully by using both landmarks and egocentric cues, but with differing results. The pelagic population from the open water zone took twice as long to learn the task compared to their benthic counterparts (Odling-Smee et al. 2008). Thus, this suggests fish living in a complex environment will have greater spatial learning abilities than those in a mundane environment. It also suggests fish will have spatial learning abilities refined to their respective microhabitats.

Cue stability is also an important aspect in successful orientation; the more unreliable a landmark is, the less likely it is that an animal will utilise it to navigate (Brydges et al. 2008). The notion of habitat stability influencing cue choice is supported by several studies investigating similar species, or sometimes sympatric populations of the same species, and their cue preferences. For example, ponds and rivers are considered to different habitats in terms of their stability; rivers, prone to flooding and currents are less stable so any potential landmarks acting as cues would quickly be washed away. In contrast, landmarks in ponds are likely to remain in the same location on smaller temporal scales. The influence of these

habitats on cue choice has been investigated in three-spine sticklebacks trained in a maze with different cues to find the food reward. Individuals from stable ponds preferred to use landmarks while those individuals from river habitats preferred body turn cues (Girvan & Braithwaite 1998; Braithwaite & Girvan 2003). Similarly, Panamanian bishops (*Brachyraphis episcopi*) occupying different river systems were investigated for cue preference. Those individuals sampled from unstable rivers relied on global cues to successfully navigate, while fish from the more stable rivers relied on local cues (Brown & Braithwaite 2005). Thus, gobies raised in an environment with minimal or no landmarks would be more likely to use another strategy, such as egocentric (i.e. information based on body centred methods of their turns).

Memory task

Many components play an important role in shaping the behaviour and memory capabilities of animals, including habitat stability. Though many studies recognise memory in single species (e.g. Brown 2001; Schluessel & Bleckmann 2012), comparative studies are providing insight into the role that habitat stability plays in learning and memory. For example, Brydges et al. (2008) investigated memory retention rates in three-spine sticklebacks sampled from rivers (unstable environment) and ponds (stable environment). Interestingly, the fish from the unstable habitats displayed a longer memory window (> 1 week) than their pond-dwelling counterparts (< 1 week).

It was expected that the rock pool gobies would demonstrate longer memory retention rates due to the differences in environmental stability. In comparison to sandy shores, rock pools are sturdier and less affected by everyday elements such as wave action and so provide a more stable habitat (White & Brown 2014a; 2014b). Homing is certainly an important aspect in the lives of rock pool specialists and so maintaining their cognitive map to reach their pools safely should be an important investment. This, combined with residing in a relatively stable environment with infrequent change, should allow for longer memory retention because individuals wouldn't need to update the information about their surroundings as often. However for rock pool gobies, the cost of maintaining a cognitive map may be less than constantly updating the information from their surroundings. If this is the

case, the gobies would benefit from constantly updating their cognitive map as the benefit of returning to the correct pool safely would outweigh the costs.

In contrast to rock pools, sandy shore habitats are spatially simple with few landmarks or outstanding physical cues. In addition, it is an environment constantly affected by tides and wave action, so if any landmarks (such as small rocks light enough to be carried by wave action) are available, the exposure to wave action will render them unreliable (Gibson 1999). Thus, for the sand dwelling species, which occupy an exposed environment with minimal landmarks and demonstrate no homing behaviour, maintaining their cognitive map would likely produce more costs than benefits. As a result of the more stable nature of their environment, it was predicted that the rock pool species would retain the information gained in the learning trials for longer, and thus choose the correct side more frequently than sand species. Surprisingly, the sand species performed similarly to (and in some cases better than) the rock pool species, particularly in the one and two week treatments.

The results for the one month treatment were as expected; neither of the species performed well, suggesting that any spatial information gathered is probably outdated and overwritten with new information. Despite the expected outcome for the other two treatments, the results indicated *F. lentiginosus* chose the correct side significantly more than all other species. In the one week treatment, one sand species *F. lentiginosus* and one rock pool species *B. krefftii* showed a one hundred percent success rate. There was a significant difference between the two rock pool species, with only half of *B. cocosensis* individuals choosing the correct side. This may be related to the slight differences between collection sites of these two species. While *B. cocosensis* individuals were collected from a large pool with a shellgrit substrate, *B. krefftii* individuals were samples from rock pools embedded on a large rocky platform with large shell fragments as a substrate. While *B. cocosensis* showed consistent results across the three treatment periods, *B. krefftii* showed a decrease in performance as the time lapse increased, though not significantly so.

The sand species showed a significant difference in performance in the two week treatment, with *F. lentiginosus* choosing the correct side more often than *I. hoesei*. However both species performed similarly in the one week trial and no significant difference was

found. Although the results follow the prediction that the rock gobies would retain the information longer, I also expected that *I. hoesei* would choose the correct side more often than *F. lentiginosus* in the memory trial just as they did during the learning period, which was not the case. Like the rock pool goby *B. krefftii*, both sand species demonstrated the general trend of gradually decreasing success as the time period between training increased.

Body Condition

Despite all test gobies being fed daily and not subject to any food deprivation whilst in captivity, physiological data showed variations in length and weight relationships. The results showed body condition was significantly correlated to an individual's learning success in the maze, though only in 3 of the 4 species. Those individuals that weighed less than they should have for their length, scored higher correct turn averages and also higher average performance percentages over 15 days. This trend suggests that physical condition motivates an individual and that these underweight gobies were more driven to the reward than healthy gobies. To my knowledge, there is currently no publication linking body condition in fish to their success in spatial learning tasks. However, food deprivation is often a motivator used in spatial learning tasks involving rats and mice. Typically, individuals will be reared in resource rich or resource poor environments to investigate differences in time or trials needed to solve a task. (e.g. Görisch & Schwarting 2006).

In contrast to the success in learning trials, the memory trials showed the opposite effect between body condition and turn success. Those individuals that were heavier for their length performed better than lighter individuals, though not significantly. This trend may represent a species specific relationship between overall body condition and memory retention rates. White & Brown (2013) reported that body size had no effect on homing ability in the four goby species tested. This is in contrast to results found by Yoshiyama et al. (1992) who investigated homing behaviour in three intertidal sculpin species, which are also found along the intertidal zone. The authors reported that larger individuals in two of the species were more successful at homing than smaller individuals. However, field research dictates that these individuals were already experienced in the test environment, but the gobies in this investigation were all introduced to previously unexplored territory.

To conclude, spatial learning abilities and the preferred cues for navigation vary greatly between species occupying contrasting habitats. As predicted by the ecological cognition hypothesis, the cues an individual will choose to use to successfully navigate depends greatly on the environment it inhabits. Complex environments that are stable in nature will select for landmarks as a cue while homogenous, unstable habitats will select for egocentric cues. The results reported here support the hypothesis that gobies living in complex habitats have enhanced capacity for spatial learning and are more likely to use a multitude of information due to the importance of returning to safe home pools. In contrast, gobies living in mundane habitats that exhibit no homing are more likely to rely on egocentric cues due to the unstable nature of sandy shores.

The memory trial results suggest short term memory in sand species which gradually wane back to levels typical of naive fish. The rock pool species showed mixed results of good short term memory in one species and non-significant results in the other species. Thus, it may be that the latter species invests more in constant update of their cognitive map rather than maintenance of old information. The results reported here add to the ever growing body of literature that learning techniques for navigation and memory capabilities vary greatly among species from contrasting habitats. Future studies should investigate the influence of both predisposition and environmental pressures by raising rock pool gobies in spatially simple environments and sand species in complex environments.

Acknowledgements

I owe many thanks to my principle supervisor Culum Brown for invaluable guidance, assistance and inspiration throughout the length of this project. I would like to thank Gemma White for providing such a stable beginning and also taking the time to review my work and provide such helpful feedback. I'd like to acknowledge Dr. Ulysse Bove and Josh Aldridge from SIMS for their assistance in running this project. Thank you also to those who volunteered their time to assist me in collecting gobies; Nathan Bass, Timothy Ghaly, Andrew Irvine and Natalie Rodriguez. Finally, thank you to each individual goby that helped me piece together this research project; I trust you will find your way home once more.

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Appendix A: Ethics approval form.



ANIMAL RESEARCH AUTHORITY (ARA)

AEC Reference No.: 2014/003-2

Date of Expiry: 20 February 2015

Full Approval Duration: 21 February 2014 to 20 February 2017 (36 Months)

This ARA remains in force until the Date of Expiry (unless suspended, cancelled or surrendered) **and will only be renewed upon receipt of a satisfactory Progress Report before expiry (see Approval email for submission details).**

Principal Investigator:

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In case of emergency, please contact:
the Principal Investigator / Associate Investigator named above, or
Animal Welfare Officer - 9850 7758 / 0439 497 383

The above-named are authorised by MACQUARIE UNIVERSITY ANIMAL ETHICS COMMITTEE to conduct the following research:

Title of the project: Spatial Learning and Memory Retention in Fish

Purpose: 4 - Research: Human or Animal Biology

Aims: To examine how long fish from contrasting environments retain spatial information

Surgical Procedures category: 3 - Minor Conscious Intervention

All procedures must be performed as per the AEC-approved protocol, unless stated otherwise by the AEC and/or AWO.

Maximum numbers approved (for the Full Approval Duration):

Species	Strain	Age/Sex	Total	Supplier/Source
23 – Fish	Intertidal gobies	Mature adult / Any	240	Wild
		TOTAL	240	

Location of research:

Location	Full street address
SIMS	Building 19, Chowder Bay Road, Mosman NSW 2088
Biology Sea Water Facility	Building E8C, Macquarie University, North Ryde, 2109
Fauna Park	209 Culloden Rd, North Ryde, 2109

Amendments approved by the AEC since initial approval:

1. Change transport and capture methods (Exec approved 8 May 2014, ratified by AEC 15 May 2014).

Conditions of Approval: N/A

Being animal research carried out in accordance with the Code of Practice for a recognised research purpose and in connection with animals (other than exempt animals) that have been obtained from the holder of an animal suppliers licence.

Professor Mark Connor (Chair, Animal Ethics Committee)

Approval Date: 15 May 2014