

# Cognitive and behavioural plasticity in the intertidal Cocos Frillgoby (*Bathygobius cocosensis*)

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## STATEMENT OF CANDIDATE

I certify that the work in this thesis titled “**Cognitive and behavioural plasticity in the intertidal Cocos Frillgoby (*Bathygobius cocosensis*)**” has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree to any other university or institution other than Macquarie University.

I also certify that the thesis is an original piece of research and it has been written by me. Any assistance that I have received in my research or preparation of the thesis itself has been appropriately acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

The research presented in this thesis was approved by Macquarie University Ethics Review Committee, reference number: **ARA# 2014/003** beginning from **20/2/2014**. Fish collections were conducted under NSW Fisheries Scientific Collection Permit number: **P08/0010-4.6**.

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## **Statement on the contribution of others**

This thesis includes collaborative work with my principal supervisor Professor Culum Brown, as well as Joo Myun, Troy Gaston, Vincent Raoult and Jane Williamson for Chapter 2. My role in this collaboration was literature reviews, general experimental design, sample collection, preliminary data analysis and interpretation. Joo Myun and Vincent Raoult conducted data analysis in Chapter 2. Co-author Professor Culum Brown provided analysis guidance and editorial assistance in other chapters.

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## General Abstract

The age-old dichotomy of nature versus nurture continues to spark debate in the context of plastic behaviour, and not just when it comes to humans. Every year there is further evidence illustrating how individual experience shapes personality, behaviour and cognitive ability, all of which are fundamental aspects of an individual's phenotype. Ultimately, experience is intricately linked to an individual's environment, and numerous studies have focused on population differences in behaviour. Key explanatory variables include variation in predation, competition and habitat stability/physical complexity. Despite their evolutionary history being embedded in ancient lineages, and the associated popularist idea that they are 'simple and primitive,' fishes demonstrate highly flexible behaviour. Moreover, they have become one of the leading vertebrate taxa in behavioural plasticity studies using comparative approaches, not least because of the wide range of aquatic habitats they occupy.

In marine coastal environments, the intertidal zone is a highly dynamic habitat and home to one of the largest and most successful groups of fishes (>2000 spp), the family Gobiidae. This family is well-adapted to the intertidal zone morphologically and behaviourally, both aspects of which differ widely in species occupying different micro-habitats. Their sheer diversity offers exciting opportunities for comparative studies which attempt to untangle the relative influence of genes versus experience in shaping behaviour.

Despite the diversity of intertidal gobies, niche overlap is common and competition for resources plays a vital role in behaviours such as foraging, suggesting that behavioural plasticity would be beneficial when securing resources. Furthermore, food resources in the intertidal zone vary on temporal and spatial scales, so that individuals with flexible behaviour can adjust to these changes and thus reap the fitness benefits. Chapter 2 of this thesis focuses on the niche overlap of two sympatric goby species, one rockpool specialist and one sand

specialist, and how this overlap changes seasonally and ontogenetically. In addition, the trophic niche of a third allopatric species was investigated, to determine how diet changes seasonally and ontogenetically in the absence of interspecific competition. In the sympatric species, we found a seasonal switch in diet complexity, where the sand species consumed a variety of prey taxa in winter but not summer, and vice versa for the rockpool specialist. In contrast, the allopatric species showed similar diet complexity across seasons but shifted toward a specialised diet later in ontogeny.

Seasonal change has been tied to variation in cognition, whereby changes in cognitive function are linked to reproductive demands and can differ dramatically between sexes, depending on life-history strategies. Although this has been demonstrated in several mammalian species, few have investigated sexually dimorphic cognitive ability in fishes, and none in the context of reproductive strategy. Rockpool gobies demonstrate exceptional cognitive function by way of spatial learning ability so Chapter 3 focused on the male nest-guarding mating system in the intertidal goby *Bathygobius cocosensis* and how it influenced male and female cognitive abilities in a spatial learning task in each season. Males and females performed similarly in all seasons except spring, which marks the breeding season in this species. Males showed a substantial decrease in cognitive ability while females did not. I suggest that the decreased cognitive ability observed in males during the breeding season is linked to their reproductive strategy; males are site-attached whilst they guard their nests and forgo foraging excursions. This study highlights the importance of cognitive plasticity and how individuals manage the trade-off between costs and benefits associated with enhanced cognition over relatively short temporal scales.

The ecological cognition hypothesis suggests that an individual's brain and behaviour are greatly influenced by environmental characteristics such as stability and predictability. However, whether the plasticity of these aspects is finite or otherwise constrained by



inherited genetic mechanisms shaped by evolutionary pressures over multiple generations, remains unexplored in gobies. To that end, Chapters 4, 5 and 6 examined environmental drivers of spatial learning ability, anti-predator behaviour and laterality in gobies, using a wild-captive comparative framework. Previous studies have shown that rockpool gobies possess superior spatial learning abilities compared to sand specialist gobies, associated with the selective environmental pressures of living in a structurally complex habitat. However, whether this ability is fixed and innate, or flexible and shaped by experience, remains unknown. In Chapter 4, I reared juvenile gobies in 4 different habitats that varied in the degree of physical complexity and trained them to solve a simple spatial learning task. I found that gobies reared in structurally complex habitats solved the task faster than those reared in the simpler regimes, suggesting that, although spatial learning ability may have an innate component, life experience shaped by environmental heterogeneity continues to alter learning ability in later ontogenetic stages.

In addition to controlled manipulation of the physical environment, captivity also allows researchers to regulate the social environment, such as predation pressure. Wild gobies were captured as adults with experience in assessing visual and olfactory cues to ascertain predation risk and altering their behaviour accordingly, while captive gobies were captured as juveniles and reared in the absence of predation risk. In Chapter 5, wild and captive-reared gobies were exposed to a series of cues from a sympatric predatory crab species and their anti-predator behaviours observed. In addition, I paid close attention to correlations between behaviours which may indicate population-level behavioural syndromes which, in other taxa, are most often manifested in high-predation contexts. Captive-reared gobies showed little differences in behaviour, regardless of cue treatment, although larger individuals generally spent less time moving in the presence of the predator. In the wild population, large individuals spent less time moving than smaller individuals, and gobies exposed to olfactory

cues were less active than those in visual and control treatments. The relationship between activity and size emphasises the importance of body size in risk-related behaviour and the influence of captive-rearing on animal behaviour more broadly. As predicted, behavioural syndromes were only observed in wild fish when exposed to olfactory cues (olfactory cues alone or in combination with visual cues) emanating from predators, which aligns well with the existing literature.

Previous studies have shown that behavioural differences in gobies from contrasting environments are mirrored in brain morphology, exemplified by larger telencephala in rockpool specialists compared to sand specialists. The question remains whether laterality, the preferred use of one brain hemisphere over another when assessing information, is similarly influenced by habitat complexity. Existing literature suggests that laterality is influenced by both habitat complexity and predation pressure. Chapter 6 focused on whether gobies exhibit population-level laterality and if differences exist between captive and wild populations. Trials were conducted using a mirror test, where the body position of gobies was observed, and eye use preference recorded. I found no evidence of population-level laterality in either group, although there was a tendency toward stronger lateralisation with increasing size, indicating laterality remains plastic throughout ontogeny. Moreover, this data supports the existing literature which suggests that population-level laterality occurs most often in highly social species.

In summary, the research outlined in this thesis emphasises the plasticity of behaviour in a species that occupies a complex and dynamic habitat, and how the extent of this plasticity can be altered with controlled manipulation of environment in early ontogeny. It also highlights the strengths of a comparative framework, particularly when captive experience is improved with environmental enrichment to encourage natural behaviours in fishes.

# **CHAPTER 1**

## **General Introduction**

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Historically, debate has been widespread as to whether patterns of learned behaviour are via nature or nurture. Although it is widely accepted that cognition is influenced by both factors, many arguments maintain a preference for one or the other. Notable works such as Lewontin, Rose and Kamin's *Not in Our Genes* (1984) argue that the cause of intelligence cannot be quantified by our genes, and this must surely indicate the work of environmental influence. In contrast, works like Herrnstein and Murray's *The Bell Curve* (1994) and the latter's *IQ Will Put You in Your Place* (1998) suggest that the overriding component of one's cognition is based on genetic makeup formed through natural selection (Pigliucci, 2001). The nature argument often quotes studies using twins, reasoning that cognitive traits are more similar between identical twins than they are between fraternal twins or unrelated individuals, and that this must be due to identical genomes. In response, the 'nurture' side points out that in none of those cases is the confounding effect of environment (i.e. that twins are raised in the same environment and, thus, endure similar circumstances) accounted for.

When considered alone, the nature side of the argument comes up short, given that the intricacies of cognitive systems are in many ways like biological systems, with multiple external sources influencing the outcome (Hutchins, 2010). Just as biological functioning is interpreted based on an organism's relationships with other organisms and its surrounding environments (Turner, 2009), so too must the understanding of cognitive systems account for environmental influences and processes. In a brilliant thought experiment, Bateson (1972) noted the following:

*Suppose I am a blind man, and I use a stick. I go tap, tap, tap. Where do I start? Is my mental system bounded at the handle of the stick? Is it bounded by my skin? Does it start halfway of the tip of the stick? But these are nonsense questions. The stick is a pathway along which transforms of difference are being transmitted... If what you are trying to explain is a given piece of behaviour, such as the locomotion of the blind man, then for this purpose, you will need the street, the stick, the man... and so on* (1972: pp. 459; Form, Substance and Difference).

The pioneering minds behind cognitive and behavioural sciences throughout the 19<sup>th</sup> Century each added a vital element to the understanding of combined forces of nature and nurture, instilling the importance of, not only heredity, genes and instinct, but also experience, culture, development and imprinting on the formation of human behaviour (Ridley, 2003).

#### **Cognitive sciences: An overview**

Comparable cognition between man and animal was an implausible notion long into the 20<sup>th</sup> century (Ridley, 2003). Renowned philosopher and scientist René Descartes reasoned that animals were like machines, incapable of perception or feeling (Harrison, 1992). Notable supporters of Charles Darwin such as Alfred Russel Wallace (co-founder of the theory of natural selection), and psychologist William James drew the line at cognitive continuity; the human brain was just too complex to be comparable to that of animals' (Ridley, 2003). Nonetheless, Charles Darwin's ideas had begun a curiosity ripple which would reach his student, champion of modern comparative psychology, George Romanes. Prior to publishing his work *Animal Intelligence* (1883), Romanes began to collect evidence from pet owners, zookeepers and naturalists for examples of learned behaviour in animals. He suggested a comparative approach where animal intelligence could be matched to that of a human of a particular age (Budiansky, 1998). The idea was rejected by psychologist Edward Thorndike, who proposed that domesticated animals had learned their various tricks by accidental repeated exposure. Other psychologists agreed with him, even going so far as to assume animal behaviour was simply reflexive with no conscious thought: an opinion which would permeate through scientific communities and the general public by the mid-20<sup>th</sup> century (Ridley, 2003).

Towards the end of the 20<sup>th</sup> Century, no single discipline had managed to monopolise the study of animal cognition; psychology, anthropology, biology, philosophy, cognitive sciences and others, were all involved in the effort to comprehend cognitive processes in animals (Andrews, 2016). In 1973, zoologists Konrad Lorenz, Nikolaas Tinbergen and Karl von Frisch were awarded the Nobel Prize for their work on individual and social patterns in animals. This proved to be a huge step for animal cognition research, as it led to the official sanction of ethology, the comparative study of behaviour in an environmental and evolutionary framework (Thomas, 2003). Nikolaas Tinbergen's four-question framework (1963) began to pave the way in understanding why animals behave the way that they do, and in a way that would placate ecologists, psychologists and biologists alike: survival value (what is it for?); ontogeny (how does it develop during an individual's lifetime?); evolution (how did it evolve during the history of a species?) and causation (how does it work?). As appreciated now as they were then, these questions provide a complementary outline to fully comprehend a biological trait (Bateson and Laland, 2013; Nesse, 2013). Still, animal cognition continued to expand and integrate a variety of theoretical perspectives, leading to the emergence of subfields such as cognitive ethology (Griffin, 1978), behavioural ecology (Krebs and Davies, 1987), comparative cognition (Wasserman, 1993) and cognitive ecology (Real, 1993).

Although not entirely gone (e.g. Premack, 2007), the human exception in cognition has become nearer to obsolete as each subfield attempts to unravel cognitive continuity from a 'building block' perspective rather than a myopic fixation on complexity (de Waal and Ferrari, 2010). With an ever-increasing interest in the field, regardless of the title, it comes as no surprise that cognition has shifted from being a 'logical process' to a 'biological phenomenon' (Hutchins, 2010).

## Phenotypic plasticity: The merging of genotype and environment

Genotype and phenotype, although theoretically grasped by Mendel, weren't formerly named until 1911 (Johannsen). The quest to quantify the relationship between the two, sometimes referred to as the *genotype-phenotype mapping function* (Alberch, 1991), has been followed ever since. Mendel's initial mapping function was relatively simple: one gene controls one aspect of phenotype. But it wasn't long before it was realised that most traits do not follow this simplistic model (Provine, 1971). Woltreck's (1909) ground-breaking work showed that *Daphnia* clones changed morphologically in successive generations, a pattern he called *reaction norms*. This clearly showed that a genotype can produce a continually variable phenotype correlated to environmental conditions. However, it wasn't until the 1980's that the notion of environmental effects on phenotype began to gather momentum. In new models, the dichotomy of genes and environment was replaced by a fluid interaction between the two, where genes respond to environmental contexts with one of several phenotypes (Pigliucci, 2001; Sarkar, 2004). This interaction is further influenced during development, where genetic and environmental information are extracted, and specific phenotypes are portrayed as a result. Importantly, plasticity can itself be considered a phenotypic trait (Williams, 1966; Pigliucci, 2001), meaning environmental pressures select for plasticity to maximise fitness (adaptive plasticity hypothesis; Dudley and Schmitt, 1996). This then leads to the question; in what environment would phenotypic plasticity be advantageous?

Put simply, phenotypic plasticity is the genotype's ability to alter phenotype for the purposes of surviving variability (Bradshaw, 1965; Schlichting, 1986), which some environments exhibit more than others. Thus, one can extrapolate that the more variable the environment, the greater the selective pressure for plasticity, even between closely related species (Houston and McNamara, 1992). For instance, larvae of three closely related frogs

(*Hyla* spp.) metamorphose in variable aquatic habitats, from permanent swamps to temporary puddles. The two species from larger aquatic habitats metamorphose at different sizes depending on the temperature, however, the species from ephemeral pools shows no such plasticity in size between temperatures (Blouin, 1992). Perhaps the most renowned example of interspecific plasticity is the adaptive behaviour of caching in bird species as a response to unpredictable food supply (e.g. Pravosudov, 1985; Krebs and Davies, 1997). Importantly, plasticity can be induced naturally or artificially, allowing researchers to exaggerate environmental variability. For instance, shell growth of the intertidal gastropod *Littorina littorea* decreases when individuals are exposed to low pH/high temperature environments formulated in predictive models (Melatunan et al. 2013). Likewise, snakes reared in enriched captive environments demonstrate superior growth, habituation and problem-solving than counterparts reared in standard conditions (Almli and Burghardt, 2006).

Phenotypic plasticity is not only variable between species or populations; studies investigating within-population plasticity have found abundant genetic variation in natural populations, suggesting that plasticity responds to natural selection (Scheiner, 1993). Early works by Dobzhansky and Spassky (1944) showed genetic variation in viability in response to temperature increase and population density in *Drosophila pseudoobscura*. Viability was favoured in several combinations of temperature (intermediate/low) and density (high/intermediate/low) by different genotypes. Most importantly, however, no single genotype was superior to others across all temperature/density combinations. Populations of caching birds (*Poecile atricapilla*) that experience harsh winters and unpredictable food availability demonstrate superior numbers/recovery of caches, spatial memory and hippocampal volume compared to populations that experience milder winters (Pravosudov and Clayton, 2002). Therefore, as long as an environment exposes populations to variable



126 biotic and abiotic factors, no genotype will be singled out as superior and natural variation  
127 will continue in the population.

128         Despite a surge of interest in vertebrate cognition in the last five decades or so, fishes  
129 remained beneath the ‘automata’ banner for much longer than our mammalian cousins.  
130 Whether restrained by their evolution from ancient lineages or the ideology that they have  
131 remained ‘unchanged,’ fishes are undoubtedly the most underestimated vertebrate group.  
132 Having first appeared some 500 million years ago, fishes have not only diversified into  
133 numerous forms that outnumber all other vertebrate groups combined but have also radiated  
134 to occupy almost every aquatic environment (Brown et al. 2011a). In occupying a variety of  
135 habitats, fishes have evolved a gamut of morphological and behavioural adaptations specific  
136 to each niche, reflected by changes in underlying neural processes and machinery (Brown et  
137 al. 2011a; Patton and Braithwaite, 2015). The extent of these specific adaptations has been  
138 investigated with increasing diligence in recent decades, leading to a greater understanding of  
139 cognitive ability in fishes, as well as the gradual preference for fishes as an ideal model in  
140 vertebrate cognition research (Vila Pouca and Brown, 2017).

141         One needs only to skim recent reviews on the subject to find impressive and  
142 comparable cognitive feats in fishes, including navigation, numeracy skills, spatial learning,  
143 long-term memory and associative learning, among others (Brown et al. 2011a; Bshary and  
144 Brown, 2014; Patton and Braithwaite; 2015). Considering the metabolic cost in maintaining  
145 neural machinery and its processes, it is assumed that ecological requirement for such  
146 abilities must play a vital role in the fitness and survival of individuals. The ecological  
147 cognition hypothesis suggests that the environment an animal inhabits, and the obstacles it  
148 faces, plays a critical role in an individual’s cognitive ability and associated neural machinery  
149 (Dukas, 1998; Healy and Braithwaite, 2000). For instance, piscivorous African cichlids that  
150 actively hunt their prey possess a larger cerebellum and better developed optic tectum

compared to species which prey on sessile taxa (Huber et al. 1997). Similarly, fishes that inhabit structurally complex environments possess larger brain structures, namely the cerebellum (e.g. Pollen et al. 2007) and telencephalon (van Staaden et al. 1994; Kotrschal et al. 1998; Burns et al. 2009). Thus, selective pressures for neural processing appear to be more demanding in complex environments.

As a means of successful navigation, the spatial adaptation theory (Gaulin, 1992; Sherry et al. 1992) proposes a correlation between spatial ability, brain morphology and structurally complex environments. In most aquatic environments, landmarks shift spatially and temporally, so fishes have had to develop different navigational strategies (Odling-Smee et al. 2008). An innovative study by Aronson (1951; 1971) found that intertidal gobies (*Bathygobius sorporator*) could leap blindly between pools to avoid predation at low tide, suggesting the gobies formed a cognitive map of their environment during high tide, allowing them to navigate towards a goal regardless of their position (Broglia et al. 2011). A key example of navigation in fish is that of Pacific salmon (*Oncorhynchus* spp.) which migrate thousands of kilometres to spawn in their natal streams (Neave, 1964; Dittman and Quinn, 1996). Other studies report navigation via landmarks in the reef cardinalfish (*Pterapogon kauderni*; Kolm et al. 2005), rockpool resident gobies (White and Brown, 2014a) and sticklebacks from pond habitats (Girvan and Braithwaite, 1998; Odling-Smee and Braithwaite, 2003), all of which originate from a stable and structurally complex environment. By contrast, sticklebacks from fast flowing rivers and gobies from intertidal sandflats rely on egocentric cues rather than potentially unreliable landmarks (Girvan and Braithwaite, 1998; Odling-Smee and Braithwaite, 2003; White and Brown, 2014a). A different approach has evolved in the blind cavefish (*Astyanax fasciatus*), which learn the outlay of their dark environment using tactile cues from water displacement (de Perera, 2004). Thus, although spatial learning abilities for navigation purposes are narrowly

specialised to the environment in which they are used, the benefits of such abilities are widespread.

There is increasing evidence to suggest that individuals exposed to complex and simple environments show latent responses in cognitive ability to match that of their latest experience. Many studies investigating spatial learning have been conducted in the laboratory, where variability is easily controlled for and environmental conditions can be altered throughout ontogeny. However, captive environments are often barren and devoid of enrichment, leading to concerns that a lack of visual stimuli inhibits natural behaviour and cognitive development (Kotrschal et al. 1998; Brown et al. 2003; Odling-Smee et al. 2008). Alongside the betterment of fish welfare, the interest in phenotypic plasticity led to environmental enrichment in captive settings as the standard protocol to encourage natural behaviour (Williams et al. 2009). Recent studies have used this approach to investigate genetic and environmental interactions (GxE; Lynch and Walsh, 1998) on cognitive processes in wild-caught and laboratory-bred populations. For instance, Spence et al (2011) showed that zebrafish (*Danio rerio*) reared in complex environments showed consistent and superior spatial learning skills than those from the same population reared in simple environments. Juvenile trout (*Oncorhynchus mykiss*) first reared in barren conditions then switched to enriched conditions demonstrate elevated spatial learning ability compared to those reared in the opposite treatment (Bergendahl et al. 2016). Moreover, enrichment provided at specific stages during early ontogeny has lasting effects on spatial learning ability in later life (e.g. striped knifejaw *Oplegnathus fasciatus*; Makino et al. 2015).

While spatial learning facilitates navigation, equally important is the amount of time cue information should be retained before it is no longer relevant (memory window). Markel (1994) reported evidence of long-term memory in the Blackeye goby (*Coryphopterus nicholsi*) by demonstrating that naïve individuals could locate a new shelter faster than

individuals that were trained to find the shelter elsewhere. This had a two-fold implication for long-term memory formation; firstly, experienced gobies were able to hide from simulated predation faster than naïve individuals, having experienced the experimental layout. Secondly, the fact that naïve individuals benefited from the switched shelter position suggests that long-term memory carries fitness costs in environments where resources shift often. A study on sticklebacks from contrasting environments found that a species (*Gasterosteus aculeatus*, forma *aculeatus*) from an isolated lake showed longer memory retention of foraging techniques than the two other species, despite having no stimuli reinforcement (Mackney and Hughes, 1995). The authors suggest that exposure to a restricted diversity of prey in a landlocked habitat would select for a longer memory window for foraging techniques. In contrast, the two other stickleback species (*Spinachia spinachia* and *G. aculeatus* forma *trachura*) inhabit highly dynamic environments and changeable prey diversity, therefore, a rapid turnover of information through a smaller memory window would be advantageous. Similarly, a comparative study by White and Brown (2014b) found rockpool gobies were better able to locate deep pools than sand gobies, indicative of their twice daily homing journeys to certain rockpools and the associated ecological pressures for long-term memory.

Over the last few decades, the focus of cognitive studies in fish expanded toward neural functioning and the mechanisms underlying information processing from various sources (Broglia et al. 2011). Although lacking a neocortex, the neural structure in fish bears homologous components and processing abilities characteristic of mammalian brains (Broglia et al. 2005). The overlap in brain structure is a form of convergent evolution, whereby similar problems encountered in organisms is solved through a universal prototype mechanism (Chittka and Skorupski, 2011; Patton and Braithwaite, 2015). Thus, it is not surprising that the use of different hemispheres to process information (cerebral

lateralisation) is widespread in the animal kingdom (see Vallortigara et al. 1999 for a review). However, fishes differ from other vertebrates in that their visual fields produced by either eye have only a small frontal overlap (Bisazza and Brown, 2011), implying that asymmetrical patterns in eye use are directly linked to left/right cerebral dominance (Bisazza et al 1998). For instance, mosquitofish with conspecifics on their left approach predators during inspection more so than when conspecifics are on their right (Bisazza et al. 1999). In detour tests, fishes that take the left pathway monitor stimuli with their right eye (and vice versa), and this tendency is repeatable amongst individuals when exposed to a predator in their home tank (Facchin et al. 1999). Asymmetrical bias in behaviour is difficult to explain, as bias toward one side would undoubtedly leave individuals vulnerable on the other (Vallortigara and Rogers, 2005). However, the dual processing hypothesis suggests that strongly lateralised individuals have enhanced cognition because their brain can process different sources of information using both hemispheres simultaneously (Rogers et al. 2004). Although some studies support this (e.g. enhanced spatial learning and prey handling in topminnows *Girardinus falcatus*; Sovrano et al. 2005; Dadda and Bisazza, 2006a), most have used shoaling species as models, suggesting that lateralised behaviour is favourable in a social framework. There is ample evidence that not only direction of laterality, but also strength, is highly variable amongst species, populations and even individuals (Bisazza et al. 1998; 2000a; Brown et al. 2004). For instance, Bisazza et al. (2000a) found population lateralisation in several shoaling species, but in less than half of the non-shoaling species. Non-social cichlids (*Amatitlania nigrofasciata*) use different eyes to view an empty space versus a mirror, and only females demonstrate population level lateralisation when in a social context (Moscicki et al. 2011). Such differences within and between populations lead to questions regarding the heritability of lateralisation, and how influential environmental factors are in the development of cerebral asymmetry. Selectively breeding strongly lateralised pairs of

poeciliids (*G. falcatus*) led to highly correlated directional bias in their offspring (Bisazza et al. 2000b). In contrast, offspring of poeciliid *Brachyrhaphis episcopi* adults from high- and low-predation sites show inherited strength of laterality but not direction, suggesting that the plasticity of heritable traits is influenced by predation regimes (Brown et al. 2007a).

Predation is perhaps the strongest selective pressure acting on the behaviour of individuals; those that recognise predators and react accordingly survive. Naïve fish that encounter a novel object will generally show a strong avoidance response (neophobia; Sneddon et al. 2003), suggesting that a first cautious reaction to a potentially risky situation is beneficial, but does not imply a genetic predisposition to predator cues (Brown et al. 2011b). Although some evidence suggests that predator recognition in fish is innate, whether in the context of a visual (Magurran, 1990), or olfactory cue (Berejikian et al. 2003; Vilhunen and Hirvonen, 2003), the more likely scenario is that anti-predator behaviour is a combination of innate components passed between generations and learned components that remain plastic throughout ontogeny depending on the proximate selective pressures in the environment (Kiefer and Colgan, 1992; Kelley and Magurran, 2003; Brown et al. 2011b). For instance, Chivers and Smith (1994a; b) showed that fathead minnows (*Pimephales promelas*) reared in laboratory aquaria devoid of predators exhibit no recognition of olfactory or visual cues of a sympatric predator, but their wild counterparts do. Ferrari et al (2007) suggest that the propensity of learned predator recognition is on a continuum ranging from true innate recognition to learned recognition. In environments where predator density is fixed, behavioural plasticity in prey populations would be low and threat-sensitive responses negligible, thus, neophobic responses would be a population-wide anti-predator response (Killen and Brown, 2006). On the other hand, populations from environments with variable predation risk would do better to engage in fitness-related activities during periods of low predation pressure and anti-predator behaviour when risk is higher (threat-sensitive predator

avoidance; Helfman, 1989). However, anti-predator behaviour is more than a binary process of experience and naivety, and within-population variation in anti-predator responses has gained much attention in the last couple of decades.

Boldness, the propensity to take risks (Wilson et al. 1994), has been implicated in anti-predator behavioural research, as it is a characteristic of individuals that inspect novel objects or predators faster than their shyer shoal mates (Murphy and Pitcher, 1997). Of the five axes of fish personality (shyness-boldness, exploration-avoidance, activity, aggressiveness and sociability; Réale et al. 2007; Conrad et al. 2011), boldness has been researched most intensively (Magnhagen et al 2014) and is known to be shaped by predator density (e.g. Dingemanse et al. 2009) but nonetheless remains a plastic trait influenced by life experience (Brown et al. 2007b; c). Two or more of these behavioural traits may be correlated to form behavioural syndromes, suggesting that behaviour is not infinitely plastic and that some traits may not evolve freely of each other (constraint hypothesis; Bell, 2007). For instance, boldness, activity and aggressiveness tend to be favoured in populations from high-predation environments (sticklebacks *G. aculeatus*; Dingemanse et al. 2007). However, there is increasing evidence suggesting that the flexibility of traits forming behavioural syndromes are influenced by environmental and ontogenetic circumstances, thus, traits are correlated in an optimal combination (Bell and Stamps, 2004). By extension, this would suggest that naïve populations reared in captivity, with no selective pressures for trait correlations, should demonstrate no behavioural syndromes.

Another critically important aspect affecting survival is the ability to secure limiting resources in both inter- and intraspecific competitive interactions. It has now been almost four decades since the earliest observations of ontogenetic and seasonal dietary shifts in intertidal fish were made (Grossman et al. 1980; Grossman, 1986). Currently, there is growing interest in the extent of plasticity in diet and resource partitioning (e.g. Barrett et al.

2016; Vinagre et al. 2018), particularly because there is no marine environment more spatially or temporally variable than the intertidal zone (Gibson and Yoshiyama, 1999). Studies have shown elaborate community guilds of carnivorous, omnivorous and herbivorous fish (Velasco et al. 2010), switches between guilds on seasonal scales and the occupying of different guilds depending on ontogenetic stage (Muñoz and Ojeda, 1998). It has been suggested that dietary overlap between fishes would be an inconsequential aspect of intertidal life, given the relatively high abundance of invertebrate fauna (Velasco et al. 2010), however, high resource partitioning between sympatric species suggests that there are plastic behavioural strategies in play to facilitate coexistence (e.g. Barrett et al. 2016). Moreover, the adaptive benefit of behavioural plasticity is likely amplified in the intertidal zone, where top consumers must adapt foraging strategies to behavioural changes in meiofauna (Palmer and Brandt, 1981; Jones et al. 1994).

#### **How complex is complex? The intertidal zone**

The intertidal zone varies both temporally and spatially more than any other marine habitat (Gibson and Yoshiyama, 1999), making it one of the most physically stressful environments on the planet (Denny and Gaines, 2007). Organisms living amongst rock platforms endure wave action at speeds of over 90km/h at high tide (Denny and Gaines, 2007), as well as rapid increase in temperature and salinity accompanied with low oxygen levels in stagnant pools at low tide (McAllen and Taylor, 2001; Griffiths et al. 2004). Yet incredibly, the intertidal zone supports rich communities of algae, invertebrates, and fishes that make up complex communities in rockpools (Denny and Gaines, 2007).

Fish species found in rockpools are typically one of three types; residents, secondary residents or transients, all of which are categorised depending on their behaviour and what stage of their life history they spend occupying these pools (Mahon and Mahon, 1994;



Griffiths, 2003a). While secondary residents and transients move between intertidal and offshore environments, resident species spend their entire lives in these rockpools. They possess highly modified physical traits well-suited to this habitat, including small, slender bodies, mottled colouration to aid camouflage, and specialised pelvic fins which fuse to form a suction cup-like shape to better cling to the substrate (Kuitert, 1996; White and Brown, 2013). Moreover, residents exhibit highly cryptic behaviour, often hiding under rocks or in crevices, and moving between them in short, rapid bursts of movement. However, all these traits would be obsolete in a dynamic habitat like the intertidal zone without the neural mechanisms that drive complex cognitive function and behavioural plasticity. Thus, resident fishes of the intertidal zone are an ideal group to investigate this relationship.

**Study species: *Bathygobius cocosensis* (Family: Gobiidae)**

Gobies are the largest taxonomic group of intertidal resident fishes (Family: Gobiidae) and have been reported as the most abundant species in studies of rockpool communities on the south-east Australian coast (Griffiths, 2003a; b; White et al. 2015). Gobies have been a model group for several cognitive studies, all of which lend increasing credibility to the ecological cognition hypothesis; that individuals reared in complex systems demonstrate increased cognitive function relative to individuals from physically homogenous environments in a domain-specific manner. Earlier studies found compelling evidence to support this in the rockpool specialist Cocos Frillgoby (*Bathygobius cocosensis*). They possess larger telencephala, use multiple cues, show higher homing rates, retain information longer and solve spatial tasks faster than species from the homogenous sandy shores in the intertidal zone (White and Brown, 2013; 2014a; 2014b; 2015a; 2015b). However, the extent of environmental influence on cognitive and behavioural phenotypic plasticity within populations, as opposed to heritable variation, remains unknown in this species. Their high

abundance, robust nature and small size make *B. cocosensis* an ideal model species for such studies.

## **Justification**

The field of cognitive ecology has established that environmental dynamics influence not only the presence of plasticity in behavioural responses, but also the degree of flexibility. By allowing an organism to ‘fit’ its phenotype to an environment, phenotypic plasticity is selected for in populations that inhabit environments that undergo frequent change. Less investigated is the extent to which environmental pressure can alter the behavioural phenotype of an individual against the framework of inherent behaviour formulated through genetic information passed down through generations. Preliminary evidence suggests that even short exposures to variable habitat enrichment can have long lasting effects on cognitive processing, social interaction and foraging behaviour (Kotrschal et al. 1998; Brown et al. 2003; Odling-Smee et al. 2008). However, studies such as these are few, and research on the influence of GxE interactions on behavioural plasticity is still very much in its infancy.

## **Outline and aims**

The foundation of this thesis is to address the changes in behavioural phenotypes, and the underlying cognitive processes, that occur as a result of environmental influence, using the common intertidal Cocos Frillgoby *Bathygobius cocosensis* (Bleeker, 1854). The population I collected from for the purpose of these investigations occurs in the rockpools at Dee Why Beach, N.S.W, Australia.

Although most organisms demonstrate some level of niche-width plasticity to counter changes in resource abundance, the dynamic nature of coastal environments suggests that foraging plasticity, and associated changes in trophic width, would be particularly favourable

for intertidal fishes. Moreover, spatial and temporal shifts in resource availability indicate that these shifts would be over relatively short areas and time scales. This leads to questions regarding foraging plasticity in intertidal fishes, particularly in contexts with high and low interspecific competition from morphologically similar species.

A dynamic environment similarly favours heightened cognitive ability, which has been demonstrated in *B. cocosensis* (White and Brown, 2013). However, if an individual's environment plays such a vital role in cognitive function and behavioural plasticity, will a change in environment reshape both traits entirely? Or is there some evolutionary inherent component that can be conserved within one lifetime? To investigate this, I compared anti-predator behaviour, lateralisation and spatial learning in *B. cocosensis*, all within the comparative framework of wild versus captive-reared populations.

**Aim 1:** The dynamic intertidal zone exemplifies a natural habitat with temporal and spatial shifts in resource availability, which fishes often respond to using adaptive behavioural responses. In addition to resource shifts, species in sympatric associations are also faced with competition from other, often ecologically similar, species. These interactions may lead to strategic resource partitioning to maximise benefits and facilitate coexistence whilst simultaneously minimising agonistic confrontations. Chapter 2 focused on diet and trophic niche plasticity in three intertidal goby species. We investigated diet profiles and isotopic signatures in one allopatric and two sympatric goby populations across seasonal and ontogenetic gradients to evaluate plasticity of niche overlap and associated resource partitioning.

**Aim 2:** The costs of cognitive functions are such that they should only be invested in when demanded by proximal ecological challenges, such as navigating between resources

efficiently. In species where one of the sexes is nest-bound when caring for offspring, movement is decreased and thus, the required maintenance of cognitive processing reduced. The benefits of cognitive plasticity allow individuals to manage trade-off costs by minimising energetic expenditure required to maintain neural processes over relatively short periods. In Chapter 3, I investigated whether spatial learning ability is sexually dimorphic in the Cocos Frillgoby, if their performance varies seasonally, and how this might interact with their mating system.

**Aim 3:** Navigation is perhaps one of the most impressive skills demonstrated by intertidal resident fishes, demonstrated when they return to a particular ‘home’ pool at low tide following foraging excursions at high tide. Spatial learning ability varies markedly between goby species pertaining to different environments within the intertidal zone, however, the extent to which this can be influenced by experience during their lifetime remains unknown. In Chapter 4, I reared gobies in habitats with variable environmental enrichment to examine the impacts on cognitive function.

**Aim 4:** To some degree, fish are hardwired with innate predator recognition and responses, however, anti-predator behaviour often remains plastic throughout ontogeny and is greatly influenced by experience. In particular, lack of predators can change not only the way an individual perceives threat, but also the sensory modalities it uses to recognise a potential predator. Additionally, exposure to high predation contexts may result in correlations between suites of certain behavioural traits (behavioural syndromes). Chapter 5 outlines how wild and captive-reared populations of the Cocos Frillgoby respond to olfactory and visual cues of a potential, sympatric predator, and how naivety to predation risk influences the development of behavioural syndromes.

**Aim 5:** Cerebral lateralisation allows individuals to process several sources of information at once, such as remaining vigilant whilst foraging. There is evidence to suggest that elements of laterality are heritable, however, laterality direction and strength have been shown to vary within populations, suggesting lateralised behaviour remains plastic throughout ontogeny and is shaped by individual experience. Moreover, ontogenetic changes in social structure may influence changes in laterality between juveniles and adults or between sexes. In the final chapter of this thesis, I compared eye-use preference in wild and captive-reared Cocos Frillgobies when viewing a mirror image to evaluate if eye-bias differs between populations exposed to different predation and stability regimes.

## CHAPTER 2

# **Trophic niche plasticity: Investigating seasonal and ontogenetic shifts in three intertidal gobies**

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## Abstract

Resource partitioning facilitates the coexistence of sympatric species through spatial, temporal and/or trophic strategies. Fishes living in the intertidal zone demonstrate incredibly adaptive plastic behaviour, including spatial and temporal shifts in diet and micro-habitat. Although intertidal fish assemblages are influenced by inter- and intraspecific competition, few studies have compared the extent of resource partitioning between sympatric species in the context of trophic niche plasticity. Here we used complementary approaches, stomach content and stable isotope ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) analyses, to evaluate seasonal and ontogenetic shifts in trophic niche position in two sympatric (*Favonigobius lentiginosus* and *Bathygobius krefftii*), and one allopatric (*Bathygobius cocosensis*) species of intertidal goby. The results indicate high levels of resource partitioning in the two sympatric species, with almost no trophic niche overlap in summer to almost one third (~30%) overlap in winter. We found evidence of diet specialisation in later ontogeny in the allopatric *Bathygobius* species, which is likely driven by a shift in micro-habitat as individuals grow. Our findings highlight the temporal range of behavioural plasticity in trophic niche position of intertidal gobies, and suggest it is of highly adaptive value in the dynamic intertidal zone.

**Key words:** Diet, *Favonigobius*, *Bathygobius*, resource partitioning; summer; winter

## Introduction

Interspecific competition is a critical ecological driver in the formation and maintenance of community assemblages (Schoener, 1983; 1985) and an individual's survival is heavily influenced by the behavioural strategies it uses to secure competitively limited resources (Milinski and Parker, 1991). Resource partitioning can alleviate intensity of competition and facilitate the coexistence of species at similar trophic levels, which is why it is often observed in sympatric associations (Young et al 2010; Barnes et al. 2011). The way in which coexisting species partition food resources is widely considered evidence of past competition for limited resources in overlapping niches (Tilman, 1982) and includes foraging for different functional types or sizes of prey (Schoener, 1974).

Strategies for resource partitioning vary greatly depending on the species but usually include some spatial and/or temporal strategy that mirrors the natural fluctuation of resource availability (Winemiller, 1989). For instance, sympatric primates forage at different tree heights and in different substrates (spatial strategy) and exhibit different ranges of niche overlap depending on the season (temporal strategy), with minimal interactions (Singh et al. 2010). In sympatric marine mammals, a high range of overlap in space use is counteracted by a preference for different depths and substrates (Parra, 2006). In fishes, there is evidence that the greatest partitioning patterns occur in relation to food, followed by habitat, and then time (Ross, 1986).

The intertidal zone is a hostile and dynamic environment characterised by extreme fluctuations in abiotic factors (Denny and Gaines, 2007). As a relatively small environment with abundant biodiversity, it is likely that coexisting species experience high levels of competition for resources (Menge and Sutherland, 1976). Particular groups of interest are rockpool fishes, as they demonstrate incredible behavioural plasticity in response to a dynamic habitat and competition from morphologically similar species (Horn et al. 1998; Chelazzi and Vannini, 2013). Community structure in these habitats includes species that spend most of their life stages on rocky platforms (residents), species that reside in the intertidal zone during early ontogeny (secondary), and transient species which are often accidentally washed in during high tide



(Mahon and Mahon, 1994; Gibson and Yoshiyama, 1999; White and Brown, 2013). Resident species are commonly sympatric with each other, sharing valuable resources such as food, shelter and spawning sites (Nieder, 1997). In addition, residents experience short-term competition from secondary resident and transient species.

Resident intertidal species often display particular demographic distributions across the intertidal zone in relation to shore height (Gibson, 1982; Illich and Kotrschal, 1990; Macpherson, 1994), however, there is increasing evidence that species interaction plays a bigger role in abundance and distribution than previously thought (Arakaki et al. 2014). For instance, sympatric species successfully reduce interspecific competition for pools and shelter by occupying different micro-habitats (Mayr and Berger, 1992). Similarly, benthic fish assemblages in rockpools often display ontogenetic shift in habitat use (Davis, 2000; Faria and Almada, 2001), where larger individuals occupy deeper pools and thus, a different micro-habitat to their smaller counterparts. The environmental segregation between ontogenetic stages implies an associated dietary shift, which has indeed been demonstrated in several benthic species (Velasco et al. 2010). This has led to increased interest in inter- and intraspecific trophic relationships, particularly trophic plasticity and associated behaviours in response to limited resources and competition (e.g. Boyle and Horn, 2006; Castellanos-Galindo and Giraldo, 2008; Velasco et al. 2010). For instance, studies have found that morphologically similar sympatric fishes occupy different trophic niches through specialist or generalist diets (Velasco et al. 2010), and many species exhibit ontogenetic changes in habitat use (Faria and Almada, 2001; Dominici-Arosemena and Wolff, 2006) and thus, diet (Muñoz and Ojeda, 1998; Velasco et al. 2010).

Temporal diet changes in intertidal species is another strategy of resource partitioning among coexisting species. Such changes can occur across seasonal gradients and reflect shifts in productivity or availability of prey (Grossman, 1986) or intensity of competition. Similarly, a shift in prey preference toward larger taxa during ontogeny is frequently observed in intertidal species, influenced by a growing mouth gape and increasing metabolic demand (Kotrschal and Thomson 1986, Horn and Gibson 1988, Muñoz and Ojeda 1998, Norton and Cook 1999). Other

species are carnivorous or omnivorous during early ontogeny, and shift to herbivory as adults (Horn et al. 1982; Cancino and Castilla, 1988; Barry and Ehret 1993; Sturm and Horn 1998; Aldana et al. 2002; German et al. 2004; Boyle and Horn, 2006). Understanding these ontogenetic changes in resource use is particularly important from an ecological perspective, as they may assist in illustrating wholistic ecosystem functioning (Floeter et al. 2004; Hooper et al. 2005).

Traditional stomach content analyses provide data on trophic niche position; however, this approach typically provides only a snapshot view into the short-term dietary preference of an individual just prior to capture (Baker et al. 2014). Stomach content analysis fails to account for variable assimilation rates of items within the stomach (Bearhop et al. 2004), leading to discrepancies in reported diets in some species (e.g. Boyle and Horn, 2006). Moreover, this method cannot identify any long-term patterns of prey consumption.

Naturally occurring stable isotopes are a complementary tool to stomach content analysis (Peterson and Fry, 1987; Michener and Schell, 1994; Davis et al. 2012) and are frequently used to trace pathways or organic matter through food webs (Hesslein et al. 1991; Michener and Lajtha, 2008). Carbon and nitrogen ratios change predictably as organisms assimilate carbon or nitrogen into their tissues as they feed, such that predictable changes in ratios occur between consumer and source (Griffiths, 1991). In the case of carbon ( $\delta^{13}\text{C}$ ), organisms have similar isotopic compositions to their diet with little enrichment ( $\sim 1\%$ , DeNiro and Epstein, 1978; Fry and Sherr, 1984; France and Peters, 1997), thus providing insight into dietary sources of the consumer (Rau et al. 1983; Michener and Schell, 1994). On the other hand, nitrogen ( $\delta^{15}\text{N}$ ), stable isotopes are enriched  $\sim 3.4\%$  at each trophic level and so are more frequently used to estimate trophic position of consumers (DeNiro and Epstein, 1981; Peterson and Fry, 1987; Post, 2002; Richert et al., 2015). Stable isotope ratios can assist researchers in mapping niche shifts in fishes (Pinnegar and Polunin, 1999; Thomas and Cahoon, 1993; Jackson et al. 2011; de la Morinière et al. 2003), and subsequently understand how they are influenced by competition and resource availability (Faria and Almada, 1999; Gibson and Yoshiyama, 1999; Park et al. 2017). For example, many marine fish species consume macroalgae but its relative volume in diet varies

seasonally with abundance (Connolly et al. 2005) and ontogenetically as individuals shift diet preference (Velasco et al. 2010), leading to different carbon and nitrogen isotopic values in the consumer. Thus, stable isotope analysis is an ideal tool to investigate seasonal and ontogenetic dietary shifts in the dynamic intertidal zone (Grossman, 1986; Jones, 1988).

In fishes, benthic resident species are the most abundant taxa (e.g. Gibson, 1982; Faria and Almada, 1999; White et al. 2015), and the dominant top consumers in intertidal habitats worldwide (Vinagre et al. 2018). Although diet in temperate assemblages of intertidal fishes has been well documented in the northern hemisphere (e.g. Yoshiyama, 1980; Grossman, 1986; Ojeda and Muñoz, 1999; Quijada and Caceres, 2000; Angel and Ojeda, 2001; Berrios and Vargas, 2004), most of these studies have focused on the relationship between substrate complexity, trophic guilds and resource partitioning without assessing the relative impacts of spatial and temporal changes in diet, or ontogeny of the fishes themselves. Likewise, temperate intertidal communities on the east coast of Australia have been investigated from an assemblage perspective (Wilson, 1989; Lardner et al. 1993; Silberschneider and Booth. 2001; Griffiths et al. 2003; Griffiths et al. 2006; White et al. 2015), however, there remains a gap in knowledge of diet preference, shifts in diet and range of niche plasticity to facilitate coexistence in benthic fish species. The combination of high abundance, site attachment and subsequent interaction with resident and transient species at high tide suggests that benthic fishes may display a high degree of plasticity in their diet to mitigate interspecific competition through resource partitioning.

Members of the family Gobiidae are a particularly abundant group of benthic fish (Gibson, 1972; 1982; Faria and Almada, 1999; 2006; Beckley, 2000; Griffiths et al. 2003; Barreiros et al., 2004; Arakaki et al. 2014) and sympatric associations between morphologically similar goby species may be characterised by high levels of prey selectivity and resource partitioning (Magnhagen and Wiederholm, 1982). The intertidal zone along the east coast of Australia is inhabited by multiple species (White et al. 2015) and thus presents an ideal study system. We investigated the diet of three common goby species from rockpools and sandy shores along the intertidal zone of the south east coast of Australia. Diet and trophic niche were

assessed using stomach content and stable isotope analyses, and data used to compare prey preference and assimilation throughout ontogeny and between seasons. Two of the study species are sympatric, and we proposed that their overlapping distributions would play a role in niche width, which may be reflected in the context of resource partitioning between seasons. The third species occurs allopatrically, which we predicted would result in a relatively stable and wider niche width between seasons, owing to reduced competition from similar species. Finally, we predicted that all three species would show ontogenetic shifts in diet, with larger individuals consuming a wider range of prey items.

## Methods

### *Study species*

Three goby species were assessed, two of which (*Bathygobius cocosensis*; Bleeker, 1854 and *B. krefftii*; Steindachner, 1866) are resident, rockpool specialists, and the third (*Favonigobius lentiginosus*; Richardson, 1844) occurs on sandy shores. Sympatric populations of *B. krefftii* and *F. lentiginosus* occur in Chowder Bay, New South Wales (NSW), Australia. The former generally occurs in permanent rockpools but ventures out onto the sandy flats at high tide to forage. Therefore, it is expected that some trophic overlap exists between these two species. *B. cocosensis* is ubiquitous along the east coast of Australia and has been reported as the most abundant fish species in rockpool assemblages (Griffiths et al. 2004; White and Brown, 2013; White et al. 2015). This species defends resources aggressively and directly impacts the distribution of other species (Griffiths et al. 2003; Pajmans and Wong, 2017). We used a population from Dee Why, NSW, as a comparative measure to a member of the *Bathygobius* genus and an example of a species dominant in an intertidal community. Previous faunal assessments list other goby species as residents in Dee Why rockpools (White et al. 2015), however, these were not included in this study owing to small sample sizes.

*Study area and sampling*

*B. krefftii* (n = 89) and *F. lentiginosus* (n = 77) were collected from Chowder Bay, an intertidal region in Sydney Harbour, NSW, Australia (-33.840011, 151.252376). The third species, *B. cocosensis* (n = 102), was collected from the rock platform at Dee Why, NSW (-33.754931, 151.298739). Individuals were collected in summer (Dec-Jan) and winter (Jun-Jul) for two consecutive years (2015, 2016). All *B. krefftii* and *B. cocosensis* were collected using small, hand-held nets in randomly selected pools along the rock platform during low tide, while *F. lentiginosus* were collected on snorkel. Once caught, all individuals were immediately transferred to a tricaine methane sulfonate (MS222) solution and euthanised. Individuals were measured for total length (TL  $\pm$ 1.0 mm), and weight (BW,  $\pm$ 0.1mg). A portion of individuals (*F. lentiginosus* n = 39; *B. krefftii* n = 50; *B. cocosensis* n = 47) were submersed in ethanol (70%) for gut content analysis. The remainder were transferred to an ice slurry and processed for isotope analysis (Barrow et al. 2008).

*Ethical note*

Gobies were caught in compliance with NSW Fisheries (permit no. P08/0010-3.0) and Macquarie University Ethics Committee (ARA 2014/003).

*Stomach content analyses*

After removal, stomach fullness was quantified (by volume) using a stereo microscope (Olympus SD30), and the contents preserved for at least 24h in 70% isopropanol. All prey items were identified to the lowest possible taxonomic level (typically the order of family level) using a dissecting microscope and taxonomic sources. The relative contribution of each prey item was visually assessed using a grid-marked Petri dish (Park et al. 2017). Dietary data were expressed as frequency of occurrence ( $\%F = 100 \times A_i \times N^{-1}$ ) and as a volumetric percentage ( $\%V = 100 \times V_i \times V_T^{-1}$ ), where  $A_i$  is the number of fish preying on prey taxa  $i$ ,  $N$  is the total number of

fish examined (excluding those with empty stomachs),  $V_i$  is the volume of prey taxa  $i$ , and  $V_T$  is the total volume of prey taxa.

To investigate ontogenetic trends in the diets of *B. cocosensis*, *B. krefftii* and *F. lentiginosus*, volumetric dietary data for each species were aggregated into successive 10mm TL intervals, i.e. 20-30 mm, 31-40 mm, 41-50 mm, 51-60 mm and 61-70 mm. Temporal changes in diet were examined by combining the dietary data for each of the three species into summer and winter. Mean percentage contributions by volume of the various prey taxa to the diets of individuals in successive length classes were calculated for each of two seasons.

#### *Stable isotope ( $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ ) analyses*

Skinless muscle samples were taken from the lateral flank of the gobies (*F. lentiginosus*  $n = 38$ ; *B. krefftii*  $n = 39$ ; *B. cocosensis*  $n = 55$ ), with care to ensure there were no bone fragments. Samples were stored in 5 mL screw-cap tubes and immediately frozen at  $-20^\circ\text{C}$  for storage until analysis, as recommended by Davenport and Bax (2002). Samples were then dried at  $60^\circ\text{C}$  for 24 h and ground to a fine powder using a mortar and pestle. 1-2 mg of the powder was then transferred into a tin capsule. Samples were analysed for carbon ( $^{13}\text{C}/^{12}\text{C}$ ) and nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ) stable isotopes using a Europa EA GSL elemental analyser coupled to a Hydra 2022 mass spectrometer (Sercon Ltd., UK) at Griffith University (Queensland, Australia). Precision for this spectrometer is expected to be within 0.20% for  $\delta^{13}\text{C}$  and 0.10% for  $\delta^{15}\text{N}$  ratios (Raoult et al. 2015).  $^{13}\text{C}/^{12}\text{C}$  ( $\delta^{13}\text{C}$ ) and  $^{15}\text{N}/^{14}\text{N}$  ( $\delta^{15}\text{N}$ ) ratios were expressed as the relative difference per thousand (‰) between the sample and a standard.

#### *Statistical Analyses*

To examine dietary differences across ontogeny and/or season within species, dietary data for each of the species were randomly sorted into groups that contained one to three individuals within each length class in each season (depending on the sample size of that group) and the averages of the percentage volumetric data for each prey taxa were determined for each

of the resultant groups. Because volumetric data are considered to best represent the relative importance of each prey taxa, especially when different-sized prey are ingested (Hyslop, 1980), subsequent analyses were performed using volumetric data for each prey taxa. Thus, averages represented the dietary samples that were used for all subsequent analyses. Such randomization and subsequent grouping of volumetric data were designed to reduce the number of prey items in the samples with zero values, thereby increasing the effectiveness of multivariate analysis (White et al. 2004; Marshall et al. 2008). Volumetric data were square-root transformed to avoid any tendency for the main dietary components to be excessively dominant. Bray-Curtis similarity matrices were constructed for each species (Platell and Potter, 2001; Clarke et al. 2006).

The Bray-Curtis similarity matrices were constructed for each goby species and visualised via nMDS ordination. The matrices were then subjected to a series of a two-way permutational multivariate analysis of variance (PERMANOVAs) to assess where there were significant effects of size class (or habitat) and season, as well as two-way interactions. PERMANOVA is a non-parametric distance-based analysis of variance that uses permutation procedures to test hypotheses. PERMANOVA assigns components of variation (COV) of differing magnitudes to the main factors and any two- or three-way interactions between combinations of main factors included in the chosen comparison. The larger the component of variation, the greater the influence of a particular factor or interaction term on the structure of the data (Anderson et al. 2008; Linke, 2011). The component of variation attributable to a fixed factor in a given model was considered in terms of the sum of squared fixed effects (Anderson et al. 2008).

Two-way crossed analysis of similarities (ANOSIMs) were used to test for any significant differences in dietary compositions of the three species with respect to size or season based on the same factors as used in the PERMANOVA, and with the magnitude of the R-statistic indicating the relative importance of any such differences (Clarke et al. 2014). Global R-statistic values from the ANOSIM to the verified similarities (distance) within defined groups vary between 0 and 1. An R value of zero represents no differences of the average similarity

among and within groups, and an R value of 1 indicates that the composition of all samples within each group are more similar to each other than to any of the samples from any other group (Clarke and Gorley, 2006). In cases where ANOSIM detected a significant difference, pairwise ANOSIM comparisons were then used to determine which comparisons between length groups of each species or between habitats, showed significant differences.

Similarity percentage (SIMPER) was employed to determine which prey taxa typified or distinguished the diets of particular species and made the greatest contributions to any dissimilarities between the diets of those species that were identified by both PERMANOVA and ANOSIM. The SIMPER analysis gives the percentage of similarity or dissimilarity between levels of factors, and for specific levels of factors. All analyses were performed using routines in the PRIMER v7 multivariate statistics package ([www.primer-e.com](http://www.primer-e.com)) and the PERMANOVA+ add-on module (Anderson et al. 2008; Clarke and Gorley, 2015).

Differences in mean stable isotope values ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ) between species, and within species between seasons and size class were assessed using ANOVA (mixed design; Vizzini and Mazzola, 2003) with species, season and size class as fixed effects. Pairwise differences within species between seasons and between size classes were evaluated using post-hoc analyses.

To determine the trophic niche area of each species in each season, the relative overlap between the two seasons for each species, and between the two sympatric species within each season, Bayesian standard ellipse areas (SEAs) were calculated from the carbon and nitrogen isotope signatures from each individual. Standard ellipse areas are widely recognised as the optimal method of assessing isotopic niche area (Syväranta et al. 2013) and incorporating a Bayesian framework within these metrics allowed the inclusion of uncertainty and more robust subsequent comparisons between species or communities (Jackson et al. 2011). Bayesian standard ellipse areas for each species in each season (summer and winter) were calculated using the SIBER package (Jackson et al. 2011) that uses a MCMC algorithm in R (R Development Core Team, 2013) version 3.4.4. Bayesian models to estimate standard ellipse areas were run for  $2 \times 10^5$  iterations and the first  $10^4$  sets of values were discarded. Relative overlaps of those



estimated Bayesian standard ellipse areas were then estimated using the Bayesian Overlap function, with 1000 draws, a 95% confidence interval, and 1000 points per ellipse.

## Results

### *Stomach content analyses*

The stomach contents of 47 *B. cocosensis* (TL = 31-67 mm), 50 *B. krefftii* (TL = 24-47 mm) and 39 *F. lentiginosus* (TL = 27-49 mm) were examined (Table 2.1). Percentages of empty stomachs were 2.1% for *B. cocosensis*, 4.0% for *B. krefftii* and 2.6% for *F. lentiginosus*.

**Table 2.1. Summary of sampling (summer: Dec 2015 – Jan 2016; winter: May-June 2016).**

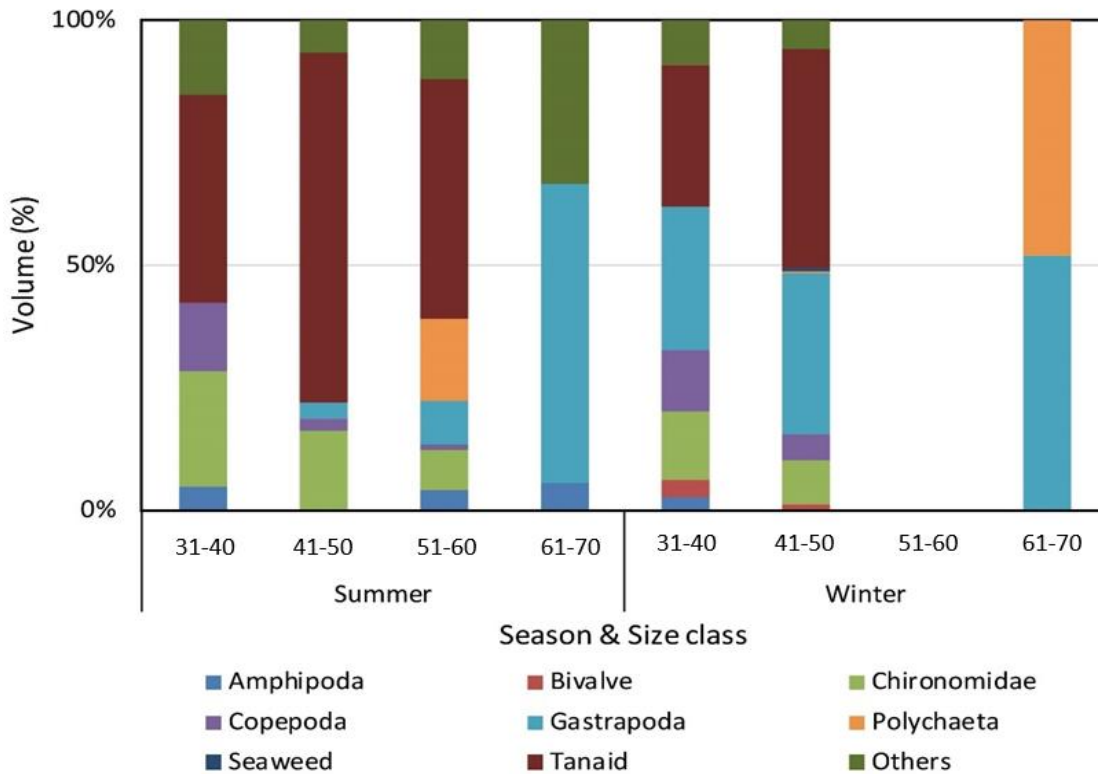
Common name	Cocos Frillgoby	Krefft's Frillgoby	Eastern Longfin Goby
Scientific name	<i>Bathygobius cocosensis</i>	<i>Bathygobius krefftii</i>	<i>Favonigobius lentiginosus</i>
Sampling area	Dee Why	Chowder Bay	Chowder Bay
Habitat	Rockpool	Rockpool	Sand beach
Number of individuals			
Summer	27	30	19
Winter	20	20	20
Total	47	50	39
Size range (TL, mean±SD)			
Summer	34-65 mm (45.6±9.0)	28-47 mm (40.2±4.7)	29-49 mm (40.2±5.6)
Winter	31-67 mm (44.5±8.8)	24-42 mm (30.6±4.1)	27-48 mm (38.3±7.3)
Total	31-67 mm (45.1±8.8)	24-47 mm (36.4±6.5)	27-49 mm (39.2±6.5)

In terms of both frequency and volume, molluscs, insects and crustaceans made an overwhelming contribution to the diets of all goby species (Table 2.2; see Appendix 1.1). In *B. cocosensis*, tanaids (crustaceans) and gastropods (molluscs) were the most important prey taxa, occurring in 76.1% and 45.7% of all stomachs and contributing 37.1% and 27.4% to the total dietary volume, respectively. Chironomid larvae (order Diptera) and copepods frequently occurred in the diets (occurring 54.3% and 56.6%, respectively), but made relatively low contributions to the diet volume (9.7% and 4.8%, respectively). *B. krefftii* diets contained at least

15 identifiable prey taxa (Table 2.2; see Appendix 1.1). Algae was the most common item at 33.3% by occurrence and 33.2% by volume. Chironomids and copepods were second in importance for this species, comprising 58.3% and 39.6% by occurrence, and 24.8% and 12.8% by volume, respectively. For *F. lentiginosus*, a total of 10 prey taxa were recorded. Most prey for this species were amphipods and polychaetes, which comprised 63.2% and 31.6% by occurrence, and 68.9% and 16.3% by volume, respectively. Copepods and gastropods were the next most abundant prey item, accounting for 26.3% and 23.7% by occurrence, and 3.7% and 2.8% by volume, respectively.

*Size-related and seasonal trends in dietary compositions: Bathygobius cocosensis*

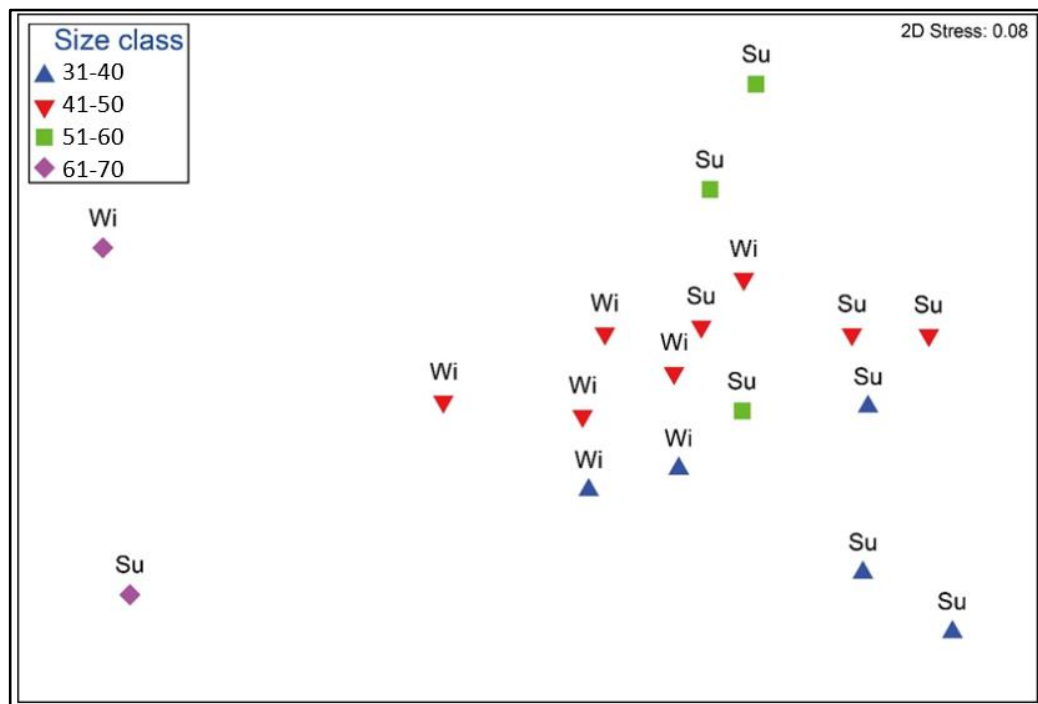
Volumetric dietary data for each species were examined by size class in each season. During both summer and winter, smaller *B. cocosensis* fed mainly on tanaids, gastropods and chironomids, the latter of which declined in importance as body size increased (Fig 2.1). In contrast, volumes of tanaids and gastropods became greater with increasing body size for *B. cocosensis*. The diet of the largest size class contained mostly gastropods, followed by polyplacophorans in summer and polychaetes in winter.



**Figure 2.1:** Dietary profile of *B. cocosensis* by volume, for season and size class. Note: lack of data (51-60mm) during winter denotes no gobies collected pertaining to that size class.

Dietary samples for *B. cocosensis* on the nMDS ordination plot displayed discrete groups of samples across both season and size class (Fig 2.2). Smaller size classes (31-40 mm) lay at the lower right corner of the plot, with a gradual shift toward the upper right axis in mid-size classes (41-50 and 51-60mm) while the largest size class (61-70 mm) lay on the left axis area of the plot (Fig 2.2). Dietary compositions of *B. cocosensis* differed significantly with size and season (PERMANOVA;  $p = 0.001$  and  $0.003$ , respectively), but did not interact significantly between these two factors ( $p = 0.065$ ). The components of variation (COV) was higher for size class than season. Two-way crossed ANOSIM showed that both size class and season ( $p = 0.002$  and  $0.014$ , respectively) significantly differed in the dietary compositions of *B. cocosensis*, and that the  $\bar{R}$ -statistic values were also similar (global  $R = 0.545$  and  $0.476$ ) for size class and season, respectively. Pairwise ANOSIM tests revealed significant differences between smaller (31-40 and 41-50mm) and the largest (61-70 mm) size classes. SIMPER emphasised that gastropods and

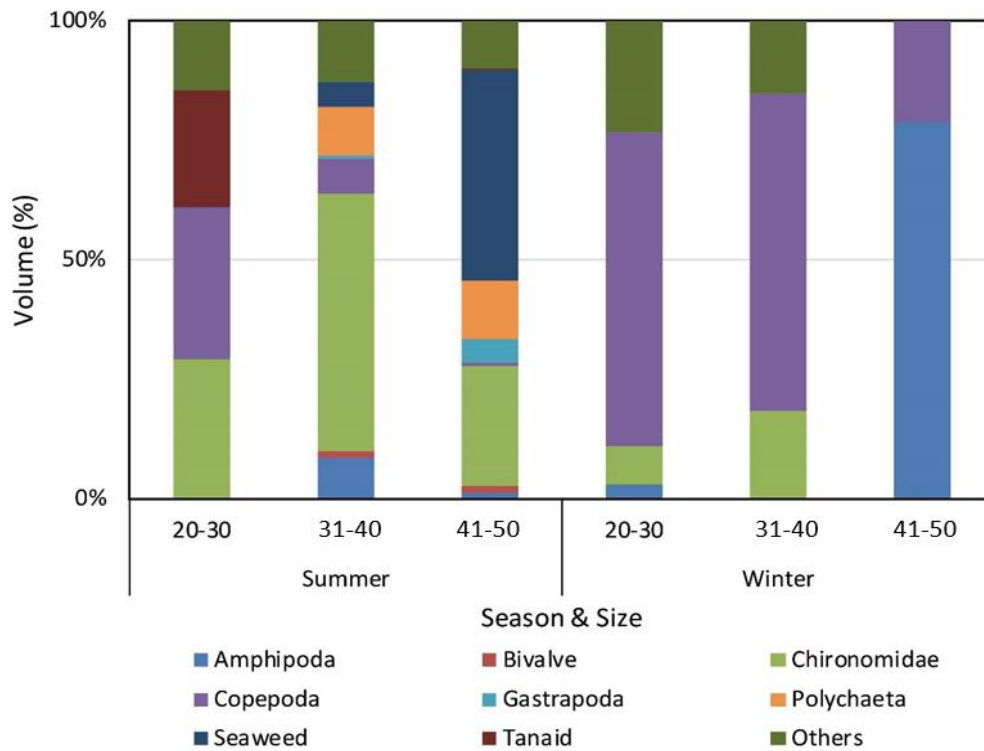
320 tanais contributed greatly to the diet dissimilarities between smaller and larger *B. cocosensis*.  
 321 The diet of *B. cocosensis* in winter contained a greater volume of gastropods and lesser volume  
 322 of chironomids compared to summer.



323  
 324 **Figure 2.2:** nMDS ordination of the dietary composition constructed from Bray–Curtis  
 325 similarity matrices of diet between four size classes of *B. cocosensis* during summer (Su) and  
 326 winter (Wi).

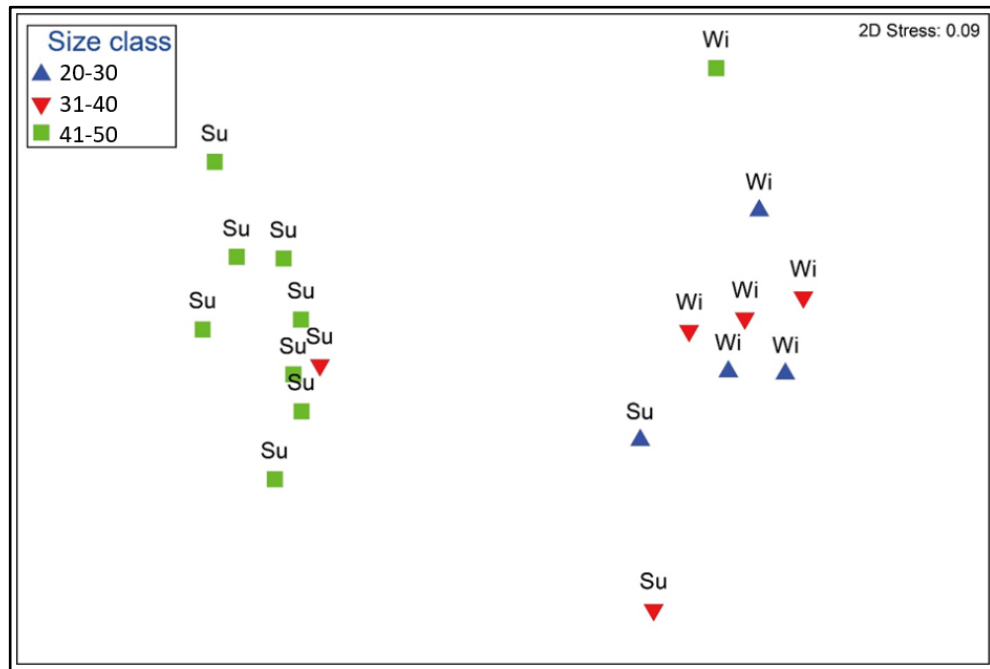
327 *Bathygobius krefftii*

328 During summer, the diet of small (20-30mm) *B. krefftii* was dominated by copepods,  
 329 tanais and chironomids. The frequency of copepods and tanais tended to decrease with body  
 330 size, while chironomids generally increased with size of this species (Fig 2.3). Most (~65%) of  
 331 the dietary volume of small size classes (20-30 and 31-40 mm) in winter was comprised of  
 332 copepods, but this contribution declined to 21.2% in larger size classes. Amphipods contributed  
 333 78.8% in the diets of the largest size class (Fig 2.3).



**Figure 2.3:** Dietary profile of *B. krefftii* by volume, for season and size class.

For *B. krefftii*, the nMDS ordination plot showed the diets of larger individuals (41-50mm) sampled in summer formed a discrete group on the left side of the plot, while those sampled in winter formed a group on the upper right side of the plot (Fig 2.4). Diets of smaller (20-30 and 31-40 mm) *B. krefftii* were highly overlapped on the nMDS plot compared to the larger size group (41-50mm; Fig 2.4). PERMANOVA showed that dietary compositions of *B. krefftii* differed with season ( $p = 0.001$ ), but no such differences were observed with size ( $p = 0.104$ ) or in interactions between size and season ( $p = 0.069$ ). COV value was two times greater for season than size class. Two-way crossed ANOSIM showed that the dietary compositions differed significantly with size class (global  $R = 0.543$ ,  $p = 0.029$ ) and season (global  $R = 0.883$ ,  $p = 0.006$ ). Pairwise ANOSIM showed that these ontogenetic differences were significant only between smallest (20-30 mm) and mid-range (31-40 mm) size classes. SIMPER analysis revealed that the diets of smaller *B. krefftii* were typified by copepods, while algae and chironomids were found in greater volume in larger size classes. Copepods contributed greatly in the winter diets, whereas summer diets had greater volumes of algae and chironomids.

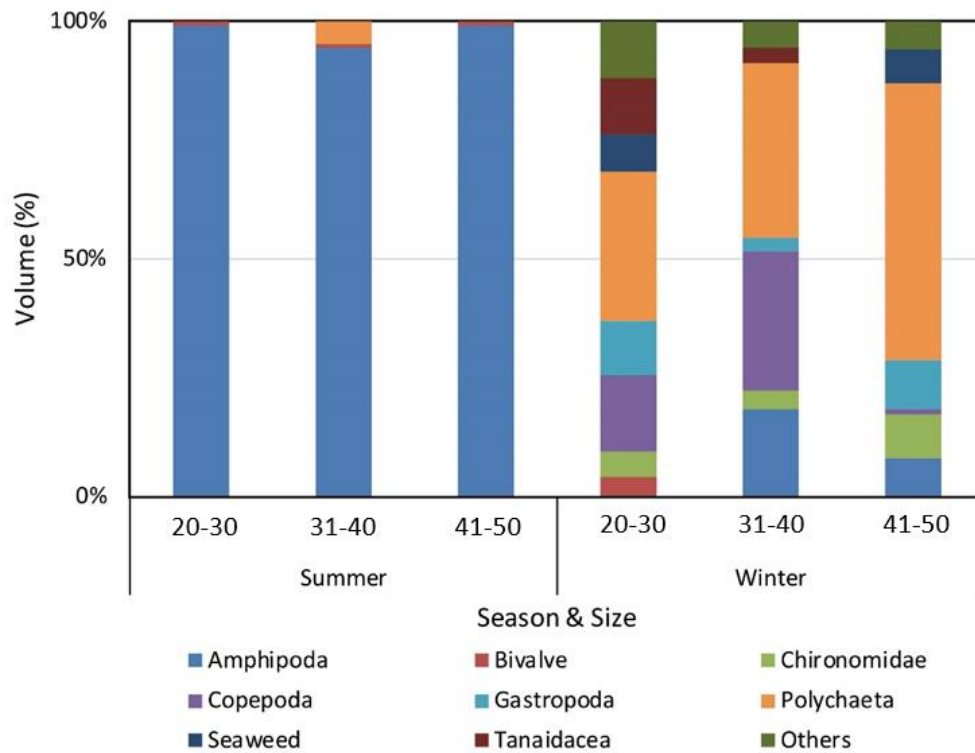


**Figure 2.4:** nMDS ordination of the dietary composition constructed from Bray–Curtis

similarity matrices of diet between four size classes of *B. krefftii* during summer (Su) and winter (Wi).

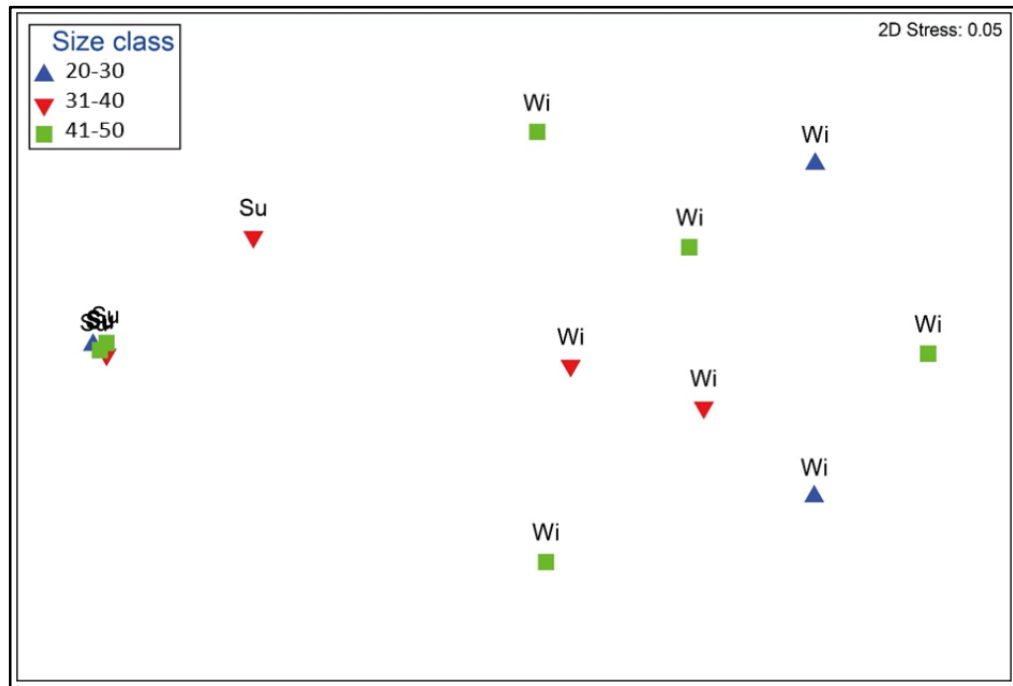
#### *Favonigobius lentiginosus*

During summer, amphipods constituted greater than 94% of diet volume in all size classes. During winter, volumetric contributions of copepods and tanaids tended to decline as body size increased (Fig 2.5). In contrast, polychaetes contributed < 37% volume to the diets of individuals in the smaller size classes (20-30 mm and 31-40 mm), but increased in importance with body size, to 58.1% in the largest size class (41-50 mm).



**Figure 2.5:** Dietary profile of *F. lentiginosus* by volume for season and size class.

The nMDS ordination plot of the dietary compositions for *F. lentiginosus* shows a clear separation of diets between season, whereby samples from summer appear on the left side of the plot and winter samples on the right (Fig 2.6). In terms of size class, summer data points were highly overlapped, while winter data points were scattered on the nMDS ordination with no clear separation among size classes. The dietary composition of *F. lentiginosus* differed significantly with season (PERMANOVA,  $p = 0.001$ ), but not between size classes, nor was there an interaction between these two factors ( $p = 0.612$  and  $0.808$ , respectively). The dietary composition of *F. lentiginosus* was shown to differ significantly with season (two-way crossed ANOSIM;  $p = 0.003$ ), but not size class ( $p = 0.397$ ), with the global R-statistic value being greater for season than size class (global R = 1.000 versus 0.058, respectively). According to the SIMPER analysis, amphipods and polychaetes typified the diet of *F. lentiginosus* in summer and winter, respectively. These two prey taxa greatly contributed to the dissimilarity between seasons.



**Figure 2.6:** nMDS ordination of the dietary composition constructed from Bray–Curtis similarity matrices of diet between four size classes of *F. lentiginosus* during summer (Su) and winter (Wi).

#### Isotope results

A total of 132 fish belonging to three species were sampled in summer and winter for isotope analyses. On average, individuals sampled in summer were larger than those collected in winter. Mean TL and  $\delta^{13}\text{C}/\delta^{15}\text{N}$  values for each species can be found in Table 2.3.

The highest carbon ( $\delta^{13}\text{C}$ ) mean value was found in *B. cocosensis* and the lowest in *F. lentiginosus* (Table 2.3).  $\delta^{13}\text{C}$  isotope ratios were significantly different between species ( $F = 87.024, p < 0.001$ ) and season ( $F = 12.981, p < 0.001$ ), however, no significant interaction was observed between the two factors (Table 2.4; Fig 2.7). Between seasons,  $\delta^{13}\text{C}$  values were significantly different in all species (*B. cocosensis*;  $t = 1.674, p < 0.05$ ; *B. krefftii*;  $t = 1.687, p < 0.001$ ; *F. lentiginosus*;  $t = 1.688, p < 0.001$ ). Between size classes,  $\delta^{13}\text{C}$  values were significantly different as, generally, assimilation decreased with body size, but there was no interaction between size class and season/species (Table 2.4; Fig 2.8). Pairwise comparisons between size classes for each species may be found in Table 2.5.



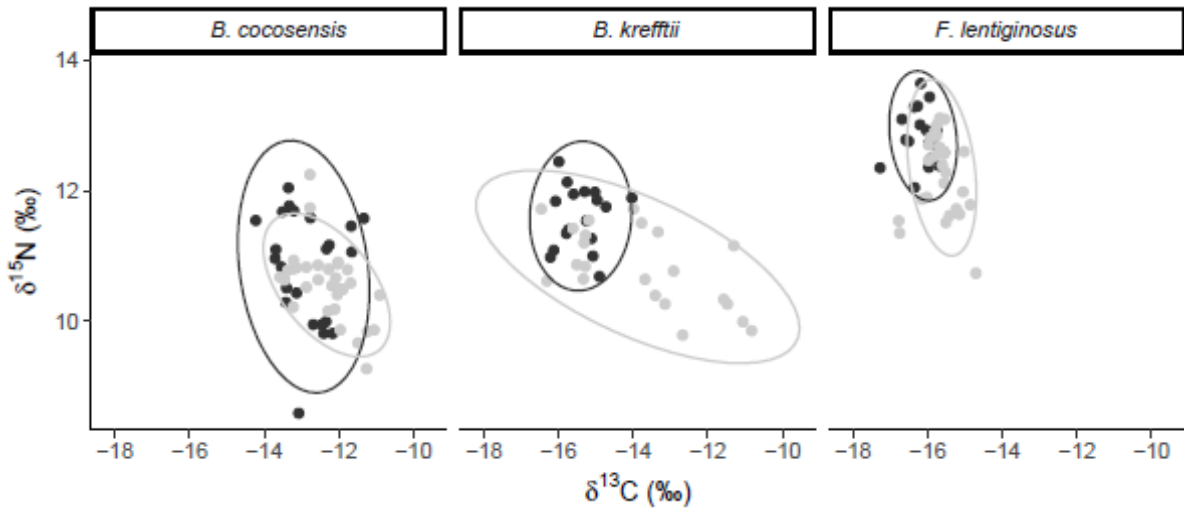
On average, nitrogen ( $\delta^{15}\text{N}$ ) isotope values were most depleted in *B. cocosensis* followed closely by those of *B. krefftii* in winter, while *F. lentiginosus* had the most enriched levels (Table 2.3).  $\delta^{15}\text{N}$  values were significantly different between species ( $F = 84.188$ ,  $p < 0.001$ ) and season ( $F = 5.795$ ,  $p = 0.018$ ), and there was a significant interaction between the two ( $F = 3.017$ ,  $p = 0.053$ ; Table 2.4; Fig 2.7). Between seasons,  $\delta^{15}\text{N}$  values were significantly different in the two sympatric species (*B. krefftii*;  $t = 1.687$ ,  $p < 0.001$ ; *F. lentiginosus*;  $t = 1.688$ ,  $p < 0.001$ ), but not the allopatric species (*B. cocosensis*;  $t = 1.674$ ,  $p = 0.09$ ). Values also varied significantly by size class, as  $\delta^{15}\text{N}$  assimilation tended to increase with body size in all species (Table 2.4, Fig 2.9). Pairwise comparisons between size classes for each species may be found in Table 2.5.

**Table 2.3.** Number of individuals sampled from each species in each season, range and mean  $\pm$  standard deviations (SD) of total length (mm) and means ( $\pm$ SD) of stable carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotopes (‰) between seasons.

Species	n/Season	TL (mm)	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$	
		Mean $\pm$ SD	Mean	SD	Mean	SD
<i>Bathygobius cocosensis</i>	Summer (n = 24)	34-65 (43.1 $\pm$ 2.5)	-12.9	0.73	10.8	0.8
	Winter (n = 31)	31-68 (41.9 $\pm$ 8.5)	-12.3	0.73	10.6	0.5
<i>Bathygobius krefftii</i>	Summer (n = 17)	30-47 (40.6 $\pm$ 4.6)	-15.4	0.57	11.6	0.5
	Winter (n = 22)	24-51 (33.8 $\pm$ 6.2)	-13.8	1.74	10.8	0.6
<i>Favonigobius lentiginosus</i>	Summer (n = 11)	41-49 (44.6 $\pm$ 2.5)	-16.4	0.36	12.9	0.5
	Winter (n = 27)	31-48 (37.6 $\pm$ 5.0)	-15.6	0.40	12.2	0.6

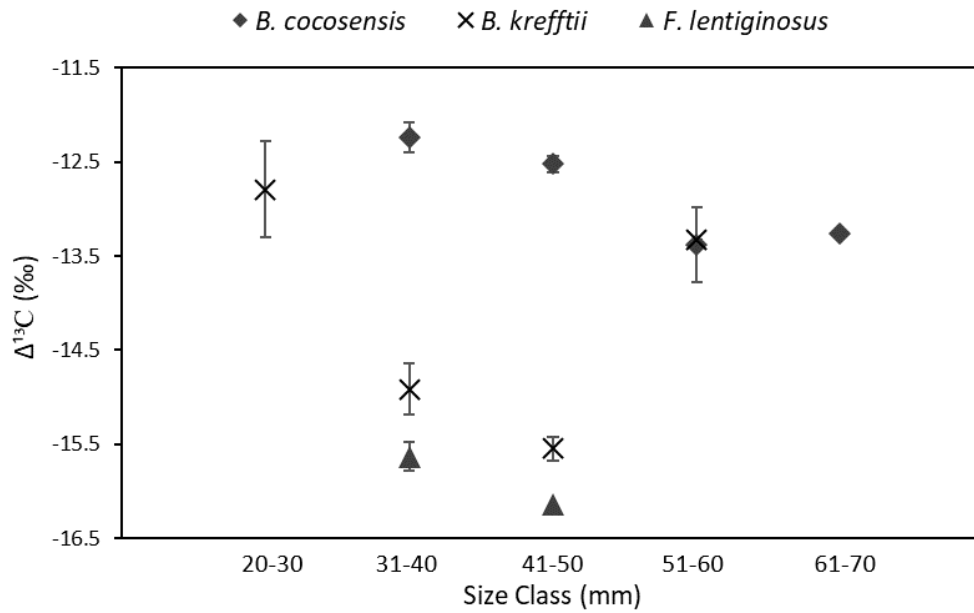
**Table 2.4.** Summary of ANOVA results ( $df$  = degrees of freedom; MS = means square,  $F$  = Fischer's;  $p$  = significance level) between species (*F. lentiginosus*, *B. krefftii* and *B. cocosensis*), season (summer and winter) and size class on carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) stable isotopes;  $p < 0.001$  = \*\*\*;  $< 0.01$  = \*\*;  $< 0.05$  = \*; NS = not significant.

Source	$df$	MS	$F$	$p$	sig.
$\delta^{13}\text{C}$					
Species	2	55.389	87.024	<0.001	***
Season	1	8.262	12.981	<0.001	***
Size class	4	2.348	3.689	<0.01	**
Species*season	2	0.161	0.253	0.777	NS
Season*size class	4	0.878	1.380	0.245	NS
Species*size class	3	1.181	1.855	0.141	NS
Species*season*size class	1	1.433	2.252	0.136	NS
Error	114	0.636			
$\delta^{15}\text{N}$					
Species	2	27.633	84.188	<0.001	***
Season	1	1.902	5.795	0.018	*
Size class	4	1.351	4.116	0.004	**
Species*season	2	0.990	3.017	0.053	*
Season*size class	4	0.412	1.255	0.292	NS
Species*size class	3	0.617	1.879	0.137	NS
Species*season*size class	1	0.14	0.041	0.840	NS
Error	114	0.328			

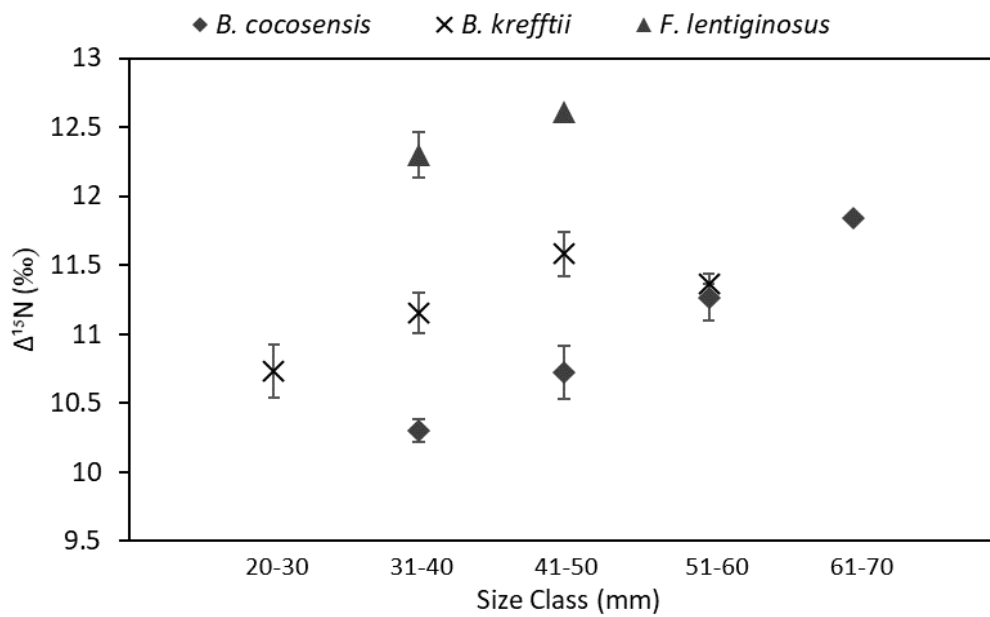


**Figure 2.7:** Individual specimen  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopic values for each species of goby.

Standard ellipse areas (non-Bayesian) estimate the trophic niche spaces for *B. cocosensis*, caught at Dee Why, and sympatric species *B. krefftii* and *F. lentiginosus*, caught at Chowder Bay, during summer (●) and winter (●).



**Figure 2.8:** Mean ( $\pm$ SE)  $\delta^{13}\text{C}$  isotope values per size class (mm) in *B. cocosensis*, *B. krefftii* and *F. lentiginosus*.

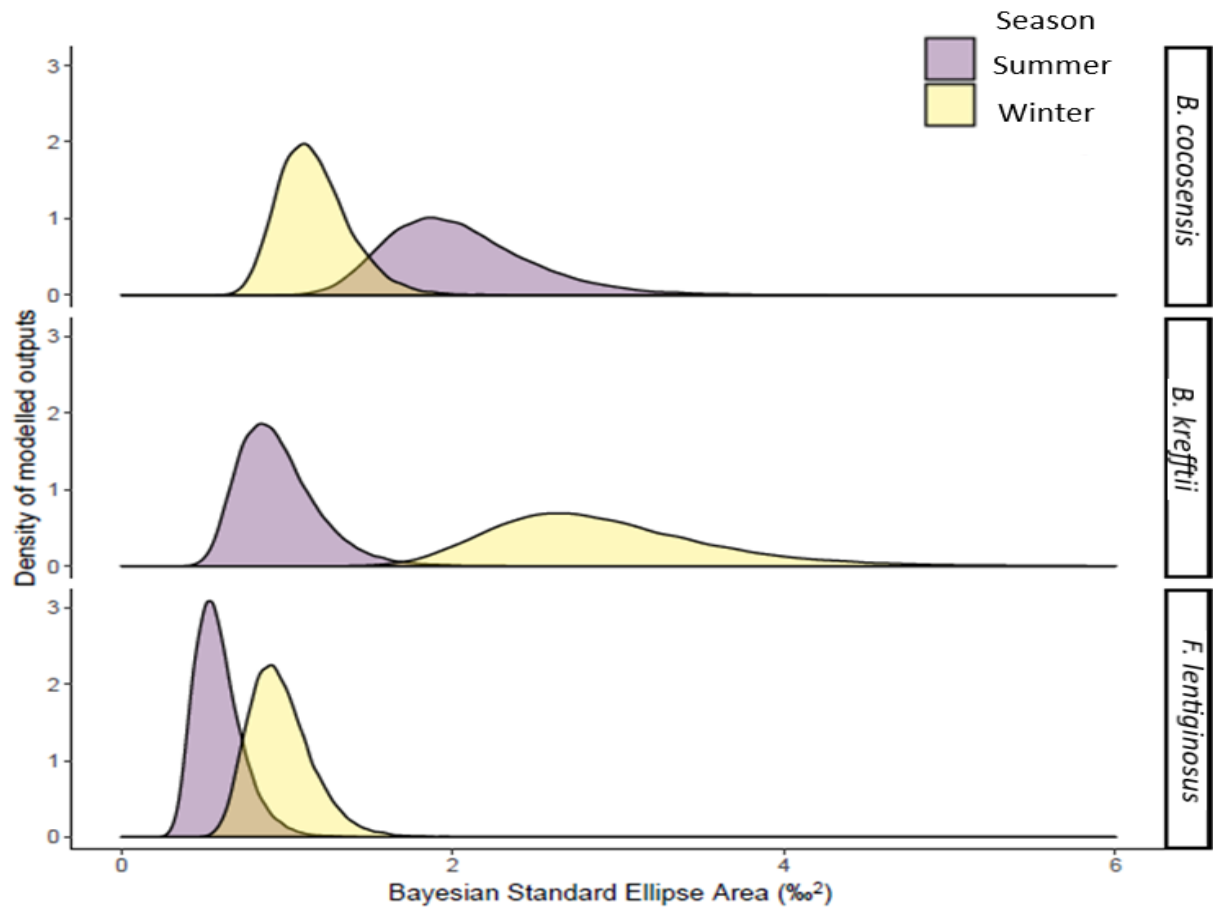


**Figure 2.9:** Mean ( $\pm$ SE)  $\delta^{15}\text{N}$  isotope values per size class (mm) in *B. cocosensis*, *B. krefftii* and *F. lentiginosus*.

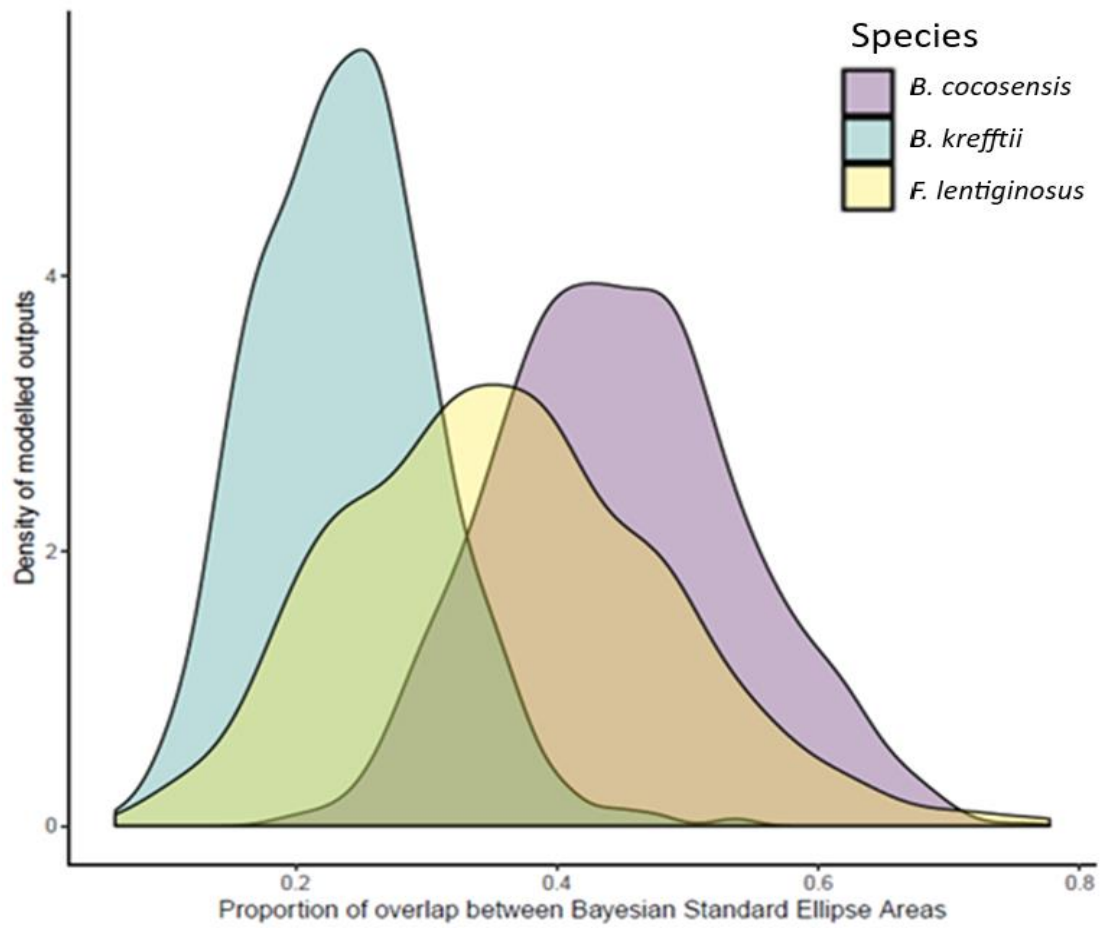
**Table 2.5:** Post-hoc pairwise differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values between size classes per species. BC = *Bathygobius cocosensis*; BK = *Bathygobius krefftii*; FL = *Favonigobius lentiginosus*. \* denotes significant differences between size classes.

		Size class (mm)				
Spp.		20-30	31-40	41-50	51-60	61-70
BC ( $\delta^{13}\text{C}$ )	31-40	-	-	-	-	0.019*
	41-50	-	0.189	-	-	0.091
	51-60	-	<0.001*	0.004*	-	0.784
BC ( $\delta^{15}\text{N}$ )	31-40	-	-	-	-	<0.001*
	41-50	-	0.038*	-	-	0.004*
	51-60	-	0.002*	0.042*	-	0.346
BK ( $\delta^{13}\text{C}$ )	31-40	<0.001*	-	-	-	-
	41-50	<0.001*	0.199	-	-	-
	51-60	0.674	0.214	0.092	-	-
BK ( $\delta^{15}\text{N}$ )	31-40	0.929	-	-	-	-
	41-50	0.004*	0.088	-	-	-
	51-60	0.335	0.740	0.741	-	-
FL ( $\delta^{13}\text{C}$ )	31-40	-	-	-	-	-
	41-50	-	0.004*	-	-	-
	51-60	-	-	-	-	-
FL ( $\delta^{15}\text{N}$ )	31-40	-	-	-	-	-
	41-50	-	0.149	-	-	-
	51-60	-	-	-	-	-

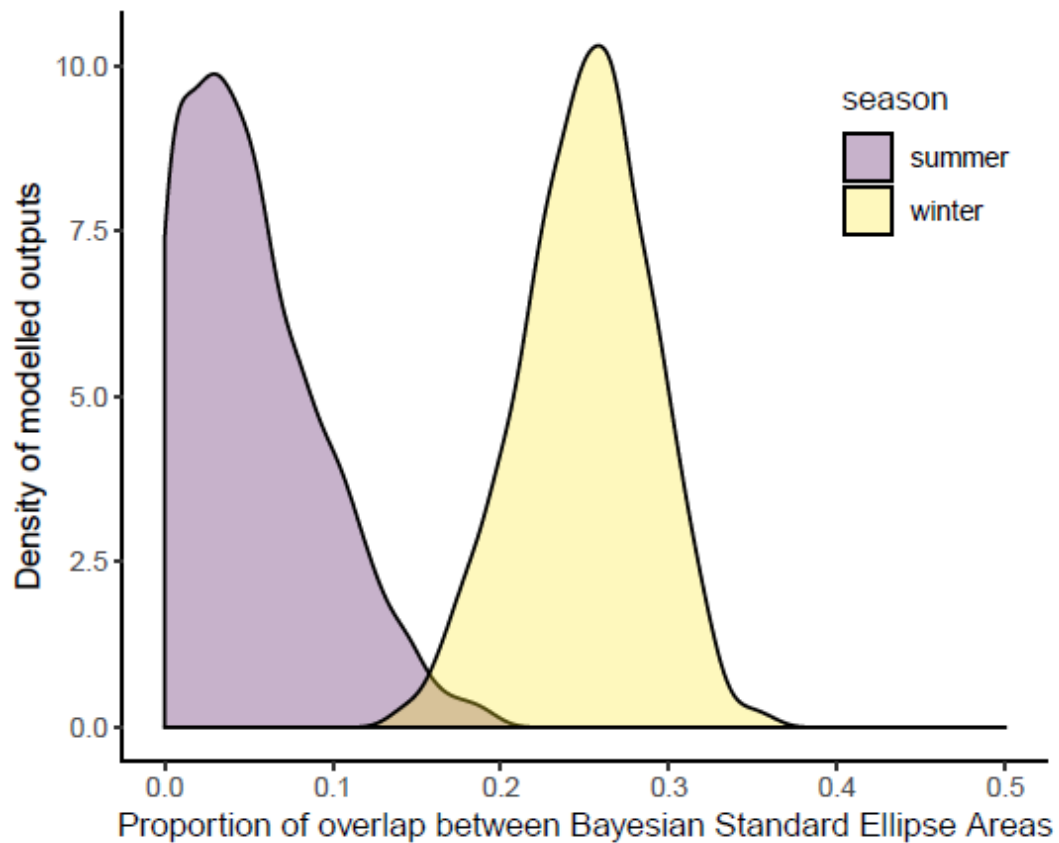
Trophic niche area was largest for *B. krefftii* in winter, while *F. lentiginosus* had the smallest trophic niche in winter (Fig 2.10). Trophic niche area for *B. krefftii* and *F. lentiginosus* increased in winter but decreased for *B. cocosensis*. Relative overlaps of Bayesian SEAs for each species between seasons suggest that *B. cocosensis* had the most stable trophic niche (~50% stable) while *B. krefftii* had the most variable trophic niche (~25% stable; Fig 2.11). Within these relative overlaps, *F. lentiginosus* had the widest range of estimates, ranging from a 10% to 80% overlap. The two sympatric species (*B. krefftii* and *F. lentiginosus*) had almost no trophic niche overlap during summer (~5%) but an increase in overlap to ~30% in winter (Fig 2.12).



**Figure 2.10:** Bayesian standard ellipse areas (SEAs) depicting relative trophic niche areas for *B. cocosensis*, *B. krefftii* and *F. lentiginosus* between summer and winter. Relative overlap between summer and winter were calculated from  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotope values.



**Figure 2.11:** The overlap of summer and winter Bayesian standard ellipse areas (SEAs), for each study species, relative to the total area of both seasonal SEAs.



**Figure 2.12:** Bayesian standard ellipse areas (SEAs) depicting the proportion of trophic niche area overlap for sympatric species *B. krefftii* and *F. lentiginosus* between summer and winter.

## Discussion

Our study explored the interspecific seasonal and intraspecific ontogenetic diet shifts in three intertidal goby species, and the underlying complexities of resource partitioning in sympatric versus allopatric associations. The results from the stomach analyses here are consistent with previous reports (Norton and Cook, 1999; Velasco et al., 2010; Compaire et al. 2016) that amphipods, copepods, polychaetes and gastropods form the majority of the diet of rocky intertidal fishes. The results suggest that allopatric species *Bathygobius cocosensis* has a relatively stable trophic niche between seasons, whereas the two sympatric species vary between very low (~5%) to almost one-third (~30%) niche overlap in summer and winter, respectively. Previous work has suggested that higher niche plasticity in diet would be beneficial in a dynamic environment such as the intertidal zone (Grossman et al. 1980), and this has been an area of recent research interest (e.g. Compaire et al. 2016; Barrett et al. 2018; Vinagre et al. 2018). Here,

we found contrasting strategies of coexistence through seasonal resource partitioning in two sympatric species, and ontogenetic dietary shifts in an allopatric species.

#### *Dietary Shifts - Seasonal*

As expected, the sympatric species *Bathygobius krefftii* and *Favonigobius lentiginosus* exhibited some overlap in prey preference, but stomach content and isotope analyses suggested some seasonal resource partitioning. *F. lentiginosus* fed almost entirely on amphipods during summer, regardless of size class, while *B. krefftii* consumed a wider variety of prey (chironomids, copepods, algae, polychaetes) but excluded amphipods from its diet. The high relative volume of chironomids in the diet of *B. krefftii* during summer suggests the importance of terrestrial contributions to intertidal food webs for this rockpool dweller, as discussed elsewhere (Mathieson and Nienhuis, 1991; Raffaelli and Hawkins, 1996). During winter, a reverse dietary trend was observed. *F. lentiginosus* consumed a greater variety of prey types (polychaetes, copepods, gastropods, chironomids, tanaids), while *B. krefftii* preyed predominantly on taxa less common in the diet of *F. lentiginosus* (copepods and amphipods). In contrast, the diet of the allopatric rockpool resident *B. cocosensis* comprised several different taxa in both seasons, with an increased presence of tanaids in summer and gastropods in winter. Similar seasonal shifts in diet observed here, such as a preference for polychaetes in winter for *F. lentiginosus* and *B. cocosensis*, and an increase in chironomids in summer in *B. krefftii*, have also been described for intertidal gobies from the Baltic Sea (Zander, 1990) and Gulf of Cadiz (Compaire et al. 2016).

Selective foraging for the most abundant taxa can decrease competition for food resources (La Mesa et al. 2008). Although the differences in diet profiles between *F. lentiginosus* and *B. krefftii* may reflect resource availability altered by sediment parameters (e.g. Yodnarasri et al. 2008), sympatric populations of *Favonigobius* spp. demonstrate little resource partitioning in restricted soft-sediment environments, despite a high diet overlap (Chargulaf et al. 2011). Seasonal micro-niche partitioning is common in temperate intertidal fishes (Davis, 2000), and



we suggest that the seasonal diet discrepancies in *F. lentiginosus* and *B. krefftii* may be explained by seasonal habitat partitioning and opportunistic foraging in rockpools by *B. krefftii* in summer. During this time, the most common prey type was chironomid larvae, which occur in high abundances in intertidal rockpools during warmer months of the year (Colbo, 1996). At the same time, *F. lentiginosus* preyed almost entirely on amphipods, suggesting little competition on the sand flats from *B. krefftii*. Alternatively, differences in diet may be explained by different mouth morphology and thus feeding strategy. *B. krefftii* selectively feeds from the top of the substrate or from the water column, while *F. lentiginosus* forages by scooping up mouthfuls of sand and filtering prey items through gill-rakers (Chargulaf et al. 2011). Like Chargulaf et al. (2011), we found traces of sand in the stomachs of *F. lentiginosus*, though not in high enough volumes to warrant the ‘ballast’ function they described. However, it may serve a digestive purpose by grinding down the integument of invertebrates, homologous to the gizzard in birds. As predicted, seasonal shift in diet was not observed in *B. cocosensis*, which may be explained by a combination of factors including their high abundance, low levels of interspecific competition (White et al. 2015) and a highly aggressive nature (Griffiths et al. 2003).

#### *Dietary shifts - ontogenetic*

Ontogenetic dietary shifts have been reported in many fish species (Preciado et al. 2006; Gning et al. 2008), however, they generally occur in tangent with other changes such as habitat, morphology or prey availability (Nunn et al. 2012). Here, we found notable dietary changes throughout ontogeny in two of the species studied. In *B. cocosensis*, stomach content analyses suggested a change from generalist to specialist diet in larger individuals, which consumed a high volume of gastropods in both seasons and polychaetes in winter. Polychaetes burrowed in the sediment may be difficult for smaller fish to extract, which may explain why they were only present in the gut of larger individuals which were, presumably, more experienced (Chargulaf et al. 2011). Alternatively, the preference for polychaetes in larger individuals may be linked to micro-habitat shifts during ontogeny (Zander and Hagemann, 1989). Large *B. cocosensis* tend to

occupy larger, deeper pools further down the platform (Malard et al. 2016), whereas smaller individuals are restricted to the higher pools with rock substrate where polychaetes cannot burrow. The largest size class of *B. krefftii* consumed different prey types compared to smaller classes. There was an abrupt decline in tanaids between the smallest size class to the two larger size classes. Although winter diets were relatively specialised in all size classes compared to summer, large *B. krefftii* predominantly consumed amphipods. In contrast, the two large size classes in summer favoured chironimds and algae respectively, the latter of which suggests they subsidise high tide foraging excursions with local foraging in their rockpools. Collection occurred in the last month of spring, which is the season during which *Bathygobius* species breed (Taru et al. 2002; Thia et al. 2018), so the high intake of algae in large individuals suggests they were feeding on available resources without compromising nest guarding. In contrast to both *Bathygobius* species, all size classes of *F. lentiginosus* consumed the same taxa but in different proportions, as described in other species (Grossman et al. 1980). Most notably, the largest size class preferred polychaetes, while the two smaller classes preyed on polychaetes and copepods, the latter of which is a common prey taxon for juveniles in many goby species (Grossman et al. 1980; Chargulaf et al. 2011).

We also found high levels of resource partitioning between the two sympatric species in all size classes, with very little overlap of prey type. Conversely, Velasco et al. (2010) reported a significant dietary overlap in sympatric gobiids and bleniids, however, they argue that it is inconsequential due to the relatively high abundance of benthic invertebrate fauna.

#### *Isotopic Niche Space – Seasonal and ontogenetic shifts*

Occupied trophic niches imply resource use, however, niche width can change rapidly in response to competition and prey abundance which, in turn, are influenced by ecological drivers embedded in evolutionary processes (Bearhop et al. 2004). Seasonal changes in a consumer's trophic niche width reflect isotopic changes in diet due to increased range of prey, prey from different habitats, or both (Newsome et al. 2007; Pool et al. 2017). In this study, seasonal shifts

in diet were mirrored in  $\delta^{13}\text{C}/\delta^{15}\text{N}$  isotopic values between and within species. *F. lentiginosus* exhibited greatest carbon ( $\delta^{13}\text{C}$ ) depletion and nitrogen ( $\delta^{15}\text{N}$ ) enrichment in both seasons, however, variation in trophic niche space was the lowest of the three species. Nonetheless, the trophic niche of *F. lentiginosus* increased marginally during winter, with a greater range of prey and likewise in  $\delta^{15}\text{N}$  isotopic values.

*B. krefftii* showed a much larger niche range in winter; individuals varied from combinations of depleted  $\delta^{13}\text{C}$ /enriched  $\delta^{15}\text{N}$ , to the inverse.  $\delta^{13}\text{C}$  isotope levels decreased with body size and larger *B. krefftii* had the most depleted levels of all size classes in summer, indicative of higher algae consumption (Horn et al. 1982). Niche overlap in sympatric species *B. krefftii* and *F. lentiginosus* was higher during winter, where the latter broadened their prey intake and/or utilised a greater range of habitat type. Although benthic meiofaunal community structure in this region has been correlated with upwelling and wave action (Dexter, 1983), these factors do not necessarily vary on a seasonal scale (Short and Wright, 1981; Dexter, 1984). Thus, the trophic overlap in winter between *F. lentiginosus* and *B. krefftii* may not be a function of seasonal changes in benthic community, but rather a shift in dietary profile of the latter. During this time, it is likely that larger *B. krefftii* individuals occupied a higher trophic niche through foraging on the benthos rather than in rockpools.

In contrast to the sympatric species, *B. cocosensis* showed a decreased trophic niche width during winter compared to summer. In both seasons, *B. cocosensis* had enriched carbon ( $\delta^{13}\text{C}$ ) and depleted nitrogen ( $\delta^{15}\text{N}$ ) levels compared to *F. lentiginosus* and *B. krefftii*. Combined with similar dietary preferences, the stable trophic width in *B. cocosensis* suggests a lack of competitive constraint on food resources and consequently little shift in trophic position (Layman et al. 2007). Muñoz and Ojeda (1998) argue that ontogenetic diet shifts mean that individuals occupy different guilds in their lifetime, however, we found no shift from herbivory to carnivory here, nor the inverse (herbivory >50% algae; Horn, 1989; Horn and Ojeda, 1999). Other authors suggest that a species/sex/size interaction in isotope values likely reflects high levels of inter- and intraspecific resource partitioning (Vizzino and Mazzola, 2003), while our

results suggest that it is driven by resource partitioning at the interspecific level between sympatric *B. krefftii* and *F. lentiginosus* and at the intraspecific level in the allopatric *B. cocosensis*.

#### *Foraging plasticity*

Grossman et al. (1980) reported yearly and seasonal dietary shifts in the Californian estuary goby *Lepidogobius lepidus*, as well as high overlap in prey taxa between size classes, though in different proportions. Here, we found a seasonal diet shift in sympatric species and ontogenetic shift pattern in the allopatric species *B. cocosensis*, which is likely associated with micro-habitat shifts (Malard et al. 2016). Importantly, the plasticity in diet and trophic niche area (and, by extension, foraging strategy) is likely highly selected for in a habitat as dynamic as the intertidal zone (Grossman et al. 1980). Moreover, the variation of trophic niches in intertidal zones suggests plasticity in resource partitioning is critical to the coexistence of benthic fish species. For instance, Velasco et al. (2010) found carnivory, herbivory and ontogenetic shifts in either direction in 16 species of benthic fishes. Despite a high overlap in prey taxa, coexistence was facilitated by high exploitation of abundant prey coupled with specialised use of limited resources between species, both strategies of which enabled reduced competition (Velasco et al. 2010). Furthermore, flexible foraging strategies undoubtedly aid consumers in overcoming fluctuating prey abundance as well as anti-predator behaviours in prey. Copepods, for example, vary in abundance within the substrate and water column depending on tidal fluctuations (Palmer and Brandt, 1981), and physically alter their micro-habitats to facilitate escape from predators (Jones et al. 1994).

To conclude, behavioural plasticity in diet preference and niche occupation plays an important role in highly adaptable and successful intertidal goby species. Resource partitioning appears to play a pivotal role in the coexistence of two sympatric species and facilitates minimal interspecific competition. This contrasts with an allopatric species which demonstrates stable trophic niche width between seasons but high levels of intraspecific resource partitioning

604 between size classes to minimise agonistic encounters with conspecifics. We have highlighted  
605 that niche plasticity remains adaptive throughout ontogeny and between seasons, however,  
606 residing in the dynamic intertidal zone probably means that niche plasticity extends over shorter  
607 temporal gradients to overcome associated challenges, such as changes in meiofaunal  
608 communities between tidal cycles (Dexter, 1984). Thus, comparison of diet between high and  
609 low tides may provide further insight into the extent of trophic niche plasticity in intertidal  
610 gobies.

## **CHAPTER 3**

### **Sexually dimorphic spatial learning is seasonally driven in the intertidal Cocos Frillgoby (*Bathygobius cocosensis*)**

This chapter has been submitted to

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## **Abstract**

Spatial learning is an important cognitive function found across a multitude of species. Natural selection can act to enhance specific cognitive abilities depending on species ecology, but under certain conditions, spatial learning is also known to vary between sexes according to reproductive status. Despite abundant studies of spatial learning across animal taxa, those focusing on sexually dimorphic spatial learning have been largely limited to rodents. Here, we show that spatial cognition varies across seasons in an intertidal goby and varies between the sexes. In general, we found individuals tested in winter reached learning criteria significantly more slowly compared to those tested in other seasons. Males and females show similar cognitive abilities in all seasons except spring, during which males demonstrated a dramatic reduction in performance relative to females. Spring marks the beginning of the breeding season for this species, when females move between nests to choose a suitable mate. Males, in contrast, remain relatively immobile as they guard their nest until the eggs hatch. This study presents the first evidence of seasonally influenced and sexually dimorphic spatial learning in fish, and we suggest the changes in cognitive ability are driven by differences in reproductive behaviour dictated by the mating system.

**Key words:** cognitive plasticity; reproduction; goby; energetic cost; learning

## Introduction

Spatial learning is the process through which individuals collect information about the layout of their environment to locate required resources efficiently (Floresco, 2014). Every day, animals engage in tasks essential to their survival including searching for food, shelter or mates, whilst simultaneously avoiding predators. Every animal will benefit from direct and purposeful movements around their environment to minimise energy expenditure (Odling-Smee et al. 2006), so it's advantageous for them to learn the spatial layout of their habitat (Healy, 1998; Giraldeau, 1997). Owing to the obvious fitness advantages, spatial learning is widely observed in varied capacities across the animal kingdom including mammals (e.g. Garber, 1989; Galea et al. 1996; Warren and Juraska, 1997; Lacreuse et al. 1999,) reptiles (e.g. Day et al. 1999; Noble et al. 2012; Carazo et al. 2014), birds (e.g. Krebs et al. 1990; Brodbeck, 1994; Roth et al. 2012), fish (e.g. Hughes and Blight, 1999; Broglio et al. 2003) and invertebrates (e.g. Boal et al. 2000; Jozet-Alves et al. 2008). Because of its ubiquity, spatial learning is a useful tool for probing the cognitive ability of animals in a comparative framework.

Interspecific variation in spatial learning ability can be predicted by the demands of the animal's environment (Healy and Jones, 2002). Similarly, the evolution of intraspecific variation, specifically between sexes, can also be understood in this context. Males and females are known to differ in their cognitive abilities (Halpern, 1991; Kimura, 1999), and it is often the case that spatial learning skills in males exceed those of females, especially in mammals (e.g. Dawson, 1972; Einon, 1980; Mishima et al. 1986; Gaulin and Fitzgerald, 1986; 1989; Galea et al. 1994; Kavaliers et al. 1996; 1998; Lacreuse et al. 1999). Several hypotheses have attempted to explain sex-biased variation in mammalian spatial ability (reviewed in Jones et al. 2003). Gray and Buffery (1971) proposed that mating systems influence spatial learning ability. They argue that males of polygamous species show greater spatial ability than females as a result of moving across large areas to breed with multiple



females and to maximise their reproductive success (Gaulin, 1995). For example, males in the promiscuous meadow vole (*Microtus pennsylvanicus*) have larger ranges and solve spatial learning tasks faster than females (Gaulin and Fitzgerald, 1986; 1989). In monogamous species, where both parents tend to be tied to single nest locations, there are no differences in spatial learning between the sexes (e.g. prairie vole, *M. ochrogaster*); (Gaulin and Fitzgerald, 1986; 1989). This hypothesis is not only supported by a substantial number of mammalian studies, but also others in reptiles (Noble et al. 2012; Carazo et al. 2014) and birds (Astie et al. 1998; Gonzalez-Gomez et al. 2014), which attribute sexually dimorphic spatial learning ability to selective pressures emanating from the roles each sex plays in the mating system.

Silverman and Eals (1992) proposed that differences in spatial ability between sexes is brought about by variation in dispersion. Male rhesus monkeys (*Macaca mulatta*) disperse further than females and appear to have greater spatial learning abilities (Drickamer and Vessey, 1973; Kaplan et al. 1995; Lacreuse et al. 1999). The fertility and parental care hypothesis proposes that females display decreased spatial ability during reproductive periods as a result of reduced mobility and changes in hormones while weaning offspring (Sherry and Hampson, 1997). For instance, female deer mice (*Peromyscus maniculatus*) show decreased spatial acquisition during the breeding season compared to males, but no differences are seen outside of the breeding season, suggesting that hormone changes associated with reproduction can influence spatial learning skills in females (Galea et al. 1994).

Fish are often used as models to understand the evolution of spatial learning (Odling-Smee et al. 2011). Despite the many and varied mating systems in fish, surprisingly few studies have investigated either sexually dimorphic or seasonally affected spatial learning in this taxon (Costa et al. 2011; Lucon-Xiccato and Bisazza, 2017). Sovrano et al. (2003) reported slight superior male performance in the Redtail splitfin (*Xenotoca eiseni*) when tested in a reorientation task. Contrary to expectations, in the freshwater blenny (*Salaria*

*fluviatilis*), males learned a two-choice maze faster than females, despite having smaller home ranges (Costa et al. 2011; Fabre et al. 2014). Females are the mobile sex in this species, whereas males defend nest territories and remain sedentary in sole parenting duties until the eggs hatch (Wickler, 1957; Vinyoles and Sostoa, 2007). More recently, male zebrafish (*Danio rerio*) were reported to make fewer errors than females when searching for food in a maze task, though both sexes solved the task in similar time frames (Roy and Bhat, 2017). Thus, support for the hypothesis that mating systems influence variation in spatial learning in fishes remains equivocal.

Gobies encompass a notable part of fish diversity (Thacker, 2009). Estimates for the total number of goby species varies, but likely lies between 2000-2250 (e.g. Nelson, 2006; Thacker, 2009; Agorreta et al. 2013) with new species still being discovered (Suzuki and Senou, 2007; Larson et al. 2017). Owing to their diversity, gobies are an exceptional model to investigate the evolution of spatial learning in a comparative context. Gobies are capable of rapid spatial acquisition in new environments (Markel, 1994) and return to their home rockpools after being displaced (Griffiths, 2003a; White and Brown, 2013). For instance, early experiments showed that the Blackeye goby (*Bathygobius sutor*), can leap blindly into neighbouring rockpools at low tide to evade simulated predation based on the spatial information they gather at high tide (Aronson, 1951; 1971), referred to as a ‘cognitive map’ (Broglia et al. 2011). Species occupying structurally dissimilar habitats show variation in spatial learning ability (White and Brown, 2014b) as well as a preference for different cues when navigating their environment (White and Brown, 2015b).

Despite these studies, sexually dimorphic spatial learning in gobies remains largely unexplored. Mating systems in this group are highly diverse, including male parental care/female choice (e.g. Lindström, 1988; Magnhagen, 1990; Forsgren et al. 1996; Mazzoldi et al. 2000; Järvenpää and Lindström, 2004; Lindström et al. 2006), male sneaker strategies

(Magnhagen, 1995; Mazzoldi et al. 2000; Jones et al. 2001), sex reversals cued by social factors (e.g. Kuwamura et al. 1994; Munday et al. 1998; Lorenzi et al. 2006; Rodgers et al. 2007) and monogamy (Kuwamura et al. 1994; Takegaki and Nakazono, 1999; Takegaki, 2000). Thus, if spatial learning abilities are sexually dimorphic, then sexual selection for spatial ability should reflect the mating system, and gobies provide an exceptional model system to investigate this.

The Cocos Frillgoby (*Bathygobius cocosensis*) inhabits the highly dynamic intertidal zone, with hourly changes in temperature, salinity and dissolved oxygen, as well as wave action and tidal fluctuations (Gibson, 2003; Gonçalves et al. 2015; Malard et al. 2016). In complex environments like this, fast and flexible spatial learning ability enhances likelihood of survival, and thus is predicted to be favoured by natural selection (Dukas, 1998; Healy and Rowe, 2010; Noble et al. 2012). The mating system of *Bathygobius* has been studied in two species from the genus (*B. fuscus* and *B. sorporator*). Both breed during spring and display male competition for nest sites and female-choice. Furthermore, male *B. fuscus* alternate between nest-holding or sneaking behaviour based on their social status (Magnhagen, 1992; 1994; 1995). Typically, larger males hold nest sites, while those smaller than 55mm tend to engage in sneaker strategies (Taru et al. 2002). In this genus, therefore, we might expect females to have greater spatial learning skills than males because they move between locations evaluating the quality of the males and their nests. Males, on the other hand, are tied to their nest location and so would show decreased spatial awareness during this time. Thus, we expect to observe the biggest difference in spatial learning skills between males and females during the breeding season. Here, we investigated both sexually dimorphic and seasonal variation in spatial learning ability in the intertidal Cocos Frillgoby (*B. cocosensis*), using a spatial t-maze and behavioural conditioning paradigm.

## Methods

### *Test subjects*

*B. cocosensis* is commonly found along the rockpools and reefs in the intertidal zone along the east coast of New South Wales (NSW), Australia. While its breeding system has not yet been formally described, our observations suggest females are the choosy sex while males guard nest sites, as seen in other members from the genus. Throughout 2015-2016, individuals were collected from Dee Why (33.7502° S, 151.2991° E), during the fifth week of each season (ca 16 gobies per season). All individuals were collected during low tide using dip nets. Captured gobies were transported in a 10L, aerated bucket to the Seawater Facility at Macquarie University. Transportation time in each instance was less than 1 hour.

Once in the lab, the gobies were separated into two groups comprised of similar size individuals to minimise aggressive behaviour and housed in opaque 70L white plastic tubs (64.5 x 41.3 x 27.6cm). The system was run at ambient temperature, and water temperature never fell below 19°C or rose above 23°C. The sea water circulated in the system was first filtered through a 100um sand filter and 80W UV steriliser to eliminate bacteria and protozoa. The water in this system (1000L) was run through a recirculating loop from the sump, through a 100um filter bag, a biofilter and a 40W UV steriliser. The water was then pumped through 13mm valves and into the holding tanks at a maximum rate of 5L per min and water depth was maintained at 25cm. A fixed 25mm out-flow pipe in each tub was covered with 3mm mesh to prevent gobies escaping. To keep the gobies from jumping out, large (65 x 42cm) plastic grids (1cm x 1cm) lined with 3mm mesh were placed over each tank.

To account for evaporation and maintain constant salinity levels, aged freshwater was added to the holding tanks according to hydrometer readings. Water temperature was recorded daily (mean 21.0°C) for the duration of the project and lighting was kept to 10 hours

daily (from 08:00) under full UV spectrum lights. To mimic the gobies' natural environment, the substrate in the housing tubs was a combination of fine sand and larger shell grit pieces. Each tub also had several artificial shelters (12cm halves of 25mm white, non-reflective PVC) to encourage the gobies to seek protection and to help familiarise them to the shelters which would be used in the spatial task. The gobies were housed in these tanks for a period of 5 days, during which they were also introduced to frozen *Artemia* for food.

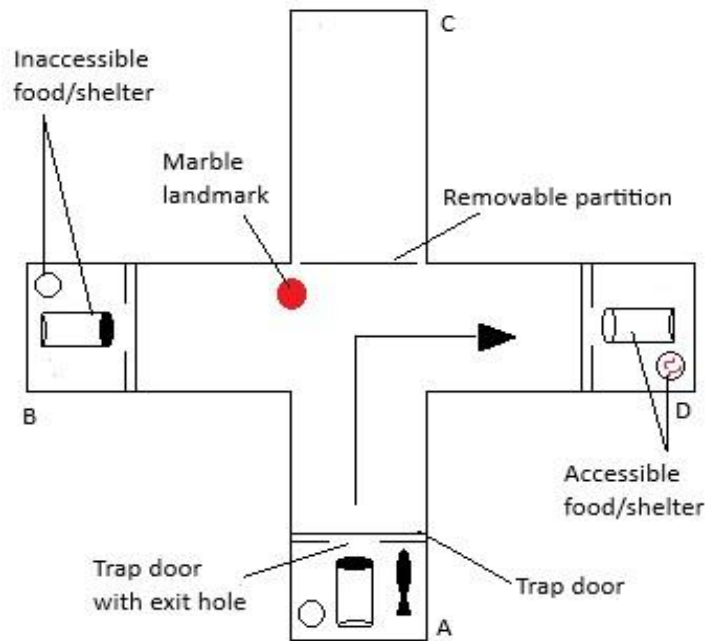
### *Tagging*

After the adjustment period, the gobies were lightly anaesthetised in a 1L bath of sea water with 50mg/l of tricaine methane sulfonate (MS222), buffered with sodium bicarbonate, for 30-60 seconds. Once sedated, individuals were tagged with a Visible Implant Fluorescent Elastomer tag (VIE: Marine Technology, Inc. 2008) for unique identification (White and Brown, 2013). Three colours of VIE tags (orange, green or blue) were implanted beneath transparent scales in one of six possible locations along the individual's dorsal surface for easily visible identification to an overhead observer. In all instances, this process took less than 2 minutes per fish and all gobies recovered to their upright positions and began swimming normally within 5 minutes of being tagged. Gobies were then returned to the holding tubs for another 5 days to allow for full recovery, during which their foraging and swimming behaviour were closely observed.

### *Test apparatus*

The test apparatus in the form of a two-arm maze was based on a design used previously by White and Brown (2014a) and adapted from Odling-Smee and Braithwaite (2003) and Odling-Smee et al. (2008). Two duplicate mazes (total LxW 50cm; Fig 3.1) made of 3mm PVC plastic were submerged in identical rectangular tubs (100x50x18cm) of aerated

178 sea water. The water level was approximately 10cm deep and the base of both mazes was  
179 lined with fine sand. A small (10x16cm) PVC screen could be easily slid in and out of  
180 grooves at each corner in the centre of the maze, to form a T structure. The middle bar of the  
181 t-maze was the starting point for every trial, however, the middle partition was rotated after  
182 each trial to alternate the outlay of the maze, preventing the gobies from relying on extra-  
183 maze cues. The far end of each arm of the maze was fitted with an additional PVC screen,  
184 approximately 10cm from the edge, to form a box-like structure (A – D; Fig 3.1). These  
185 screens had a small (2x4cm) opening cut into the base for the gobies to swim through as they  
186 were leaving the start box towards the junction of the maze. Additionally, there were grooves  
187 approximately 1cm behind the permanent partitions for temporary screens, to stop the gobies  
188 leaving their box between trials. Within each start box was a clear, glass dish (3cm in  
189 diameter x 1.5cm deep) in which a food reward could be placed, and a shelter like those in  
190 the holding tanks (halved 25mm white, non-reflective PVC) to help reduce exposure stress.  
191 All arms were made to look identical, so the gobies could not differentiate between them,  
192 except for an inaccessible shelter in the incorrect arm, which was fitted with a clear plastic  
193 film on both ends. This shelter was rotated into the unrewarded arm before each trial. A video  
194 camera was mounted above both mazes and all trials recorded onto a hard drive.



**Figure 3.1:** Layout of the spatial learning task. The letters indicate the location of the start box in sequence of three trials. The arrow indicates the correct path a fish needed to follow to obtain a food and shelter reward.

### *Procedure*

Prior to commencing trials, each group of gobies was introduced into the maze for a familiarity period of 24 hours (Brown, 2003). The maze was completely open during this time with no partitions, and all start boxes accessible with shelters. After 8 hours, the food dishes were filled with *Artemia* to encourage foraging behaviour from a previously unfamiliar object. After the familiarity session, all gobies were returned to their housing tanks for another 24 hours to maximise hunger while minimising loss of familiarity with the test environment. For the trials, half the gobies were randomly assigned to right-turn training, and the other half were left-turn trained. Each goby was tested individually for three consecutive trials per day, and the start box was randomised each day.

For each trial, one individual was gently netted from the home tank and introduced to the test apparatus. After a 5-minute settlement period, the temporary partition was removed, and the goby was free to explore the maze, the objective being to locate the reward arm. The

exit time from the start box in the maze was judged as being when a goby had half or more of its body outside of the start box, and this time was used to indicate how motivated the gobies were to engage in the choice process. Each exit time value was recorded in seconds, and three exit times were averaged for a daily mean per individual. This process was repeated for the first 10 days of experiment. A small rock was placed off-centre in the junction of the maze as a landmark to guide the gobies to the correct arm; individuals had to turn away from the rock if they were to choose the correct direction. If the test goby chose the correct arm, they were rewarded with shelter and food; 2 individual *Artemia* delivered from a clear 3ml pipette into the food dish. To minimise olfactory cues in the maze, food was only given after the task was complete and removed if not eaten before the next trial. If a goby chose the incorrect arm, the escape door was closed, and the goby held inside for 3 minutes without a food reward or access to the shelter, before being gently ushered into the correct arm. Gobies were given a 5 min rest interval between trials, after which the maze was reset; the reward arm then became the start box and the landmark was shifted to the new layout. This procedure ensured the gobies relied on the landmark provided or egocentric information to solve the maze rather than extra-maze cues (Girvan and Braithwaite, 1998). Ten litres of water were removed and replenished with clean salt water at the end of every trial.

Each goby was trialled three times per day until they achieved 3 correct turns each day for 5 consecutive days. In the event that an individual chose incorrectly in one trial, the day count would be re-started from the trials the following day. Once an individual achieved 5 consecutive days with 3 correct scores (i.e. 15 correct choices), training ceased. During the first 5 days of the trial period, if gobies took longer than 5 min to leave the start box they were marked as having failed the trial. Further, if they chose the wrong side, they were given a food reward after they were encouraged into the correct side. After the 5<sup>th</sup> day, gobies were encouraged out of the start box after 5 minutes and received no food reward if they made an



incorrect choice. Each trial was recorded from the over-head camera and behaviour noted, including emergence time, side chosen, completion time and whether the individual returned to the start box.

#### *Ethical note*

Gobies were caught in compliance with NSW Fisheries (permit no. P08/0010-3.0). Husbandry and experimental conditions were approved by the Macquarie University Ethics Committee (ARA 2014/003). At the end of the experiment, all gobies were released at the site of capture.

#### *Statistical analyses: Days to reach criteria*

In all cases, data were normally distributed and analysed using parametric tests. We used ANOVA to investigate effects of seasonality on spatial learning performance, using the number of days to reach criteria as the dependent variable with season and sex as fixed factors. Post hoc analyses were used to determine the pair-wise differences between the four seasonal treatments.

#### *Daily scores*

Daily score was based on the number of correct choices out of three trials per day, converted to a daily percentage. As the experiment continued, individuals reached criteria and were omitted from the daily averages as they were no longer included in the trials. We used a repeated measures ANOVA with mean daily score on increment days of 5 between days 1 and 25 (i.e. days 1, 5, 10, 15, 20 and 25) per treatment group as the dependent variable with season and sex as fixed factors. Score differences between trial days were evaluated using post-hoc pairwise analyses.

## Motivation and Learning

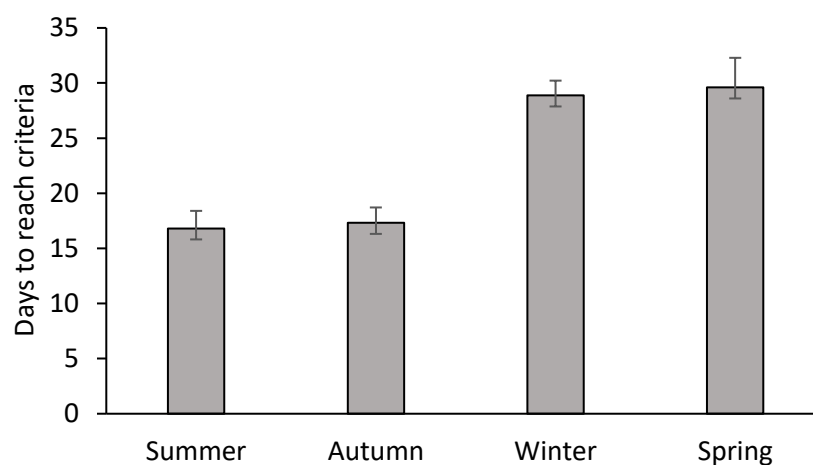
Emergence time was averaged per goby from three trials per day for a daily mean.

The daily mean for days 1, 5, 10, 15, 20 and 25 were then analysed using a repeated measures ANOVA with season and sex as fixed factors. Total trial time was calculated as the time each goby spent in the maze, from the moment of leaving the start box to the moment they chose a side. This time was also averaged per individual from three trials per day, as an indicator for learning rate, and analysed using the same techniques. All analyses were performed using StatView Version 232 5·0·1 (SAS Institute Inc. 1998).

## Results

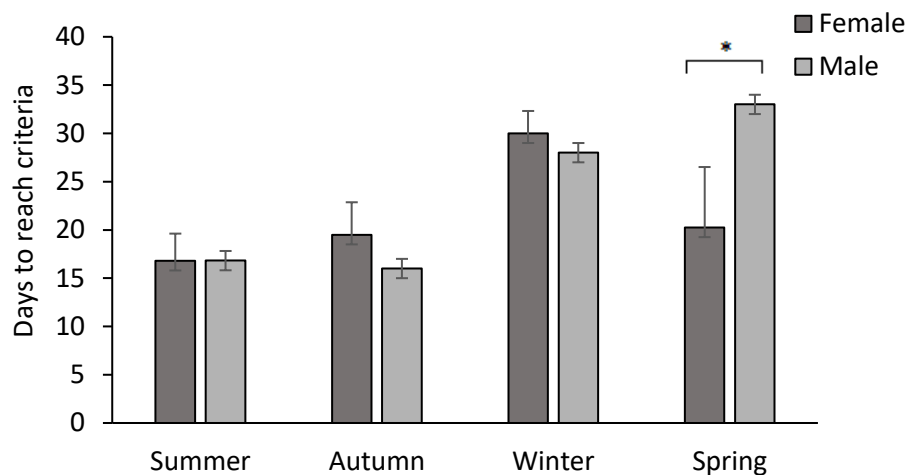
### *Days to reach criteria*

There was a significant difference between season in the average number of days to reach criteria ( $F_{3,53} = 12.211$ ,  $p = <0.0001$ ; Fig 3.2) with gobies completing trials faster in summer and autumn compared to winter and spring (Fisher's PLSD;  $p < 0.001$  in all cases). There was no significant effect of sex on the number of days to reach criteria ( $p > 0.05$ ), however there was a significant interaction between season and sex ( $F_{1,3} = 3.568$ ,  $p = 0.020$ ; Fig 3.3).



**Figure 3.2:** Mean ( $\pm$ S.E.) number of days gobies required to reach criteria per season.

Post-hoc analyses revealed a significant difference between males and females only in spring (Fisher's PLSD;  $p = 0.015$ ) with females reaching criteria faster than males. Within sexes, there was a significant difference between seasons in the number of days to reach criteria in females ( $F_{3,18} = 3.163$ ,  $p = 0.049$ ), with those in winter requiring significantly more days to reach criteria compared to females tested in summer (Fisher's PLSD;  $p = 0.013$ ) and autumn (Fisher's PLSD;  $p = 0.032$ ). Similarly, males showed a significant effect of season in the number of days required to reach criteria ( $F_{3,37} = 21.34$ ,  $p < 0.0001$ ) with males in summer and autumn reaching criteria faster than winter and spring participants ( $p < 0.001$  in all cases).

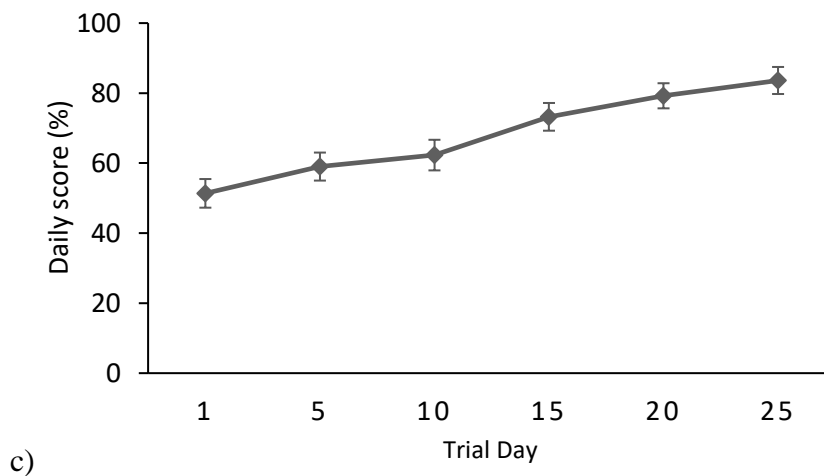
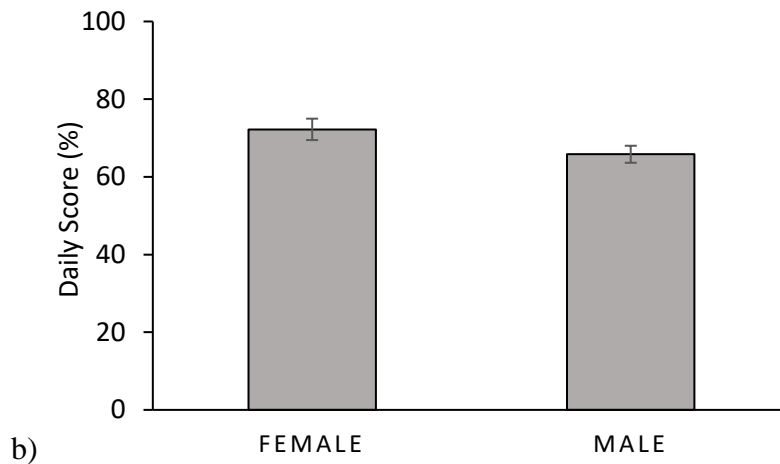
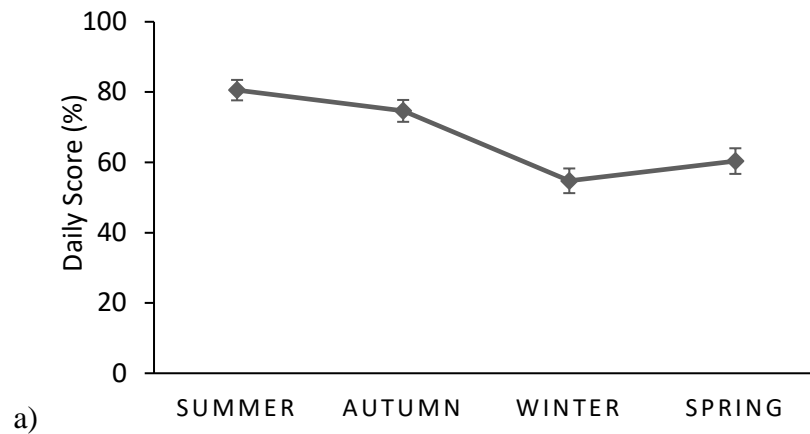


**Figure 3.3:** Mean ( $\pm$ S.E.) number of days to reach criteria in males and females per season.

Significantly different results between sexes are marked by (\*).

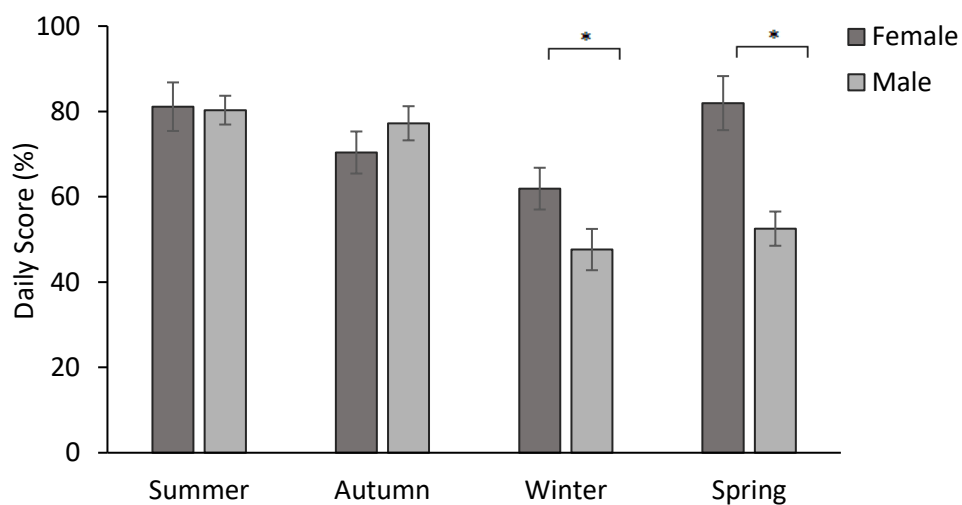
#### *Daily scores*

There was a significant effect of season on daily score ( $F_{3,53} = 8.634$ ,  $p < 0.0001$ ; Fig 3.4a), with scores being higher in summer and autumn than winter and spring. Females had higher daily scores than males ( $F_{1,53} = 6.081$ ,  $p = 0.034$ ; Fig 3.4b). There was also a significant effect of trial day; in general, fish improved their scores as training went on ( $F_{5,265} = 10.832$ ,  $p < 0.0001$ ; Fig 3.4c).

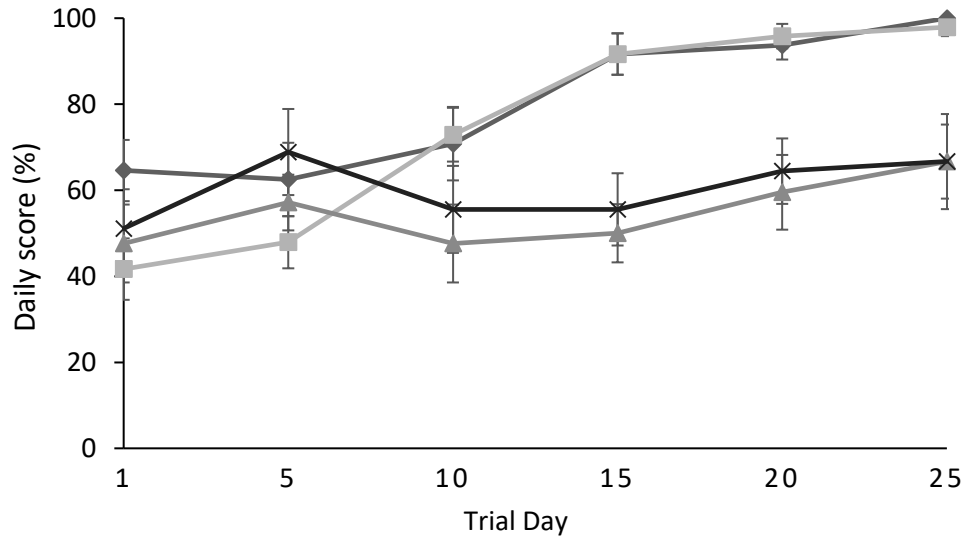


**Figure 3.4:** a) mean ( $\pm$ S.E.) daily score (%) between season treatment groups, b) mean ( $\pm$ S.E.) daily scores (%) of males and females, c) mean ( $\pm$ S.E.) daily performance scores (%) of all gobies across days 1 – 25.

There was a significant interaction between season and sex ( $F_{3,53} = 4.210, p = 0.010$ ; Fig 3.5) as well as season and trial day ( $F_{15,265} = 2.747, p < 0.001$ ; Fig 3.6). All other interactions were non-significant. Performance generally improved over time during summer and autumn, but not in winter and spring. Within sexes, females showed little differences between seasons in their average daily performance ( $F_{3,18} = 1.916, p = 0.163$ ) however, males showed highly significant differences, with higher scores in summer and autumn compared to winter and spring ( $F_{3,35} = 16.341, p < 0.0001$ ).



**Figure 3.5:** Mean ( $\pm$ S.E.) daily performance scores (%) between days 1 – 25 for males and females between treatment groups. Significantly different results between sexes are marked by (\*).

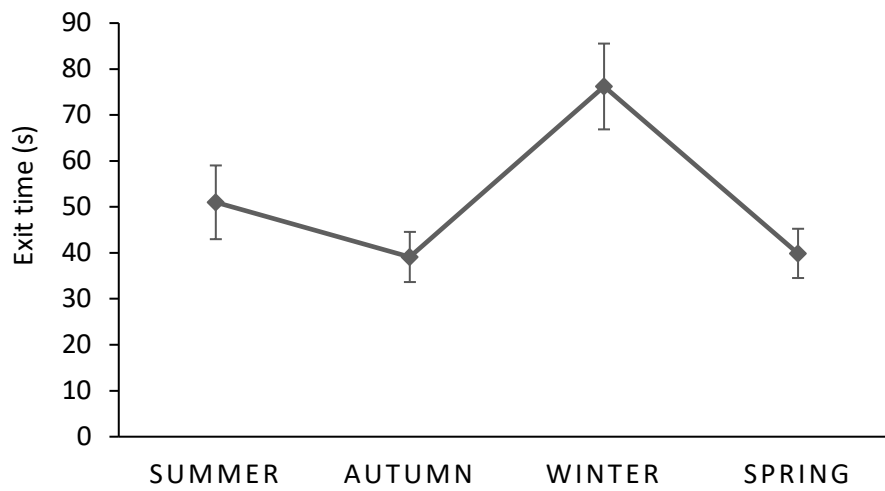


**Figure 3.6:** The mean ( $\pm$ S.E.) combined daily performance scores (%) of all gobies in each treatment group (♦ summer, ■ autumn, ▲ winter, x spring), shown for days incremented by 5. Note: for analysis purposes, gobies that had reached criteria before the 25<sup>th</sup> day were assigned a score of 100% for consecutive days.

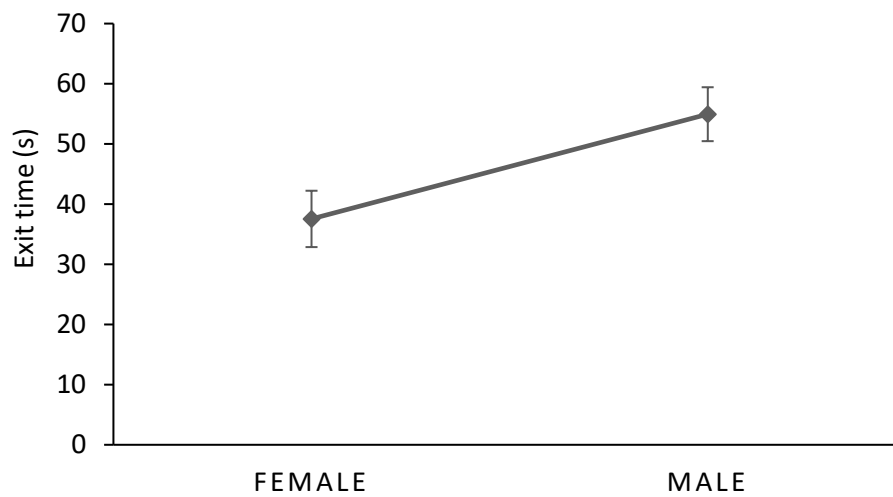
### Motivation

We focused on average exit time as a measure of motivation and how involved gobies were in the choice process. There was a significant effect of season ( $F_{3,53} = 3.208$ ,  $p = 0.030$ ; Fig 3.7) and males were less motivated than females ( $F_{1,53} = 4.106$ ,  $p = 0.048$ ; Fig 3.8) in terms of average exit time. Gobies were particularly poorly motivated to commence the task in winter. However, individuals emerged from the start box faster as trial days went on, suggesting increased motivation as they learned the task ( $F_{5,265} = 17.721$ ,  $p < 0.001$ ; Fig 3.9). There were no significant interactions.

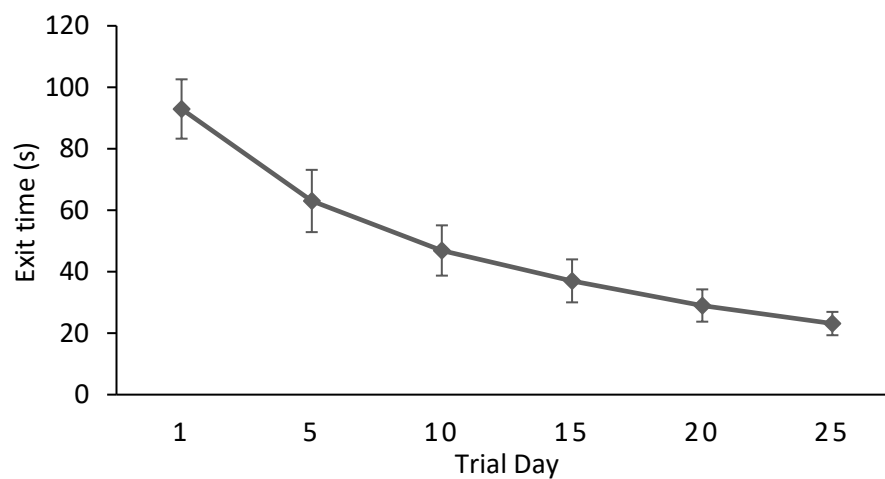
Post-hoc analyses showed a significant difference in average exit time between sexes only in autumn and summer (Fisher's PLSD;  $p = 0.031$  and  $p = 0.039$ , respectively). Both sexes showed significantly faster exit times in summer, autumn and spring compared to winter (Fisher's PLSD;  $p < 0.001$  in all cases).



**Figure 3.7:** Mean exit time ( $\pm$ S.E.) for days 1 – 25 per treatment group.



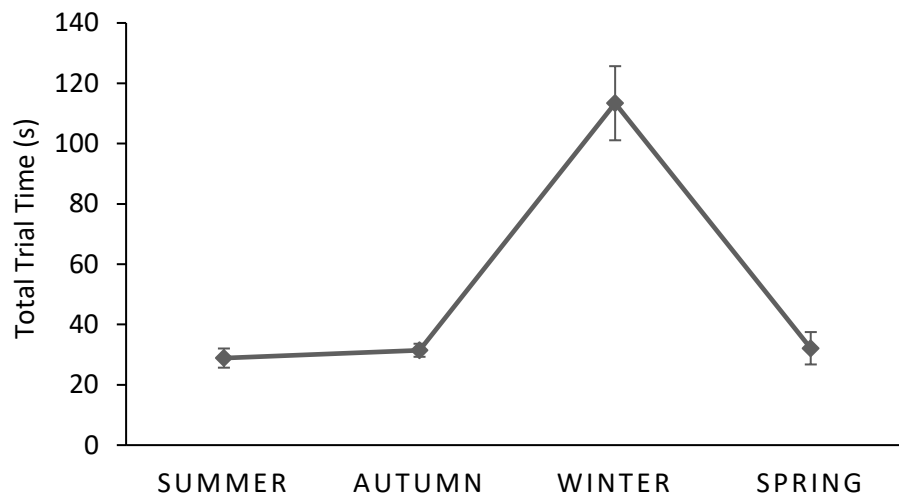
**Figure 3.8:** Mean ( $\pm$ S.E.) exit time per season in females and males.



**Figure 3.9:** Mean ( $\pm$ S.E.) exit time of all gobies across trial days 1 – 25.

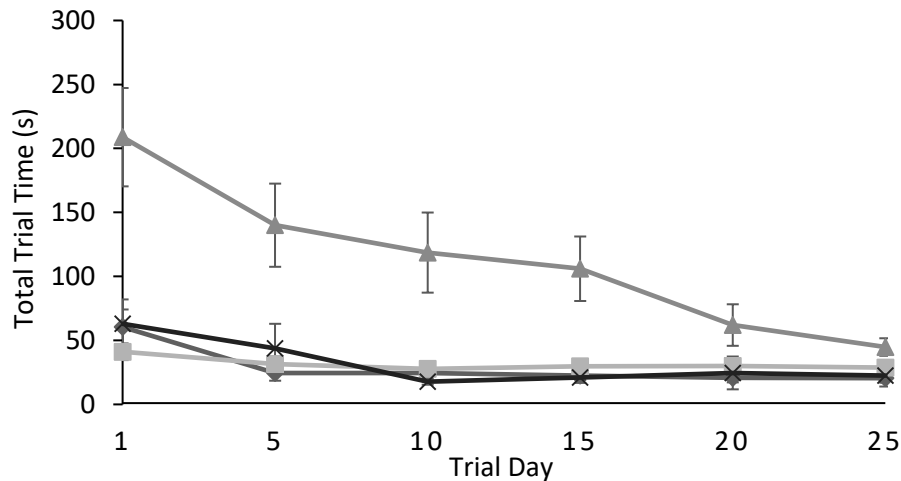
### Total trial time

We analysed average total trial time based from the time each goby left the start box to the time it took for them to enter either the correct or incorrect box. There was a significant effect of season with trial time being particularly long in winter ( $F_{3,53} = 16.435, p < 0.0001$ ; Fig 3.10). There were no differences between sexes, nor was there a significant interaction between season and sex ( $p > 0.05$  in both cases). Total trial time decreased with increasing trial number ( $F_{5,265} = 11.554, p = < 0.001$ ). There was also a significant interaction between trial number and season ( $F_{15,265} = 3.932, p = < 0.001$ ) with the greatest improvement over time observed in winter (Fig 3.11).



**Figure 3.10:** Mean ( $\pm$ S.E.) total trial time between days 1 – 25 per treatment group.





**Figure 3.11:** Mean ( $\pm$ S.E.) total trial time (s) across days 1 – 25 per season treatment group (♦ summer, ■ autumn, ▲ winter, x spring).

## Discussion

We found that spatial learning abilities in *B. cocosensis* fluctuated between seasons and that males and females performed similarly to each other in all seasons except spring. While there are few differences between males and females during summer, autumn and winter, marked differences in spring potentially implicate reproductive behaviour of both sexes as dictated by their mating system of nest guarding and female choice. Males are confined to their nests during spring and showed a clear decrease in cognitive performance during this time. In contrast, female performance during spring mirrored summer/autumn results, as during this time they visit multiple nests to choose a suitable male whilst simultaneously foraging and avoiding predators. This variation in life-history priorities between sexes favours a reduction in male cognitive ability likely achieved through phenotypic plasticity under hormonal control.

Spatial learning is ubiquitous across vertebrate taxa, and many mammalian studies have illustrated that it is influenced by mating systems (e.g. Gaulin and Fitzgerald, 1986; 1989; Galea et al. 1994; Kavaliers et al. 1996; 1998), which drives variation in spatial learning between sexes. A few studies have shown sexually dimorphic spatial learning ability

can fluctuate between seasons, a trend seemingly tied to hormonal changes (Galea et al. 1994; 1996). Given the energetic demands of cognition, it makes sense to reduce costs if cognitive requirements are reduced. For example, many avian and small mammalian species show a decrease in specific brain region volume between breeding and non-breeding seasons (Yaskin, 1984; Smith et al. 1997; Tramontin et al. 1998; Tramontin and Brenowitz, 2000). Here, we found the first evidence that the intertidal Cocos Frillgoby also shows differences in spatial learning ability between sexes, and this is most notable during the breeding season. Unlike traditional mammalian systems however, the male in this species is confined to the nest and correspondingly shows the greatest reduction in cognitive ability.

Evidence for sexually dimorphic spatial learning ability is somewhat equivocal in fishes, although a number of studies have suggested that males outperform females in spatial tasks. Studies on guppies (*Poecilia reticulata*), redbtail splitfin (*Xenotoca eiseni*), zebrafish (*Danio rerio*) and freshwater blennies (*Salaria fluviatilis*) have all reported that males show enhanced performance in spatial learning (Sovrano et al. 2003; Fabre et al. 2014; Lucon-Xiccato and Bisazza, 2017; Roy and Bhat, 2017). In the case of the first three species, males tend to disperse further than females as they chase multiple mating opportunities, which is somewhat reminiscent of a typical mammalian system (Silverman and Eals, 1992). In contrast, female Azorean rockpool blennies (*Parablennius parvicornis*) move greater distances relative to males during the breeding season, and thus have a greater demand for spatial cognition to recall multiple nest locations (Carneiro et al. 2001). Correspondingly, females have larger lateral palliums compared to males.

Behavioural plasticity occurs in a wide variety of species occupying seasonal environments (Tramontin and Brenowitz, 2000). Under certain environmental conditions, some species will show adaptive behaviour in their foraging habits, associated with changes in brain morphology. For example, caching birds are capable of storing hundreds of food

items and returning to them days or even months later (e.g. Sherry et al. 1989; Krebs, 1990; Krebs et al. 1990). During winter, when food is scarce, these caches become especially important for these species. In two populations of black-capped chickadee (*Poecile atricapillus*), those residing in harsher conditions during winter showed larger hippocampal volume and greater spatial memory ability compared to the population that experienced milder winters (Pravosudov and Clayton, 2002). On the other hand, harsh environmental conditions can also be met with adaptations for lowered metabolic rates, activity levels and, consequently, decreased brain size. For instance, adult male white-footed mice (*Peromyscus leucopus*) show reduced hippocampal volume and long-term spatial memory when exposed to short photoperiods, a reliable cue to indicate the onset of winter and harsher conditions (Pyter et al. 2005). Brain and associated behavioural plasticity should be expected then, as brain functions require more energy per mass than any other tissue, and responses such as a reduction in mass could lower energetic costs (Jacobs, 1996).

While the mating system of *B. cocosensis* remains undescribed, other *Bathygobius* species are known to engage in male competition and nest holding disputes, while females are the choosy sex (e.g. Tavalga, 1954; Taru et al. 2002; Kong and Chen, 2013). In premating rituals, females actively search for potential mates, while males remain in their chosen nest site, alternatively cleaning the site and courting passing females. Once spawning occurs, females return to their home range (Taru et al. 2002) while males guard the eggs until hatching. Given that breeding in *B. cocosensis* primarily occurs in spring (Thia et al. 2018), we suggest that reduced cognitive ability in males during this time is because males are site-attached to their nest, so their need for neurologically expensive spatial ability presumably decreases. It is likely the males have reduced calorific intake during this time as well due to reduced foraging opportunities. Here, we found that males required significantly more days to reach criteria and had lower daily scores in spring compared to females, corresponding to

their nest-guarding behaviour during this time. Both sexes reached criteria in a similar time frame in all other seasons, and it is interesting to note that collection site temperatures are similar in autumn and spring, although males performed just as well as females in the former. In contrast, female performance in spring reflected summer/autumn patterns, suggesting no apparent change to their spatial learning capabilities in the breeding season when, in addition to their regular activities, they are also moving between nests.

Despite the test maze temperature kept constant across treatment groups, both sexes showed increased exit time in winter compared to other seasons. Although females were faster to leave the start box on average compared to males, the overall increased exit time may be interpreted as reduced motivation to complete the task due to lowered metabolic rate. Given that females invest heavily in egg production, it may be that they are slightly more motivated than males to search for food because of their enhanced energy requirements. Total trial time was also significantly higher in winter compared to other seasons but improved as trial days went on. This is likely a reflection of the fact that fish were adjusting their behaviour to the routine of the maze and securing the reward more quickly. It should be noted, however, that daily scores of both sexes remained stable throughout winter, suggesting a reduction in cognitive mapping ability when metabolic demands are lower.

In summary, this study presents the first evidence that spatial learning in fish varies between seasons and may be influenced by a mating system where males and females play contrasting roles. While differences in spatial learning between sexes is well documented in mammalian species, it remains poorly studied in fishes. Future studies should consider the underlying physiological mechanisms behind this phenomenon which may include hormonal influences on brain plasticity.

## CHAPTER 4

### **Environmental enrichment influences spatial learning ability in captive-reared *Bathygobius cocosensis***

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## Abstract

Behavioural plasticity is an advantageous trait for animals living in dynamic environments and can be induced through learning. While some behavioural traits are innate, others are framed by experience and learning during an individual's lifetime. Many studies have investigated cognitive abilities in species from contrasting environments, but the relative contribution of natural selection versus behavioural plasticity in cognitive variability remains equivocal. Further, rearing conditions in laboratories are often mundane, failing to encourage natural behaviour in the species used in these studies. Here, we captured juvenile gobies (*Bathygobius cocosensis*) from intertidal rockpools and raised them in captivity under varied environmental enrichment treatments that mimic variation observed in coastal habitats. When tested in a simple spatial learning task, individuals from complex rearing treatments (rock or oyster substrate) reached learning criteria faster than those reared in less complex (seagrass) and homogenous environments (sand substrate). Interestingly, gobies reared in complex environments demonstrated longer latencies to start the task than gobies in homogeneous treatments. Our results indicate that cognitive ability is strongly shaped by individual experience during early ontogeny, and exposure to reduced environmental complexity leads to reduced cognitive abilities in intertidal gobies.

**Key words:** cognition; plasticity; structural complexity; captivity; goby

## Introduction

Adaptation to environmental change is an important driving force of evolution (Darwin, 1859) and often driven or buffered by behavioural plasticity (Brown, 2012). Phenotypic plasticity is central to dealing with short-term environmental change (Price et al. 2003; reviewed in Ghalambor et al. 2007; Nussey et al. 2007), whereby individuals can adopt new and beneficial responses better suited to contemporary conditions, resulting in increased fitness through novel and plastic behaviours (Bradshaw, 1965; West-Eberhard, 1989; Pigliucci, 2001; Dukas, 2013). In cases where genotype expression is greatly influenced by biotic or abiotic factors of the environment in which they exist (GxE interactions; Lynch and Walsh, 1998), individuals with identical genotypes can exhibit different phenotypes depending on experience. Thus, individuals with similar genotypes raised in different environments, particularly early in life, often differ in their behaviour (Rosenzweig and Bennet, 1996).

Typically, behavioural repertoires are underpinned by neurological substrates in the brain such that brains and behaviour evolve and develop codependently. Individuals raised in homogeneous or otherwise predictable environments tend to have reduced cognitive capacity and smaller brain size or brain regions compared to those raised in dynamic and structurally complex environments (Clayton and Krebs, 1994; Healy et al. 1996; Matthews et al. 2005; Kihlslinger et al. 2006). For instance, caching avian species demonstrate enlarged hippocampus' relative to their non-caching cousins, having to deal with increased visuospatial demands of recalling hidden caches (e.g. Krebs, 1990; Krebs et al., 1996; Shettleworth, 1995; Shettleworth and Hampton, 1998). Further, within-population differences between hippocampal volume likely relate to a gradient of climatic conditions, including unpredictable food availability (Pravosudov and Clayton, 2002; Roth and Pravosudov, 2009). Similar findings have been reported in fish (reviewed in Kotrschal et al. 1998) where size of

the telencephalon (analogous to the hippocampus), shows a positive correlation with structural complexity of environmental origin. In guppies (*Poecilia reticulata*), for example, the telencephalon in lab reared individuals showed a 19% size decrease compared to those from wild populations (Burns et al. 2009). Sympatric goby species occupying a gradient of micro-niches, from homogeneous sand shores to complex rockpools, demonstrate varying telencephalon volumes depending on the physical complexity of their habitat (White and Brown, 2015a).

As environmental complexity influences brain morphology, it will invariably lead to associated improvements in cognitive capability, and one such way this is demonstrated is through spatial learning. Every day behaviours such as foraging and predator avoidance require an intricate knowledge of the spatial distribution of resources and shelters (Dodson, 1988; Odling-Smee and Braithwaite, 2003), and this should favour an ability to learn. This is especially critical in aquatic environments such as the intertidal zone, where resources may shift, and so the need to keep spatial information updated is crucial. Fish occupying structurally complex environments develop enhanced spatial learning abilities when compared to individuals raised in barren settings. For example, zebrafish (*Danio rerio*) reared in heterogeneous environments learn food locations faster than those reared in homogenous environments (Spence et al. 2011; Roy and Bhat, 2017). Likewise, sticklebacks (*Gasterosteus aculeatus*) from the structurally complex littoral zone demonstrate superior spatial learning skills, and a preference for different cues, compared to their sympatric, pelagic counterparts (Odling Smee et al. 2008). This variation in cue preference is likely due to the availability of landmarks in the littoral zone compared to the homogenous, featureless pelagic zone where individuals must navigate using egocentric information.

Similar findings have been reported in intertidal gobies, where rockpool residents solved a spatial task faster, and with fewer errors, compared to sand specialist species (White



and Brown, 2014b). In addition to spatial learning ability, environmental complexity plays a role in cue preference. White and Brown (2014a) demonstrated that rockpool and sand gobies used landmark and egocentric cues, respectively, to solve a simple spatial assay. This difference in cue preference is likely due to the stability of landmarks in protected, relatively stable rockpools compared to the exposed and variable sand flats, where landmarks would shift and, thus, be unreliable.

In many cases, the impact of environmental demand on cognitive function and behavioural plasticity has been investigated in laboratory conditions. As captivity can greatly affect the physiology and behaviour of animals (Kelley et al. 2005), it is often suggested that complexity or enrichment be added in housing to facilitate individuals' growth while discouraging abnormal behaviour (reviewed in Brown and Day, 2002; Brydges and Braithwaite, 2009). Environmental enrichment can be defined as deliberate manipulation to environmental complexity (Näslund and Johnsson, 2016), which exposes individuals to greater sensory, motor and cognitive demands, while encouraging learning through exploratory behaviour (Dinse, 2004, Leggio et al. 2005; Harburger et al. 2007; Strand et al. 2010; Salvanes et al. 2013). Enrichment during early ontogeny positively influences learning and problem-solving skills later in life (Rosenzweig and Bennet, 1996) and can affect cerebral morphology, regardless of the amount of enrichment time (Rosenzweig and Bennet, 1996, Brown et al. 2003; Bergendahl et al. 2016). As changing environments select for behavioural plasticity, the same can be extended to enriched captive environments. For example, rodents display both behavioural (Sackett et al. 1999, Zimmermann et al. 2001, Görisch and Schwarting, 2006, Harris et al. 2009) and cerebral (Varty et al. 2000, Van Praag et al., 2000, Griñan-Ferré et al. 2016) improvements when raised in enriched conditions.

Fish have become increasingly common in laboratory-based investigations; however, laboratory aquaria are generally devoid of complexity, often leading to abnormal and

inflexible behaviour (e.g. Brown et al. 2003). Some comparative cognitive studies suggest habitat enrichment can help increase cognitive ability to levels seen in wild populations (Brown and Day, 2002; Braithwaite and Salvanes, 2005; Odling-Smee et al. 2008).) For instance, learning ability in striped knifejaw (*Oplegnathus fasciatus*) improved when individuals were raised with submerged structures to enhance environmental complexity (Makino et al. 2015), while mahseer (*Tor putitora*) show significantly higher exploratory behaviours and anti-predator responses when reared in enriched conditions (Ullah et al. 2017). There is little doubt that fish show high capacity for both behavioural and neurophysiological plasticity to changing environments, thus, even brief exposure to enrichment can dramatically alter behaviour (Brown et al. 2003).

Owing to the energetic requirements of associated underlying neural mechanisms, the costs of learning are such that they should only be invested in if required by ecological demands (Robinson and Dukas, 1999; Mery and Kawecki, 2003; Odling-Smee et al. 2008). As phenotypic plasticity is a beneficial adaptation in changing environments, species that inhabit a range of environments make ideal research candidates to investigate the relationship between environmental complexity and cognition. The intertidal zone is one such environment with a number of diverse niches that vary in stability and complexity. For example, intertidal rockpools are highly dynamic, and individuals' coordinate their movements with the changing tides (Martins et al. 2017). The rockpools themselves are structurally complex but relatively stable, while sandy beaches are largely featureless and prone to substratum shifts with the tides.

The family Gobiidae is an extensive group of benthic fishes commonly found along the intertidal zone and in the pools amongst rocky platforms (Thacker and Roje, 2011). Early investigations on this group showed they have incredible navigation abilities (Aronson, 1951; Wickler, 1957; Markel, 1994) and subsequent studies have revealed a wide range of

behavioural and life-history differences depending on phylogenetic origin (Thacker and Roje, 2011). We have previously shown that gobies collected from varying environments differ in their spatial learning skills, the cues they use and the underlying brain morphology (White and Brown, 2014a; 2014b; 2015a; 2015b). Although comparisons between species highlight the impact of environmental influence on cognitive function, they cannot differentiate between inherent versus acquired traits.

Here, we investigated the influence of exposure to variation in early rearing environment on the spatial learning capabilities of a ubiquitous marine goby species found along the east coast of Australia. A single species was collected from one location and reared under different enrichment regimes to elucidate the degree of behavioural plasticity in the context of spatial learning. Each of the rearing environments mimicked the main micro-habitats where this species is found in the intertidal zone and vary in their degree of physical complexity: sandflats, sea grass beds, oyster beds and intertidal rockpools. We predicted that early-life exposure to these different habitats would result in variable cognitive skills, such that those fish reared in more complex habitats would develop enhanced spatial learning capabilities.

## Methods

### *Test subjects*

The goby species *Bathygobius cocosensis* is ubiquitous along the New South Wales (NSW) coastline but is particularly abundant amongst the rockpools in the intertidal zone. Individuals of all life stages can be found in the naturally occurring pools along the rocky platform. Juvenile gobies were collected from Dee Why, NSW, Australia, using small dip-nets in early (Jan-Feb) 2017. A total of 56 juveniles were collected, ranging from 7-10mm, and transferred to the Sea Water Facility at Macquarie University in a large bucket (10L) of

aerated seawater. Once there, they were slowly acclimatised to a 70L opaque-white, plastic holding tub (64.5 x 41.3 x 27.6cm) linked to a recirculating system with a 3L/min flow rate. The tub had a 15mm hose inlet and a 25mm PVC outlet, covered with 200 $\mu$  mesh to prevent gobies escaping. The young gobies were acclimated in this housing tub for 4 weeks, during which they were introduced to a diet of frozen *Artemia* infused with powdered Polylab Nano Food Roids. They were also given finely crushed commercialised *Artemia* flakes to encourage foraging in smaller individuals.

### *Housing*

After the settling period, the gobies were randomly assigned to a micro-habitat type (n = 14 per treatment) and introduced to a tidal home tank. This tank was made of 6mm glass (144cm (L) x 50cm (W) x 40cm (H)), and divided into five parts, four of which were 33cm long, separated by four black acrylic partitions (50cm (L) x 0.5cm (W) x 45cm (H)). Each partition had three holes (diameter 5cm) covered by 200 $\mu$  mesh, fine enough to stop gobies from passing through, but coarse enough to allow water flow between sections. Each of the four sections formed a micro-niche rearing chamber. Two chambers represented relatively homogenous habitats (fine sand substrate with and without seagrass *Zostera muelleri*) and the other two rocky platform habitats (a mixture of live oyster formations and broken oyster fragments and a makeshift rockpool on a bed of coarse shell grit, surrounded by larger stones). These chambers mimicked the most common coastal habitats along the NSW coastline: open sandflat, seagrass beds, oyster reefs and intertidal rockpool habitats, respectively. A smaller chamber (12x50x40cm) on one end of the housing chambers contained the drainage mechanism used to simulate tides. This chamber was fitted with a PVC outlet pipe (5cm D x 25cm H) which, at high tide, emptied directly into the sump below (144x50x40cm). An additional three holes were fitted with 20mm solenoids alongside the

main outlet, also draining to the sump below. These solenoids were controlled by an automated sprinkler system (Hunter Pro-C 16 Station Modular Controller). The automated system was set to open all solenoids at a set time and drain the tank for a period of 6 hours, after which the water level dropped to 15cm deep. At high tide, when the solenoids were closed, the water level gradually rose to a depth of 35cm. The tank was kept under laboratory conditions that remained constant for 3-month blocks, such that water temperatures had subtle changes to mirror the seasons (mean temp.; summer, 24°C; autumn, 22°C; winter, 19°C; spring, 21°C). To lessen the impact of movement in the lab on the gobies, and to insulate against noise, the tidal tank was covered with 10mm polystyrene and black tarp.

Food was provided automatically. An automatic feeder (Jebao DP-4) with four separate pumps was arranged with each pump outlet leading to one of the four sections of the tank. A 1L flask with a mixture of 800ml of saltwater, 200ml of aged freshwater and approximately 25g of commercial aquarium foods (Ocean Nutrition Frozen *Artemia* and Marine Mix) was fed into the pumps. The mixture was kept aerated and agitated to allow easy flow through the pumps. Each pump was programmed to release different amounts of the pre-prepared mix twice daily in the morning and afternoon at the changeover between low (35ml) and high tide (55ml).

### *Tagging*

The gobies were kept in this tank for 12 months until they reached between 30-40mm in length and could be tagged with ease. Each group of gobies was assigned a different tagging colour and sequence according to the micro-habitat they were housed in. For the tagging procedures, each goby was placed in a bath of tricaine methane sulfonate (MS222) buffered with sodium bicarbonate (50mg/l per 1L saltwater) for 30-60 seconds until equilibrium was disrupted. They were then tagged on one of six possible sites beneath

transparent scales along their dorsal surface with elastomer ID tags (VIE: Marine Technology, Inc. 2008) for individual identification. The gobies were also measured, weighed and sexed, then placed in an aerated bucket of saltwater for recovery. This process took less than 2 minutes per goby, and each individual recovered within 5 minutes. They were then returned to the tidal tank for one week for full recovery.

### *Test Apparatus*

The “plus” maze used was of the design described by White and Brown (2015a) adapted from Odling-Smee and Braithwaite (2003) and Odling-Smee et al. (2008; see Chapter 3, Fig. 3.1) and contained a fine sand substrate approximately 1cm thick. The maze was submerged in a large fibreglass tub (120x75x19cm) and water maintained at 12cm deep. Four large black barriers were erected around the maze to discourage the gobies from using external cues. Each of the four ends of the maze had a clear food dish (3cm diameter, 1.5cm deep) and a shelter made from halved PVC piping (7cm long, 1.25cm radius). One of these shelters was fitted with clear plastic over both ends to block access and was used as the incorrect choice shelter during the trials. The shelter appeared functional, but the plastic prevented the fish from entering. A camera was mounted above the maze on a steel frame to record all trials, and the footage uploaded to a hard drive. Each group of gobies was introduced to the maze for a 24-hour acclimation period where they had full access to the maze and food inside the food dishes. They were then returned to their respective micro-habitat for another 24-hours until testing began.

### *Trials*

To account for possible population level lateralisation bias (see Chapter 6), each goby was randomly assigned to left- or right-side training prior to commencing trials (Brown and

Braithwaite, 2004). Each goby was tested alone, three times per day, beginning with a 3-minute introduction to the start box. For orientation purposes, a landmark (small marble, 1.2cm diameter) was placed in the junction of the maze. The use of a marble ensured none of the subjects were familiar with the landmark and thus neither group could be at a disadvantage from others. The landmark was placed slightly to the left in trials where the goby was assigned to choose the right-hand arm, and vice versa. This way, the goby had to turn away from the landmark to choose the rewarded arm.

Once the individual was settled in the start box, the separation door was lifted, and the trial began. The exit time, the side of choice and total trial time were all noted. The exit time was considered to have occurred when at least half of the individual's body was outside of the start box. Trials were finished when a goby entered either the left or right reward box, or a maximum time of 10 minutes passed. In cases where gobies did not exit in 10 minutes, they were considered to have failed the trial. When gobies chose the correct side, the removable partition was added to prevent their escape, and they were immediately rewarded with frozen *Artemia*, delivered via pipette into the food dish. When the incorrect choice was made, gobies were kept isolated for 3 minutes in their choice box with no food reward and no access to the shelter. They were then gently ushered into the correct reward arm to access the shelter. Once in the correct box, the gobies were rewarded with food, though this practice was discontinued after the first five days of trials. When gobies chose the correct side for three trials across five consecutive days successfully, they were considered to have learnt the task and were returned to their micro-habitat. Between each trial, the reward location became the new start location, which means the fish could not rely on extra-maze cues to solve the spatial task; rather, they had to use the landmark or egocentric information (i.e. turn direction).

*Ethical note*

Gobies were caught in compliance with NSW Fisheries (permit no. P08/0010-3.0). Husbandry and experimental conditions were approved by the Macquarie University Ethics Committee (ARA 2014/003). At the end of the experiment, all gobies were returned to the housing tank for further studies.

*Statistical analyses*

In most cases, data were normally distributed and analysed using parametric tests, or log transformed to achieve a normal distribution. Exit time from the start box was used as a measure of task motivation and the gobies' involvement in the choice process. Each goby had three exit times per day, which were then averaged for a daily exit time per individual. We used data from the first, fifth and tenth day of the spatial task and the data were log transformed to achieve a normal distribution. Size class was based on total length (TL), where  $< 35\text{mm}$  were small and  $> 35\text{mm}$  were large according to a natural break in size distribution. We used a repeated measures ANOVA to analyse exit time against treatment environment and size class as independent variables. The same protocol was applied when analysing total trial time, calculated from the time between a goby leaving the start box and entering either the correct or incorrect box.

We used ANOVA to examine the effects of micro-habitat type and size class on the number of days to reach criteria and a Fisher's post-hoc test to determine the pair-wise differences between the four environment treatments.

To analyse performance trends as influenced by environmental enrichment, each goby was given a binary score (0 or 1) based on correct or incorrect choice in the maze. After three trials per day, each goby was assigned the average of this score for a daily score. We analysed effect of environment and size class on daily scores for the first, fifth and tenth day

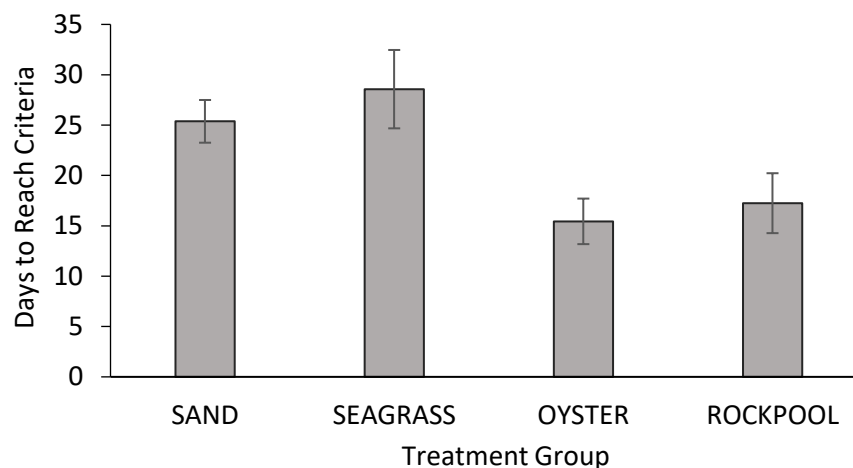


of trials using a repeated measures ANOVA. By the tenth day of trials only 2 individuals had reached criteria and were given scores of 100% for analyses purposes. All analyses were performed using StatView Version 232 5·0·1 (SAS Institute Inc. 1998).

## Results

### *Days to reach criteria*

There was a significant effect of rearing environment on the number of days to reach criteria ( $F_{3,24} = 3.804$ ,  $p = 0.023$ ; Fig 4.1). Body size had no effect on the number of days to reach criteria and there was no interaction between rearing environment and size ( $p > 0.05$ ). Post-hoc analysis showed that fish reared in rockpool and oyster bed environments reached criteria faster than those reared in seagrass (Fisher's PLSD;  $p = 0.014$  and  $0.004$ , respectively). Gobies reared in the oyster bed environment also reached criteria significantly faster than those in the sand environment (Fisher's PLSD;  $p = 0.02$ ).



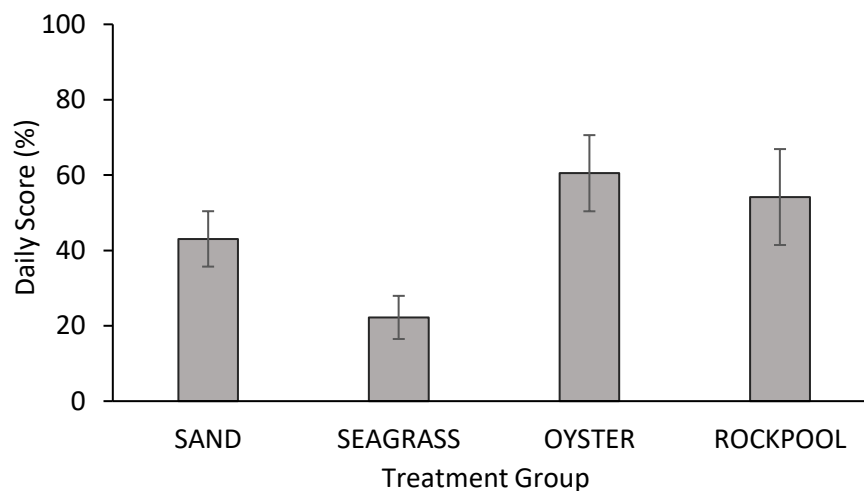
**Figure 4.1:** Mean ( $\pm$ S.E.) number of days to reach criteria per treatment group of captive gobies.

### *Daily scores*

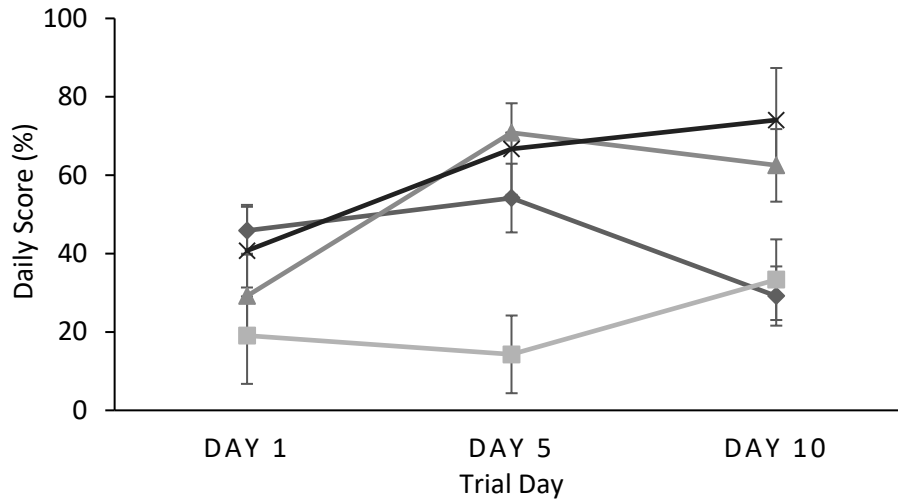
Daily scores are an indicator of how many correct choices the fish made during the day. There was a significant effect of rearing environment on daily score ( $F_{3,24} = 4.881$ ,  $p =$

0.009; Fig 4.2). Body size was not significant, nor was the interaction between rearing environment and body size ( $p > 0.05$  in both cases). There was a significant effect of trial day on mean daily score ( $F_{2,48} = 6.362$ ,  $p = 0.004$ ); scores generally improved with increasing trial number. We also found an interaction between trial day and environment ( $F_{6,48} = 3.159$ ,  $p = 0.011$ ; Fig 4.3) suggesting the rate of learning over time varied depending on which rearing environment the fish were exposed to.

Post-hoc analyses showed that daily score means on day 1 were similar across treatment groups (Fisher's PLSD;  $p = 0.310$ ), but there were highly significant differences on days 5 ( $p < 0.001$ ) and 10 ( $p = 0.009$ ). In general, fish reared in rockpools and oyster beds showed the greatest improvement over time (Fisher's PLSD;  $p = 0.043$  and  $0.010$ , respectively). Those reared in sand or seagrass showed little improvement, or in some cases poorer scores, over time ( $p > 0.05$  in both cases).



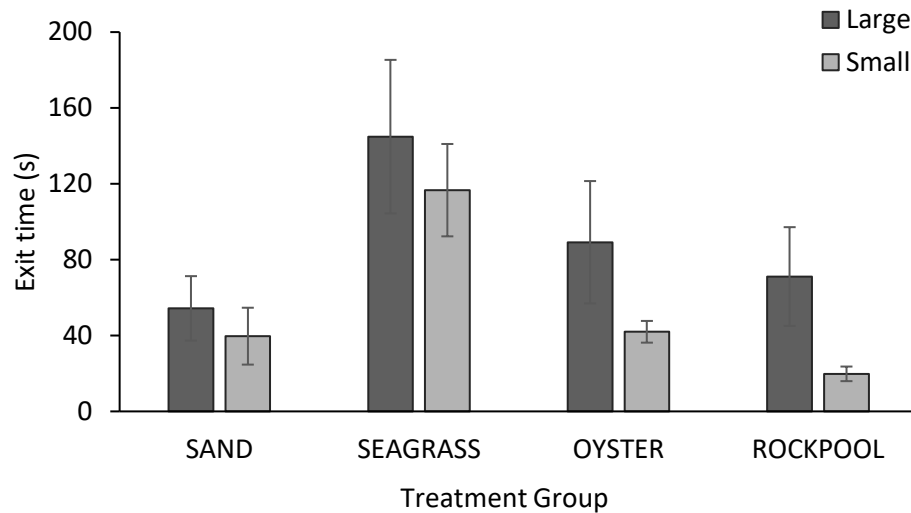
**Figure 4.2:** Mean ( $\pm$ S.E.) daily scores for all trials on days 1, 5 and 10 per treatment group.



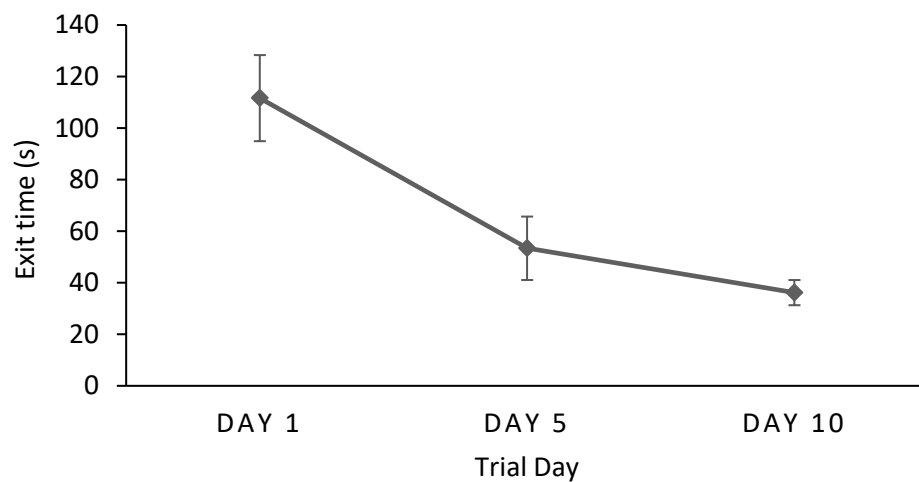
**Figure 4.3:** Mean ( $\pm$ S.E.) daily scores for all trials on days 1, 5 and 10 per treatment group ((♦) sand, (■) seagrass, (▲) rockpool, (×) oyster) of captive gobies.

### *Motivation*

We used the time to leave the start box as an indicator of motivation. There was a significant effect of rearing environment ( $F_{3,24} = 7.701, p < 0.001$ ), with gobies reared in the seagrass environment exiting significantly slower than fish from all other environmental treatments (Fisher's PLSD;  $p < 0.02$  in all cases). Larger gobies were slower to exit the start box than smaller gobies ( $F_{1,24} = 4.419, p = 0.046$ ; Fig. 4.4). The interaction between rearing environment and body size was not significant ( $p > 0.05$ ). Trial day also had a significant effect on exit time as gobies exited the start box faster with increasing trial number ( $F_{2,48} = 22.013, p < 0.001$ ; Fig 4.5).



**Figure 4.4:** Mean ( $\pm$ S.E.) exit time for large and small gobies per environmental treatment across all trials.



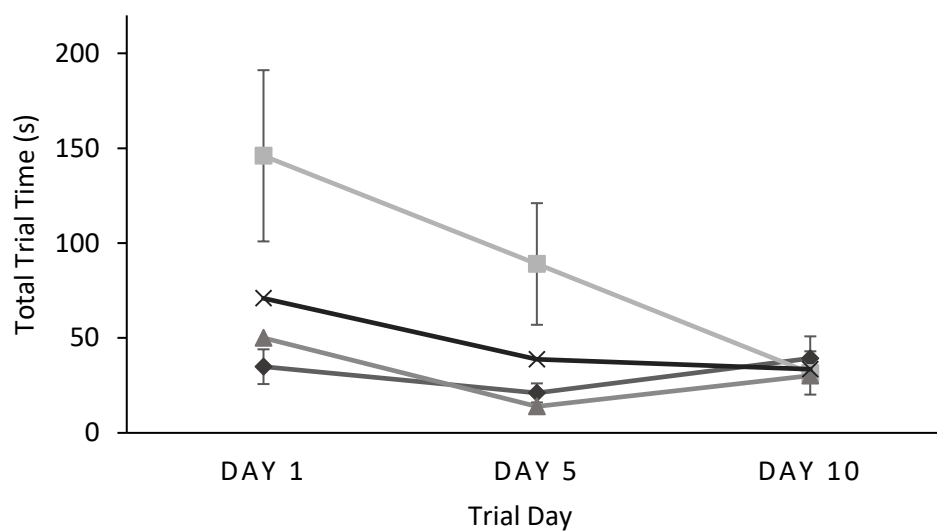
**Figure 4.5:** Mean ( $\pm$ S.E.) exit time for all gobies for trial days 1, 5 and 10.

#### *Total trial time*

Total trial time was based on the time an individual spent in the t-maze from leaving the start box to choosing either the left or right side. There was no significant effect of rearing environment or size class on total trial time ( $p > 0.05$  in both cases). The interaction between rearing environment and size class was also not significant ( $p > 0.05$ ). Trial day had a significant effect on total trial time ( $F_{2,48} = 7.000$ ,  $p = 0.002$ ) such that the time to complete

the maze declined over time for all fish. There was also a significant interaction between trial day and environmental treatment ( $F_{6,48} = 2.528$ ,  $p = 0.033$ ; Fig 4.6). There were no other significant interactions.

Post-hoc analyses revealed gobies reared in the rockpool and seagrass environments showed the greatest improvement over time to complete the task (Fisher's PLSD;  $p = 0.021$  and  $0.035$ , respectively). Those reared in oyster and sand environments showed little improvement over time ( $p > 0.05$  in both cases).



**Figure 4.6:** Mean ( $\pm$ S.E.) total trial time for days 1, 5 and 10 between treatment groups: ((◆) sand, (■) seagrass, (▲) rockpool, (×) oyster) of captive gobies.

#### *Treatments and body length*

The total length of the fish reared in each of the treatments was not significantly different ( $F_{3,28} = 0.494$ ,  $p = 0.689$ ) nor did they differ in body weight ( $F_{3,28} = 0.171$ ,  $p = 0.915$ ). However, gobies in the oyster treatment group showed reduced variance of sizes compared to other treatment groups.

## Discussion

Previous experiments have illustrated dramatic differences in the spatial learning skills of fish inhabiting contrasting environments (e.g. Odling-smee and Braithwaite 2003, White and Brown 2014a), but it is unclear to what extent that variation is a result of natural selection operating over generations, or behavioural plasticity resulting from individual experience during ontogeny. Here, we collected *Bathygobius cocosensis* fry from a rockpool environment and reared them in four structurally different habitats common along Australian coastal environments for 12 months. We found that rearing gobies in these contrasting habitats had a profound impact on their ability to solve a novel spatial learning task. Gobies reared in more complex habitats, oyster reef and rocky reef, took fewer trials to reach learning criteria and made more correct choices than those reared in less complex environments (sandy shore or seagrass). Fish reared in complex environments also took longer to leave the start box which indicates their motivation in the task as well as their propensity to take risks (boldness; e.g. Brown and Braithwaite, 2004; Toms et al. 2010).

Environmental enrichment has long been associated with changes in the nervous system (Will et al. 2004), by posing an increased demand in sensory, motor and cognitive functions (Dinse, 2004; Leggio et al. 2005; Harburger et al. 2007, Strand et al. 2010; Salvanes et al. 2013). Physical complexity also aids in reducing stress (Braithwaite and Salvanes, 2005; Millidine et al. 2006; Kistler et al. 2011; Näslund et al. 2013) and encourages exploratory behaviour (Camacho-Cervantes et al. 2015). Here we report significant changes in the cognitive abilities of the intertidal resident *B. cocosensis* following rearing in environments of varying complexity from early ontogeny to ca. 12 months of age. Individuals reared in the more structurally complex environments (oyster bed and rockpool) reached learning criteria significantly faster than those reared in more homogenous environments (sand and seagrass). These results agree well with similar enrichment studies (e.g. Salvanes

and Braithwaite 2005; Salvanes et al. 2007; Spence et al. 2011). The rockpool treatment group was modelled after a rockpool setting from which the juveniles were collected from, thus this may be the closest representation of how wild caught individuals would perform in a spatial task at the same developmental stage as those used in this study. Interestingly, the seagrass group required more days to reach learning criteria than all other treatments, despite being in a moderately enriched habitat compared to those in the sand treatment. This is likely because it took them a long time to move to the reward zone once they exited the start box, which may reflect the fact that sea grass blades move in the current and while increasing physical complexity, they also increase landmark instability.

Daily scores (i.e. the proportion of correct choices made during their three daily trials) increased significantly in all treatments over the length of the experiment, however there were also significant differences between fish reared in the various environments. Fish in all treatment groups performed similarly on the first day of trials, but daily scores diverged significantly on days 5 and 10 as oyster and rockpool groups performed better than sand and seagrass groups. These differences between learning abilities, derived from the change in habitat complexity in early ontogeny, indicate that different experiences play a critical role in the formation of flexible behaviour in later life (Rosenzweig and Bennet, 1996; Kotrschal and Taborsky, 2010). A study on juvenile trout reported that individuals demonstrated greater spatial learning and problem-solving behaviour when exposed to an enrichment treatment, followed by individuals that experienced homogeneity early in life then were switched to enriched settings. Individuals in two other treatments of early enrichment/late homogeneity and complete homogeneity behaved similarly, indicating that the more recent the enrichment experience, the greater the role in developing flexible behaviour (Bergendahl et al. 2016). Similarly, Atlantic salmon briefly exposed to live prey and structural enrichment showed significantly improved responses to novel, live prey (Brown et al. 2003). Neurologically, this

402 makes sense, as a plain environment would have minimal use for learning. If an individual's  
403 environment doesn't demand it, there's little point investing energy into neurological  
404 structures required for cognitive processes.

405 Survival in wild conditions requires the collection and interpretation of environmental  
406 information (Galef and Laland, 2005), which can be enhanced in individuals with bold or  
407 exploratory behavioural traits, enabling them to collect this information rapidly (Braithwaite  
408 and Salvanes, 2005). Naturally, boldness may enhance fitness through longer foraging trips,  
409 however there is also an increased risk of predation (Sih et al. 2004). We found that smaller  
410 gobies were faster to leave the start box than larger gobies on the first day of trials, however  
411 the larger fish showed a decrease in latency by the fifth day, which was maintained until the  
412 tenth day. Previous studies have also identified that smaller individuals tend to be bolder than  
413 large individuals when emerging from cover into a potentially dangerous environment  
414 (Brown and Braithwaite, 2004). Small fish have high metabolisms and emphasise feeding to  
415 reach sizes where they can avoid gape-limited predation and increase their intra-specific  
416 competitiveness. In addition, larger fish arguably have more to lose given their greater long-  
417 term investment in growth (asset protection principle; Clark, 1994). It is interesting to note  
418 that as the fish became familiar with the test environment, the larger gobies' emergence times  
419 converged with that of the smaller individuals, suggesting they no longer perceived the arena  
420 as dangerous.

421 Brydges and Braithwaite (2009) suggest that sticklebacks from enriched treatments  
422 should display lower levels of neophobia and greater levels of boldness compared to  
423 individuals from homogeneous tanks (e.g. Sherwin, 2004; Braithwaite and Salvanes, 2005;  
424 Fox et al. 2006). These patterns were reported in other studies, for example cod exposed to  
425 spatial heterogeneity during rearing were bolder, however they are also faster at seeking  
426 shelter than fish reared without enrichment (Salvanes and Braithwaite, 2005). Bergendahl et



al. (2016) admit that while exit time may be a better indicator of motivation rather than learning, their experiment showed that trout reared in enriched treatments learned and exited faster than their homogeneous counterparts. Similarly, mahseer (*Tor putitora*) raised in enriched conditions were less neophobic, emerging from a start box faster than those reared in impoverished environments (Ullah et al. 2017). Our results agree with these results to some extent, as shown by the slower exit times in the seagrass treatment group, followed by those in the complex treatments (oyster and rockpool groups), perhaps due to their unfamiliarity with open, unsheltered areas in the spatial learning test. It should be noted, however, that this trend is only observed in the larger gobies. It is likely that they perceived open areas as potentially risky because they were accustomed to hiding in crevices while in their home tanks. In contrast, smaller gobies in the rockpool treatment were fastest to exit the start box. Despite the fact that larger gobies tended to emerge later than those fish from the less complex sand environment, they still learned the task substantially more quickly. This was not because they moved more quickly through the maze once they had exited the start box, but because they tended to make good decisions when deciding which arm of the maze housed the reward.

Although rearing environment influences exploratory traits (Kelley and Magurran, 2003), the reasons behind motivation are often difficult to interpret (Braithwaite and Salvanes, 2005). It's possible that hunger would have been one motivating factor to seek out the reward (Colgan, 1993), but the cryptic nature of gobies likely influenced their motivation to seek out shelter also. In early trials, motivation was perhaps twofold with the reward being food and shelter, demonstrated when gobies would first move into the shelter and only explore the food dish after some time. After several trial days, and presumably when gobies became accustomed to the maze and lack of predators, individuals immediately searched for a food reward upon entering the correct arm. This may explain why the fish reared in complex

environments solved the tasks more rapidly: they were initially highly motivated to seek shelter.

Numerous studies have reported variable growth rate in fish reared in different environments (Salvanes and Braithwaite, 2005; Spence et al. 2011). The lack of difference in the mean length and weight between treatments was rather unexpected. Interestingly, the oyster treatment showed reduced size variance in both small and large fish, compared to other treatments. Fish reared in the oyster treatment showed a narrow range of smaller individuals and some much larger individuals. Variation in the spatial distribution of food can drive individual variation in foraging success via competition (see Chapter 2). For example, juvenile walleye Pollock grew slower when food was clumped, probably because they spent more time swimming, searching for enriched areas (Ryer and Olla, 1997). Moreover, aggressive individuals may be able to defend food patches which would lead to increased variance in growth rate. Adding complexity to the environment can facilitate the establishment of small territories which are visually isolated from one another (Imre et al. 2002; Höjesjö et al. 2004; Kadry and Barreto, 2010). Given the cryptic and territorial nature of this goby species (Griffiths, 2003a; b; White and Brown, 2013), we suggest that selective pressures during development in the oyster bed environment may have divided the fish into one of two strategy groups; a) growing large enough to defend a shelter or b) remaining small enough to avoid aggression from larger individuals, which would then likely reduce foraging activity, thus directly impacting growth.

To conclude, environmental changes drive genetic variation in innate behaviours and, as environments undergo shifts in complexity, so too do behavioural phenotypes change such that no phenotypic trait remains completely optimal over time (Mery and Burns 2010). In cases where species experience temporal or spatial heterogeneity on a regular basis, flexible and reversible plasticity is a favourable trait (e.g. Bloch and Robinson; 2001, Relyea, 2003;

477 Nussey et al., 2007). Kotrschal and Taborsky (2010) suggest that disturbed regimes in early  
478 ontogeny solidify cognitive abilities of individuals, perhaps because it signifies that the  
479 individual lives in a dynamic world. Fishes make great models to investigate behavioural  
480 plasticity because they show high levels of neural flexibility throughout their lifetime  
481 (Ebbesson and Braithwaite, 2012). Here, we exposed juveniles derived from the same micro-  
482 habitat (rockpools) to variations in rearing environment and found considerable differences in  
483 their abilities to solve a spatial learning task. Gobies reared in more complex environments  
484 were better at solving the task and it is likely that these environments favour enhanced spatial  
485 skills. Gobies reared in the seagrass and sandy treatments showed stunted cognitive function,  
486 as a result of experiencing a low-demand habitat from early ontogeny. Our results indicate  
487 that many of the behavioural variations observed in populations of animals collected from  
488 contrasting environments are largely the result of behavioural plasticity maintained during  
489 ontogeny.

**Note:** following the completion of these trials, the four chambers of the captive treatment tidal tank were combined into one; the sand, seagrass and oyster micro-niches were remodelled after the rockpool habitat from which captive juveniles were collected. Gobies were acclimated to the new setup for 6 months prior to any predator or laterality tests (see Chapters 5 and 6).

## **CHAPTER 5**

### **Evidence of behavioural syndromes in wild but not captive-reared Cocos Frillgoby (*Bathygobius cocosensis*)**

To be submitted to *Ethology*

Authors: *Penelope Carbia and Culum Brown*

## Abstract

Predator recognition has innate and learned components, and exposure to predation threat in early ontogeny greatly influences anti-predator behaviour. Several studies have suggested that exposure to predation can result in the formation of correlations between key behavioural traits, known as behavioural syndromes. Observing anti-predator behaviour in populations reared with high and low predator density thus provides an ideal opportunity to investigate the plasticity of this behaviour, as well as the development of behavioural syndromes between boldness and activity in a predation-risk context. Behaviour around predators and behavioural syndromes have been well-investigated in fishes, however, research on the synergistic link between the two is lacking. Here, we exposed wild and captive-reared populations of the intertidal goby *Bathygobius cocosensis* to visual and/or olfactory cues from a sympatric, predatory crab species, *Plagusia chabris*. Predator-inspection behaviour was similar in both populations, as was the probability of exiting the start box, time to exit the start box (boldness) and time to commence foraging. Time to commence foraging increased with body size, while the inverse was observed with activity levels. In the olfactory treatments, wild gobies that were faster to exit the start box (i.e. bold individuals) were also more active. In contrast, there were no correlations between exit time and activity levels in captive-reared gobies in any treatments. Our results highlight the importance of body size on risk-related behaviours and the influence of captive rearing on animal behaviour. Importantly, we show that behavioural syndromes were only manifested in fish from high predation locations and only under high risk contexts restricted to specific sensory modalities.

**Key words:** anti-predator behaviour; learned recognition; cues; visual; olfactory;

## Introduction

Predation is a dominant selective force influencing the life-history, morphology and behaviour of prey species often through decision making processes (Sih, 1987; Lima and Dill, 1990). Individuals capable of rapid detection and response to predation risk survive to reproduce, while slower individuals do not. There is, however, an important trade-off between the amount of time spent reacting to possible predation threat versus the time better spent on alternative fitness-affiliated activities (Lima and Bednekoff, 1999; Wisenden, 2000a). For example, balancing predation risk with foraging requirements can be achieved by selectively feeding in micro-habitats that offer easy escape, close access to refuges or facilitate crypsis (Lima and Dill, 1990; Lima, 1998). Honing these assessment skills involves a complex process of receiving, interpreting and reacting to reliable information from cues in the local environment through multiple sensory modalities.

Although prey species use multiple senses for predator detection (e.g. visual, tactile), there appears to be several advantages to using olfactory cues (Kats and Dill, 1998). This is especially true in aquatic habitats, where olfactory cues disperse widely and easily (Wisenden, 2000b), and thus carry vital information over wider distances. Moreover, chemosensory mechanisms may be used to interpret cues emanating from predators (reviewed in Kats and Dill, 1998), as well as alarmed or injured conspecifics (reviewed in Chivers and Smith, 1998; Wisenden, 2000b; Ferrari et al. 2010).

Anti-predator behaviour in fishes in the context of olfactory cues was observed in early studies from the 1950's, when Brett and McKinnon (1952; 1954) reported that populations of Pacific salmon (*Oncorhynchus spp.*) had ceased upstream migrations as a result of mammalian skin contamination in their home streams. There has since been a plethora of studies added to the growing body of literature that fishes are capable of detecting predators through chemosensory mechanisms and adjust their behaviour according to the

level of risk (Brown et al. 2011b). For instance, the threat-sensitivity hypothesis suggests that predator avoidance will increase according to threat level (Helfman, 1989), and has been supported by various studies investigating anti-predator behaviour in response to predator olfactory cues (e.g. Brown and Godin, 1999; Brown et al. 2000; Smith and Belk, 2001; Ferrari et al. 2005; Ferrari and Chivers, 2006) and conspecific alarm cues (e.g. Dupuch et al. 2004; Zhao and Chivers, 2005; Brown et al. 2006a; b; Vavrek and Brown, 2009), which can be paired together to facilitate learning about novel predators (Brown et al. 2011b).

To a certain degree, predator recognition in fish is innate (Magurran, 1990; Kieffer and Colgan, 1992; Hirvonen et al. 2000; Berejikan et al. 2003; Vilhunen and Hirvonen, 2003; but see Mathis et al. 1993; Chivers and Smith, 1994; 1998). However, as with any behaviour, anti-predator behaviour has a learned component influenced by environmental pressures (Kieffer and Colgan, 1992), leading to plastic responses depending on the necessities and experience of individuals (reviewed in Brown, 2003; Kelley and Magurran, 2003). Behavioural plasticity, therefore, fine-tunes behavioural responses to optimally suit contemporary conditions. For example, wild fathead minnows (*Phoxinus phoxinus*, Cyprinidae) living sympatrically with pike (*Esox lucius*) have superior anti-predator behaviours compared to fish from pike-free waters, demonstrated through behaviours such as predator inspection and cohesive shoaling (Magurran, 1986). However, when reared in a laboratory and exposed to simulated predator attacks in early ontogeny, both naïve and experienced populations showed improvements in anti-predator responses, suggesting early experience is critical to refining behaviour around predators (Magurran, 1990). Thus, responses in naïve individuals can be improved with experience (e.g. Olla and Davis, 1989; Magurran, 1990; Kieffer and Colgan, 1992; Berejikian, 1995; Brown and Warburton, 1999). Similar learning trends have been reported in other species including sticklebacks (*Gasterosteus aculeatus*; Huntingford and Wright, 1992), rainbowfish (*Melanotaenia*

*eachamensis*; Brown and Warburton, 1997; *M. duboulayi*; Brown and Warburton, 1999), chinook salmon (*Oncorhynchus tshawytscha*; Berejikian et al. 1999; 2003) and rainbow trout (*Oncorhynchus mykiss*; Brown and Smith, 1998).

Commonly, studies that investigate anti-predator behaviour follow a behavioural trait framework by scoring fish behaviour on a continuum. Perhaps the most frequently investigated trait is boldness (Magnhagen et al. 2014), which is aligned with an individual's propensity for risk-taking behaviour, such as approaching a novel object or inspecting a predator (Murphy and Pitcher, 1997; Ariyomo et al. 2013). In ecological terms, boldness plays a critical role in fitness and survival (Colléter and Brown, 2011; White et al. 2013) as shy and bold individuals fare differently in everyday challenges such as resource competition (Dugatkin and Alfieri, 2003), habitat selection (Wilson et al. 1993; Budaev, 1997), and foraging under predation pressure (Dugatkin, 1992; Biro et al. 2006; Stamps, 2007). Furthermore, boldness can accurately indicate an individual's survival probability when confronted with a predator (Smith and Blumstein, 2010). It is no surprise then that predator density greatly influences boldness. Generally, fish populations living sympatrically with predators demonstrate higher levels of boldness compared to those in low predator density environments, even within species (Giles and Huntingford, 1984; Brown et al. 2005; Bell and Sih, 2007; Dingemanse et al. 2009), suggesting that boldness is a highly plastic trait influenced by local environmental selective pressures. In addition, boldness can be induced in shy populations with repeated exposure to predator simulation, implying that life experience also plays a role in shaping boldness (Brown et al. 2007a).

Research has shown that personality traits (boldness, exploration, activity, aggressiveness and sociability; Réale et al. 2007; Conrad et al. 2011) are influenced by individual body size and by predator regime (Brown and Braithwaite, 2004; Brown et al. 2005; 2007b; Dingemanse, 2009). Behavioural traits can be correlated in populations, so that



one trait can predict or even be constrained by another (Sih et al. 2004; Bell, 2007). When traits are correlated in this manner they are known as behavioural syndromes (Sih, 2004). Behavioural syndromes are important from an evolutionary perspective because they suggest that behaviour is not infinitely plastic and that traits may not necessarily evolve or develop freely of each other (constraint hypothesis; Bell, 2007). For instance, boldness and aggressiveness tend to be favoured in populations from high predator density environments, perhaps due to some underlying physiological mechanism such as hormone expression. Dingemanse et al. (2007) measured behavioural correlations in 12 populations of sticklebacks (*Gasterosteus aculeatus*) and found evidence of syndromes between aggressiveness, exploration and activity only in populations from large ponds containing predators. Similarly, sticklebacks exposed to predation risk developed a behavioural syndrome between boldness and aggression in a population with no previous evidence of such a correlation (Bell and Sih, 2007). Conversely, strains of predator-naïve lab- and hatchery-reared zebrafish (*Danio rerio*) show strong behavioural correlations between activity and boldness compared to a strain derived from wild populations (e.g. Moretz et al. 2007). Correlated behavioural traits have been shown to vary in an individual's lifetime, suggesting that behavioural syndromes may be formed by suites of beneficial traits influenced by environmental and ontogenetic circumstances resulting in optimal trait combinations (Bell and Stamps, 2004; Dingemanse et al. 2007). Given the implications for survival and fitness, it comes as no surprise that interest in behavioural syndromes have increased over the last decade (Vonk et al. 2017).

Owing to their robust nature and ubiquity in a variety of environments, the family Gobiidae are an ideal model taxon to investigate anti-predator behaviour as demonstrated through boldness and activity behavioural trait continuums. Investigations on anti-predator behaviour in this family are relatively rare, although Smith (1989) found that starry gobies (*Asterropteryx semipunctatus*) reduce activity when exposed to a predator olfactory cue for

the first time, while two-spotted gobies (*Gobiusculus flavescens*) require multiple exposures (Utne-Palm, 2001). In a more recent study, two coral reef-dwelling gobies (*Coryphopterus glaucofraenum* and *Gnatholepis thompsoni*) responded to visual cues of native and invasive predators, however only *C. glaucofraenum* utilised olfactory cues from conspecifics to assess predation risk (Marsh-Hunkin et al. 2013). Thus, the cues used for predator recognition appears to vary tremendously between goby species. Moreover, although anti-predator responses have been investigated in this group (Smith, 1989; 1992; Smith and Lawrence, 1991; Turesson et al. 2009), comparisons of such behaviour between populations from high and low predation sites have not been made.

In this study, we investigated the anti-predator behaviour of wild-caught and captive-reared *Bathygobius cocosensis* towards visual and/or olfactory cues from a sympatric predatory crab species. We examined several behaviours (time to exit shelter, activity and forage success) in the presence of predator cues and evaluated correlations between two key traits; boldness and activity in each context. We hypothesised that wild gobies would show stronger anti-predator behaviour than captive-reared gobies, that anti-predator behaviour would vary in response to different cues, and that behavioural syndromes would most likely emerge in high threat contexts (i.e. wild gobies). Lastly, we expected small fish to be less risk-sensitive than larger fish due to their naivety in risky scenarios.

## Methods

### *Wild test subjects*

*B. cocosensis* is found along the rocky and sandy shorelines of the coast of New South Wales (NSW). Individuals were collected on four separate occasions (n = 80, ca 20 per collection period) within six weeks of each other at Dee Why (33.7502° S, 151.2991° E) using small hand-held nets, and were transported in large (10L), aerated buckets to the Sea

Water Facility at Macquarie University. The gobies were immediately acclimatised to a recirculating system upon arrival. Each group of gobies was added to an opaque tub of approximately 70L capacity (64.5 x 41.3 x 27.6cm). Each of these plastic tubs was covered with a lid, which was fitted with a 15mm hose providing flowing water at a rate of 3L/min. On the proximal end of each tub was a 25mm outlet for drainage, covered in 2mm mesh to prevent the gobies escaping. All tubs were kept bare, with no added aeration, shelter or substrate, and the system water was maintained between 19-20°C. The gobies were kept under these conditions for 40 hours without feeding until testing began.

The predator used in this investigation was the common rock crab *Plagusia chabrus*, and, while *B. cocosensis* shares tide pools with many crab species, they are typically small individuals of a morphology suited to an opportunistic diet of molluscs and algae (*Ozium truncates*, *Leptograpsus variegatus*; Skilleter and Anderson, 1986). In contrast, *P. chabrus* is a carnivorous species known to prey on small fish (Johnston and Freeman, 2005; White et al. 2015).

#### *Captive test subjects*

Captive-reared *B. cocosensis* were collected as newly hatched juveniles (7-12mm) from Dee Why and initially reared under laboratory conditions in a tidal tank for 12 months (see *test subjects* and *housing* in Methods, Chapter 4). The micro-niches were subsequently joined and altered to mimic the rockpool environment they were collected from, and they remained in that setup for an additional 6 months prior to further experiments (see note, end of Chapter 4). Feeding was done systematically (see *housing* in Methods, Chapter 4). All individuals (captive n = 36; wild n = 80) were randomised into one of the four predator treatments (captive n = 9; wild n = 20 per treatment).

## Test apparatus and procedure

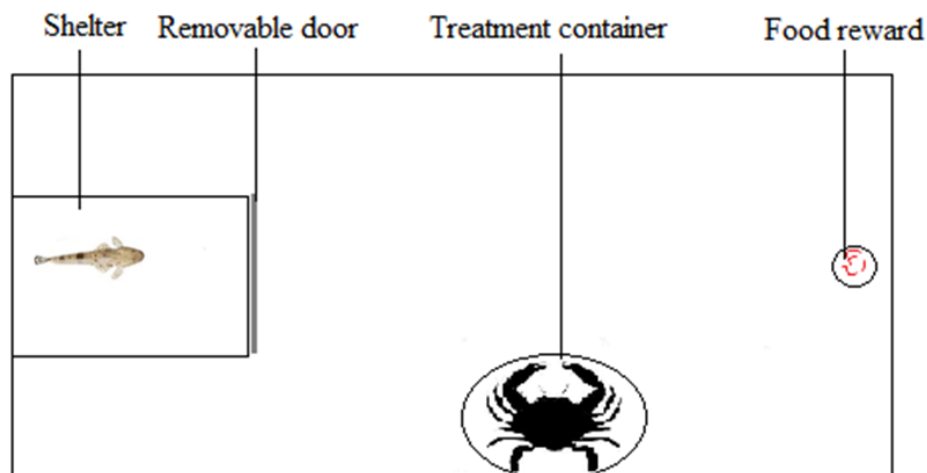
The experimental arena consisted of a white rectangular tub (60cm (L) x 50cm (W) x 35cm (H)) containing saltwater 6cm deep (30L). A clear, rectangular shelter (L30xW15xH15cm) was placed at the proximal end, with an angled removable partition on one end, which housed the gobies at the beginning of each trial (Fig 5.1). A mesh screen was erected around one end of the test tank to minimise perceived movement and possible disruption to test gobies from outside sources. For each trial, one goby was introduced to the shelter and given two minutes to settle. After this, the container holding the predator cue was introduced to the middle of the arena in a clear, circular container, either on the left or right side of the shelter (randomised between trials). For the combined olfactory and visual treatment (OV), one *P. chabrus* individual (carapace diameter 48mm) was placed in a clear container with holes to facilitate olfactory cue dispersal around the test area. For the olfactory only treatment (O), the container with holes held a sponge soaked in the scent of *P. chabrus* derived from the predator's housing tank. In the case of the procedural control (P), the container held a sponge soaked with saltwater. For the visual treatment (V), an individual *P. chabrus* was placed in an identical container with no holes so the gobies could see but not smell the predator. The gobies were given an additional two minutes of acclimation time after the predator was introduced.

A food reward (*Artemia*) was introduced at the end of the test arena opposite the shelter to encourage the fish to commence foraging behaviour. To decrease the likelihood of olfactory cues mixing, the food reward was introduced to the distal end of the tank just 10s before the test goby was released. Each goby spent a total of four minutes in the shelter, after which the partition was removed, and behavioural observations began. We recorded the time to exit the shelter, the amount of time spent moving, and whether the food reward was consumed before the end of the trial. Each goby was given a maximum of 16 min from the

time the shelter was opened to find and consume the food, after which the trial concluded. The test tank was emptied, rinsed and refilled after all trials to eliminate contamination of cues between trials. All trials were monitored via a camera mounted above the test arena, and footage was transferred to a hard drive. Each goby was exposed to one experimental treatment and one trial only, after which they were measured for total length ( $TL \pm 0.1\text{mm}$ ) and then returned to the opaque acclimatisation tubs or tidal tank. To avoid repeated capture, each cohort of wild-caught gobies was held in the lab for the duration of this study.

#### *Ethical note*

Gobies were caught in compliance with NSW Fisheries (permit no. P08/0010-3.0). Husbandry and experimental conditions were approved by the Macquarie University Ethics Committee (ARA 2014/003). Following experimental trials, all wild gobies were released at the site of capture and captive individuals were returned to the tidal tank.



**Figure 5.1:** Predator experimental setup. The treatment container to the left or right of the shelter box was a randomised process. The shelter was an opaque plastic box on the distal end and acted as a safe house for gobies prior to commencing each trial. The food reward was placed in a small, circular pocket opposite the shelter.

220 *Data analysis*

221           During trials, some individuals did not exit the shelter into the test assay. These  
222 gobies were removed from further analysis. Of those that did exit the shelter, a generalised  
223 linear model (GLM) with a binomial distribution was used to test for the effects of population  
224 and predator cue treatment on the probability of gobies consuming the food reward or not.  
225 Due to confounding issues of size between populations, TL was analysed independently  
226 using a GLM.

227           For the exit time analysis, data were square-root transformed to achieve a normal  
228 distribution and analysed using an analysis of covariance (ANCOVA). To account for  
229 confounding issues in size between wild and captive-reared gobies, each outcome variable  
230 was analysed with TL as a covariate. Fixed effects were population and experience with  
231 predator (i.e. wild: experienced; captive: naïve) and predator cue treatment. Time to forage  
232 was calculated from the time gobies exited the safety shelter to the time a) they consumed the  
233 food reward or b) the trial reached maximum time (16min). Time to forage was square-root  
234 transformed and analysed in the same way as exit time. In cases where the subject did not  
235 complete the trial (i.e. did not find or consume the food), they were given a maximum time  
236 value of 20 min.

237           The amount of time the fish spent moving was obtained by watching a slowed version  
238 of the video. Gobies move in a start-stop manner and exhibit long pauses between  
239 movements. These pauses were timed in seconds and summed. The amount of time spent  
240 moving (activity) was then extrapolated from the time to forage, converted to a percentage  
241 score and analysed using ANCOVA with population and predator treatment cue as fixed  
242 effects and TL as the covariate. Further analyses on TL and time spent active were carried out  
243 using regression analyses.

The presence of behavioural syndromes was investigated using regression analyses on time to exit (boldness) and the proportion of time fish spent active between populations and predator cue treatments.

All statistical analyses were performed using SPSS (Statistics for Windows, Version 24.0 IBM Corp. 2016), StatView Version 5.0.1 (SAS Institute Inc. 1998) and Excel version 12.2.3 (Microsoft, 2008).

## Results

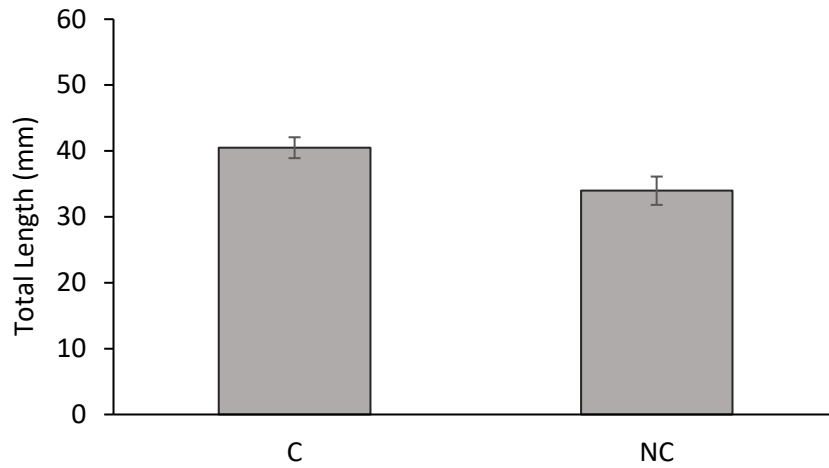
### *Time to exit shelter (boldness)*

There were no significant effects of population, predator cue treatment or TL on exit time, nor any significant interactions ( $p > 0.05$  in all cases).

### *Time to forage and probability of consuming the food reward*

There was no significant effect of population or predator cue treatment on time to begin foraging, however, there was a significant effect of TL ( $F_{1,67} = 5.094$ ,  $p = 0.027$ ); smaller individuals were faster to consume the food reward. There were no significant interactions.

Wild gobies were more likely to consume the food reward than those reared in captivity (~86% and 45%, respectively; Wald Chi Square,  $X^2_2 = 12.078$ ,  $p < 0.001$ ), but there was no effect of predator cue treatment ( $p > 0.05$ ), and there was no significant interaction between the two ( $p > 0.05$ ). Fish that consumed the food reward were larger than those that did not (Wald Chi Square,  $X^2_2 = 5.885$ ,  $p = 0.015$ ; Fig 5.2).

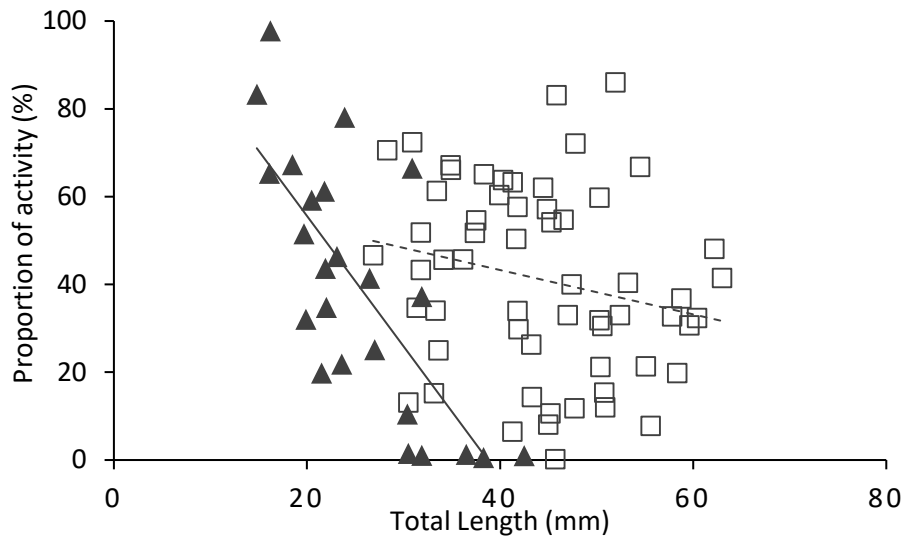


**Figure 5.2:** Mean ( $\pm$ S.E.) TL (mm) of gobies that consumed (C) and did not consume (NC) the food reward.

#### *Activity levels*

There was a significant effect of population ( $F_{1,3} = 5.886$ ,  $p = 0.018$ ) and TL ( $F_{1,67} = 27.929$ ,  $p < 0.001$ ) on the proportion of time spent active, but no significant effect of predator cue type ( $p > 0.05$ ). There was a significant interaction between population and TL ( $F_{1,1} = 12.993$ ,  $p < 0.001$ ; Fig 5.3). In both populations there was a negative association between size and the proportion of time spent active, but only significantly so in the captive-reared population (wild:  $F = 0.472$ ,  $df = 1$ ,  $R^2 = 0.091$ ,  $p > 0.05$ ; captive:  $F = 8.844$ ,  $df = 1$ ,  $R^2 = 0.287$ ,  $p < 0.01$ ).





**Figure 5.3:** Relationship between the proportion of time spent moving (%) and TL (mm) for wild (□, dashed line) and captive-reared (▲, solid line) populations.

#### *Behavioural syndrome: boldness and activity*

Regression analyses investigating for the presence of behavioural syndromes revealed a significant negative correlation between exit time (boldness) and activity in wild gobies that were subjected to the O and OV predator cues, but not V and P (Table 5.1). There were no significant correlations between exit time and activity in captive-reared gobies (Table 5.1).

**Table 5.1.** Relationship between exit time and the proportion of activity (%) between treatments per population. \* denotes significant correlation.

Population	Predator cue	F-value	R <sup>2</sup>	p-value
<b>Wild</b>	O	5.121	0.254	<0.05*
	V	3.045	0.217	0.109
	OV	4.948	0.310	<0.05*
	P	0.133	0.009	0.721
<b>Captive</b>	O	0.360	0.012	0.862
	V	0.585	0.019	0.825
	OV	0.856	0.222	0.423
	P	1.360	0.163	0.282

## Discussion

We found that wild gobies reacted to olfactory predator cues more so than to visual cues, while captive-reared gobies reacted similarly across all predator treatments. Both populations decreased activity as body size increased, and this change was most apparent in the captive population. Our results support the hypothesis that wild gobies, having constant exposure to predation risk, show stronger correlations between boldness and activity (behavioural syndrome) compared to captive-reared gobies and this was only manifested in a high-risk context: in the presence of predator olfactory cues.

### *Behavioural Syndrome: Boldness and Activity*

In wild gobies, we found a significant negative relationship between boldness and activity behavioural traits, but only in treatments that included the predator olfactory cue (olfaction alone and a combination of visual and olfactory cues), whereby individuals that were faster to exit the safety shelter also spent more time active during the trial. Exit time (boldness) and activity of wild gobies in the visual only treatment showed the same correlation, though not significantly so. There was no correlation in the control treatment nor in any of the treatments for captive-reared fish. The lack of behavioural syndromes in captive-reared *B. cocosensis* suggests that, without continual (or at least recent exposure to) predation threat, there is no selective pressure for the boldness-activity syndrome to emerge, bearing in mind that these fish were captured from the wild, albeit at a very young age. This suggest that the removal of predatory threat for long periods during ontogeny is enough to decouple these traits. This begs the question as to how much of the variability we often see between populations (e.g. Bell, 2005; Dingermanse et al. 2007) is due to ancestral, heritable variability versus plasticity during development as a direct result of individual experience. Bell and Sih (2007) measured the correlation between boldness and aggression before and

after exposure to predation, which resulted in the deaths of half the fish. Prior to predator exposure, no correlation existed, but predator exposure generated the syndrome, thus, the syndrome was created by both selective predation and behavioural plasticity in the prey. We suggest that removal of predation can cause syndromes to collapse and that this can be brought about entirely through plasticity.

In earlier studies, two major drawbacks of behavioural syndromes were outlined. Firstly, they are an evolutionary constraint because the underlying genotype gives rise to correlations which may or not be successful in a given environment and therefore may or may not be passed onto the next generation (Lande and Arnold, 1983). Secondly, if individuals tend to behave in a particular way, there is reason to believe that this would hinder adaptive behavioural change and thus, inhibit optimal behaviour (Sih et al. 2003). However, there is increasing evidence that behavioural traits are not coupled in a finite way, even in populations of the same species. Bell and Stamps (2004), for example, found that aggression and boldness in one population of sticklebacks were unstable throughout ontogeny, as was the correlation between them. In another population of the same species, behavioural traits were not stable across ontogeny but the correlations between them were, especially when individuals were exposed to predation threat (Bell and Stamps, 2004). A follow up study between 12 populations of sticklebacks showed that behavioural syndromes are greatly influenced by predator density; populations from large ponds with piscivorous predators showed stronger trait correlations than counterparts from smaller, predator-free ponds (Dingemanse et al. 2007). Thus, although behavioural syndromes can form between behavioural traits, the correlation between them is flexible in high risk contexts, such that an optimal combination is maintained in relation to risk. Therefore, while we recognise that measuring risk-taking behaviour with a predator simulation cannot quantify all boldness traits

(Toms et al. 2010), we propose that predation regime plays a vital role in the development of boldness-related behavioural traits (and syndromes) in gobies.

### *Body size*

As expected, we found that size was implicated in a number of measured behaviours, such as time spent active and time to begin foraging. In both of these frameworks, smaller gobies were less cautious, exhibiting higher activity levels with shorter pauses between movements, and this was irrespective of predator cue treatment. In contrast, larger gobies from both populations were more risk averse, spending less time active during trials of all treatment types. This mirrors the results of previous studies that showed behavioural traits remain plastic throughout ontogeny (Bell and Stamps, 2004; White et al. 2013). For instance, Brown and Braithwaite (2004) reported that juvenile poeciliids emerge from a shelter to forage faster than adults, possibly due to a higher metabolic demand and a corresponding disregard of predation pressure. Another possibility is that larger, presumably more experienced gobies, were wearier of the predator (Brown and Braithwaite, 2004) or more stringent in evaluating the level of risk in the trial prior to the commencement of foraging. It should be noted that, although present in both populations, the negative relationship between movement and size was only statistically significant in the captive-reared group.

While larger gobies appeared to be shyer as indicated by taking longer to start foraging, the inverse relationship was observed with the food consumption response; larger gobies consumed the food reward more often. While this may at first seem to contradict the metabolic hypothesis, whereby small fish with high metabolic rate trade off high risk to access food (Brown and Braithwaite, 2004; Brown et al. 2005), it is likely that once the large fish did emerge, having decided it was safe to do so, they have greater food requirements simply because they have larger body size. Almost all wild gobies (~86%) consumed the

food reward compared to less than half the captive-reared population (45%); a surprising outcome given that the food reward (*Artemia*) is presumably better recognised by captive-reared than wild gobies (e.g. Suboski and Templeton, 1989), but perhaps not an unexpected outcome from a boldness perspective.

Individual behavioural traits change throughout ontogeny (Magurran, 1990; Budaev et al. 1999; Bell and Stamps, 2004; White et al. 2013), which is likely the case with *B. cocosensis*. In shallow pools high on rocky platforms, individuals are small with relatively large eyes compared to individuals in deeper pools located lower on the platform (Malard et al. 2016). Shallow pools may pose a greater risk of predation by avian species (Yoshiyama, 1980), suggesting these gobies would take on a ‘hiding in plain sight’ approach through camouflage (Smithers et al. 2017). In contrast, larger gobies in deeper pools would likely incorporate rapid movements into their escape responses and hide amongst the rocks and crevices. Both behaviours have been observed in the Dee Why population of *B. cocosensis*, suggesting that anti-predator behavioural plasticity is influenced by shifts in micro-habitat use during ontogeny. This suggestion is further warranted by the habitat in which *B. cocosensis* is found; the intertidal zone is an ever-changing and unstable habitat, so plastic behavioural traits would be advantageous in short-term (such as changing tides) as well as long-term (e.g. seasons) contexts. Intertidal species demonstrate physiological and behavioural plasticity depending on origin (White and Brown 2013; 2014a; b; White et al. 2015). Our results further imply that intertidal gobies demonstrate intraspecific behavioural plasticity which is influenced by size/ontogenetic stage. Inconsistent behavioural traits have been demonstrated in juvenile damselfish (*Pomacentrus amboinensis*), which the authors suggest is due to favoured flexibility whilst individuals are not yet established (White et al. 2013). This may well be the case for *B. cocosensis* which has a pelagic larval stage (Thia et

al. 2018), however, a benthic lifestyle following settlement becomes no more predictable in the intertidal zone.

To conclude, despite finding few differences in the anti-predator behaviour of wild and captive-reared gobies we did find evidence of behavioural syndromes that were only manifested in predator-experienced populations in high-risk contexts (the presence of olfactory cues) which is broadly consistent with previous literature. Here, however, there is a notable difference in that the captive reared population lost the correlation between boldness and activity having been removed from predation threat. Our results once again highlight the important role of body size in shaping behavioural traits and clearly point to shifts in behaviour over ontogeny.

## CHAPTER 6

### **No evidence of population-level laterality in wild and captive-reared gobies (*Bathygobius cocosensis*)**

To be submitted to *Journal of Fish Biology*

Authors: *Penelope Carbia and Culum Brown*

## Abstract

Cerebral lateralisation, the preferential use of either brain hemisphere to analyse specific information, has considerable fitness benefits, including enhanced cognitive capacity and efficiency for behaviours such as schooling. Some fish demonstrate variation in lateralised behaviour at the population level, but little is known about the underlying mechanisms. Previous work has suggested that individual experience with environmental complexity, sociality and predation risk may shape laterality during ontogeny. However, studies investigating the relationship between these factors, and indeed lateralisation in general, has largely focused on shoaling species. Here, we observed wild and captive-reared *Bathygobius cocosensis*, a solitary benthic species, in a mirror test to investigate individual- and population-level cerebral lateralisation as demonstrated by eye-use preference when viewing their mirror image. Although some individuals demonstrated an eye bias, we observed no population-level lateralisation in either wild or captive-reared populations. However, males and females differed from one another, with females demonstrating a trend towards left-eye bias as size increased. Our results demonstrate the plasticity of laterality throughout ontogeny and are consistent with previous comparative studies that suggest sociality (schooling) is a likely strong driver of population-level lateralisation and, as such, is not displayed in rockpool gobies.

**Key words:** Cerebral lateralisation; eye bias; mirror image; non-social; ontogeny



## Introduction

Cerebral lateralisation refers to the functional partitioning of information processing in each brain hemisphere and was once considered to be a uniquely human phenomenon (Sovrano et al. 1999), however, the ubiquity of laterality in animals suggests its evolution has ancient origins (Babcock, 1993). We now know that behavioural lateralisation occurs in many mammals (Cowell and Denenberg, 2002; Wells, 2003), birds (Andrew et al. 2000; Vallortigara, 2000; Csermely, 2004), amphibians (Bisazza et al. 1996; 1998b; 2002) and fish (Bisazza et al. 2000a; Bisazza and Brown, 2011). Given its wide reach, it is unsurprising that cerebral lateralisation has associated fitness benefits (Takeuchi et al. 2010) such as multitasking (Güntürkün et al. 2000; Dadda and Bisazza, 2006a; Rogers et al. 2004; Hunt et al. 2006) and handling divided attention (McGrew and Marchant, 1999; Rogers, 2001; Dadda and Bisazza, 2006b). Strongly lateralised parrots, for example, are better problem solvers than non-lateralised parrots (Magat and Brown, 2009).

The dual processing hypothesis suggests that strongly lateralised individuals have enhanced cognition because their brain can process different sources of information using both hemispheres simultaneously (Rogers et al. 2004). For example, chicks (*Gallus gallus*) use their left eye (right cerebral hemisphere) when viewing food items and their right eye (left cerebral hemisphere) when observing predators (Rogers et al. 1985), thus enhancing fitness through better performance of two tasks simultaneously when compared to non-lateralised chicks (Rogers et al. 2004). Theoretically, the benefits of laterality would be realised at the individual level irrespective of the direction (left or right), so long as individuals predominantly use one hemisphere to analyse particular sources of information.

Having laterally placed eyes, fish perceive different fields of view and these fields are projected almost exclusively to the contralateral cerebral hemisphere (Vanegas and Ito, 1983; Vallortigara et al. 1998). As a result, information transfer between cerebral hemispheres is

low, and preferential eye use is believed to be a behavioural consequence of specialisation of the left or right hemisphere (Vallortigara, 2000), influencing social interactions and everyday behaviours (Sovrano et al. 1999). Cerebral lateralisation is well documented in fish (Bisazza et al. 1998; Vallortigara et al. 1999; Rogers, 2001; Vallortigara and Rogers, 2005), with different preferences for eye use depending on the context (see Bisazza and Brown, 2011 for a review). Among other things, cerebral lateralisation has been linked to a range of important behaviours in fishes including schooling (Bibost and Brown, 2013), decision making (Trompf and Brown, 2014), numerical skills (Dadda et al. 2015) and learning (Bibost and Brown, 2014).

While there are multiple potential benefits to laterality, there are also potential costs, and the relative trade-off between costs and benefits likely explains why variation in laterality exists at both individual and population levels (Dadda et al. 2009). For instance, a prey species that preferentially uses their right eye to view a potential predator and a leftward escape response would be at a disadvantage if predators learn to anticipate escape responses or approach prey from their left side (Vallortigara, 2006). Equally, however, any individual that behaves differently to the rest of the group may be singled out (the oddity effect; Landaeu and Terborgh 1986). Therefore, population-level lateralisation is difficult to interpret and must be formulated by a range of balanced selective forces that render predictable behaviour in individuals valuable enough to be expressed consistently by the entire population in some contexts, but not others (Vallortigara and Rogers, 2005; MacNeilage et al. 2009).

One of the leading factors used to explain population-level laterality in some species compared to others is the level of sociality, as a population bias for one side would increase cohesion and coordination in group behaviour, such as schooling to evade a predator (e.g. Bisazza et al. 2002; Brown, 2005; Sovrano et al. 2005). Lateralisation in individuals,

regardless of left or right bias, uncoupled from population-level lateralisation would likely be more prevalent in solitary species with little social interaction. In support of this hypothesis, Bisazza et al. (2000) investigated lateralisation in 16 species of fish and found that all shoaling species demonstrated a side-bias at the population level, compared to only 4 out of 10 non-shoaling species. Subsequent studies on shoaling species have shown lateralised behaviour is more strongly expressed in females, the more social sex (e.g. Bisazza et al. 1997a; b; Sovrano et al. 1999; Sovrano et al. 2001), while others have shown that population-level lateralisation is influenced by sociality throughout ontogeny, where juveniles school for protection but become increasingly solitary as adults (Moscicki et al. 2011). Thus, social construct appears to be an important factor driving the development and maintenance of lateralised behaviour in a population and may shift throughout ontogeny.

The forces shaping individual-level laterality are complex to investigate due to different experiences and stressors that may influence its development during early ontogeny (Bisazza et al. 1997a; b; 1998a). There is evidence to suggest that some component of laterality is heritable (Brown et al. 2007a), but nonetheless is influenced by experience during development. For instance, guppies reared with predator olfactory cues develop more strongly lateralised behaviour than siblings raised without cues, but this was not manifested at the population level (Broder and Angeloni, 2014). Some species demonstrate eye-use preference when viewing predators or neutral objects (Facchin et al. 1999), which may be correlated to the level of predation pressure experienced in early ontogeny (Brown et al. 2004; 2007a), while others show either a left or right side-bias depending on the stimuli or degree of familiarity (Miklosi et al. 1997; Sovrano, 2004; Bibost et al. 2013). Therefore, lateralisation may be more plastic than previously thought, and changes in environmental stimuli may influence shifts in lateral bias through familiarisation or change in the emotive value of the object, scene or context.

Here, we investigated laterality in captive-reared and wild-caught populations of the solitary intertidal goby, *Bathygobius cocosensis*, by evaluating individual eye-use preference when viewing their mirror image. Having been reared in a dynamic and high predation environment, we predicted that the wild population would show greater lateralised behaviour than the captive-reared population. Given the solitary nature of the species, however, we did not expect to find population consistency in the pattern of laterality. Moreover, we expected males and females to differ in laterality owing to varying life-history strategies and associated social interactions. Along similar lines, we also thought laterality might vary with body size since shifts in sociability through ontogeny are common in fish. Thus, body size, sex and rearing environment might influence how individuals interpret their own mirror image.

## Methods

### *Test subjects*

Captive-reared *B. cocosensis* were collected as newly hatched juveniles (7-12mm) from Dee Why (see *test subjects* Chapter 4). Wild adult gobies (n = 24) were captured from Dee Why and transported using the same protocol as with the juvenile gobies.

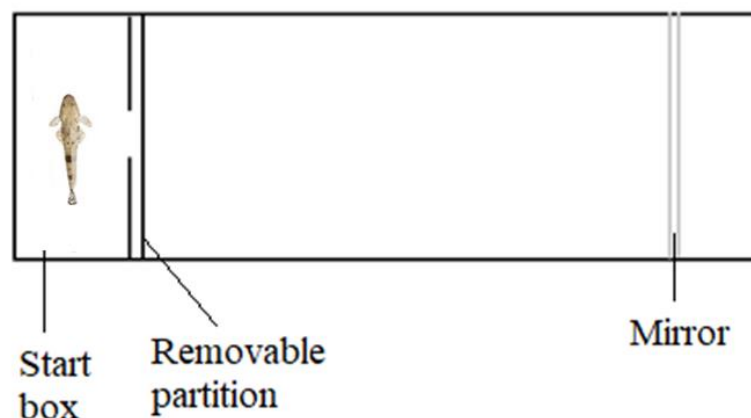
### *Housing*

Juvenile gobies were initially reared under laboratory conditions in a tidal tank for 12 months (see *housing* in Methods, Chapter 4). The micro-niches were subsequently combined and altered to mimic the rockpool environment they were initially collected from, and they remained in that setup for an additional 6 months prior to further experiments (see note, end of Chapter 4). To minimise interaction with external factors, the tank was surrounded with polystyrene foam, with four small holes (1cm D) above each chamber. Food was administered automatically (see *housing*, Chapter 4). Following the acclimation period, 30 captive-reared individuals were randomly chosen and used for the lateralisation experiment.

Wild gobies were kept in the recirculating system outlined in methods (*wild test subjects*; Chapter 5). They were randomly distributed into two of the 70L opaque housing tubs (13 per tub). Wild gobies were kept in these tubs for 36 hours for acclimation purposes and fed a mixture of commercialised frozen *Artemia* and mysid shrimp.

### *Test Apparatus*

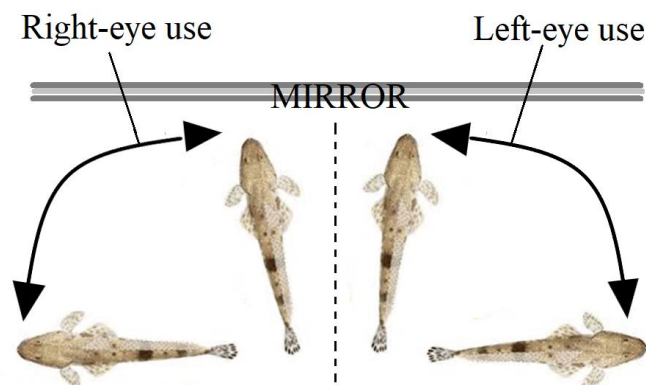
The test apparatus was modelled after Sovrano and Andrew (2006; Fig 6.1). The entire runway (50cm Lx10cm W) was constructed of 3mm PVC plastic and lined with 1cm of fine sandy substrate. One end of the runway was used as the start box, fitted with a permanent partition (10cm Wx16cm H) with a small door cut out along the base (2cm Wx4cm H) through which the gobies could exit. A removable partition covered the exit which could be remotely operated. The runway was submerged in a large fibreglass tub (120x75x19cm) and water was maintained at 12cm deep. A camera was mounted above the maze on a steel frame to record all trials and avoid observer-induced bias. For acclimation purposes, both wild and captive groups were given a 24-hour familiarity session in the test apparatus with no mirrors (Brown, 2001). They were then returned to their respective housing tanks for another 24-hours until testing began.



**Figure 6.1:** Schematic representation of runway apparatus set up for lateralisation test.

## Procedure

All gobies were tested individually. Each fish was gently transferred from an aerated container to the start box for a 5-minute acclimatisation period. The partition was then removed, and the goby was free to explore the runway for a total of 5 minutes. The footage was later analysed for preferred eye use upon first approach to the mirror as well as throughout the trial by scoring fish position every 2 s (Sovrano et al. 1999). Fish position was then used to calculate use of the right:left monocular visual field, based on the angle at which gobies were positioned relative to the mirror (Fig 6.2). In cases where gobies faced the mirror directly, or turned away at an angle greater than 90°, data were removed from analysis.



**Figure 6.2:** Schematic representation of the viewing angles of test gobies that define monocular vision (and subsequent cerebral hemisphere processing) with the right or left eye.

Following each trial, individuals were measured for total length (TL), weighed and sexed. Each goby took less than 2 minutes to process and recovery was immediate. Wild gobies were then returned to the holding tubs for another 24-hours and subsequently released at the original site of capture. Captive gobies were returned to the tidal aquarium.

## Ethical note

Gobies were caught in compliance with NSW Fisheries (permit no. P08/0010-3.0). Husbandry and experimental conditions were approved by the Macquarie University Ethics

Committee (ARA 2014/003). At the end of the experiment, all wild gobies were released at the site of capture and captive individuals retained for further experiments.

### *Statistical analyses*

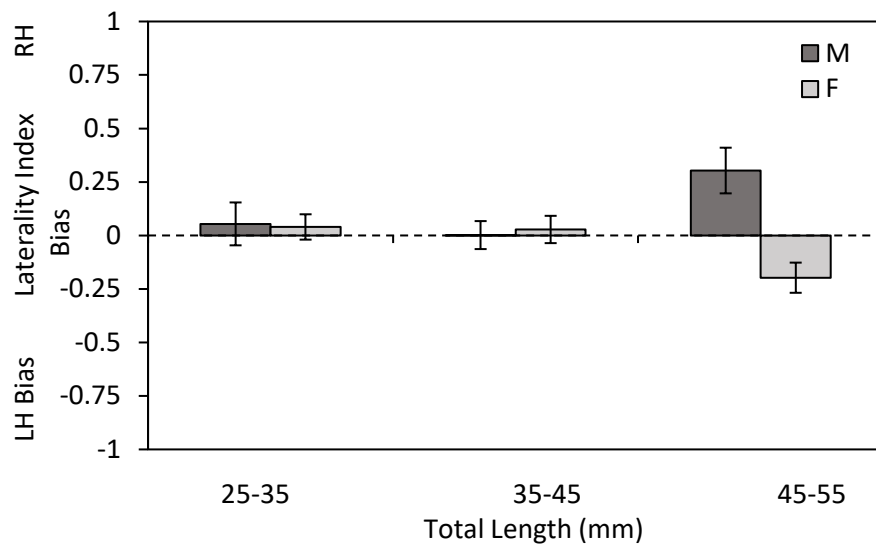
All data were tested for assumptions prior to analysis. Preferential eye-use data for each individual was converted to a laterality index score calculated as: [frequency of R eye use – frequency of L eye use] / [sum of R and L eye use] (LI; Brown et al. 2007a). Scores range from -1 (right-eye bias) to +1 (left-eye bias). Scores of 0 have no bias. We then analysed LI by examining the effects of sex and population using ANCOVA, with total length (TL) as a covariate to account for confounding issues in size between wild and captive-reared gobies.

In some cases, the LI differences between strongly and weakly lateralised individuals conceal population effects, so we analysed the absolute value of LI which indicates the strength of lateralised behaviour irrespective of direction (Brown et al. 2007a). Absolute laterality was analysed using ANCOVA, with population and sex as fixed factors, and TL as a covariate. Finally, we calculated the proportion of strongly lateralised individuals (> 80% L/R preference) in both populations.

## **Results**

The range of LI scores were normally distributed (Shapiro-Wilk:  $p > 0.05$  in all cases), suggesting there was no directional or disruptive selection. In the analyses of laterality index, there was no significant effect of population ( $F_{1,46} = 0.073$ ,  $p > 0.05$ ), sex ( $F_{1,46} = 2.339$ ,  $p > 0.05$ ) or TL ( $F_{1,46} = 0.086$ ,  $p > 0.05$ ). When grouped into size classes, the two smaller groups showed no sex bias for eye use (two-sample t-test; class 25-35mm;  $t = 0.350$ ,  $p > 0.05$ ; class 35-45mm;  $t = 0.081$ ,  $p > 0.05$ ; Fig 6.3). The largest size class exhibited slight

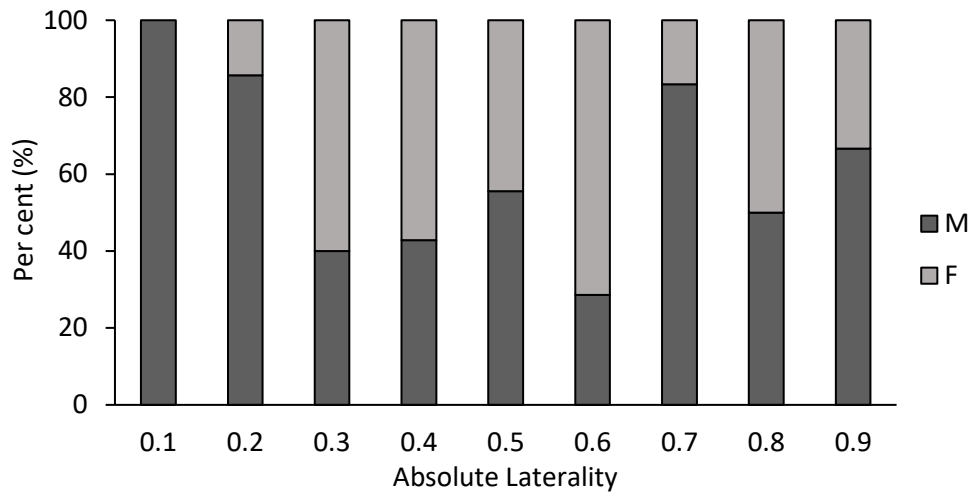
but non-significant eye-use preference; right-eye bias in males and left-eye bias in females (class 45-55mm;  $t = 0.847$ ;  $p > 0.05$ ; Fig 6.3). There were no significant interactions.



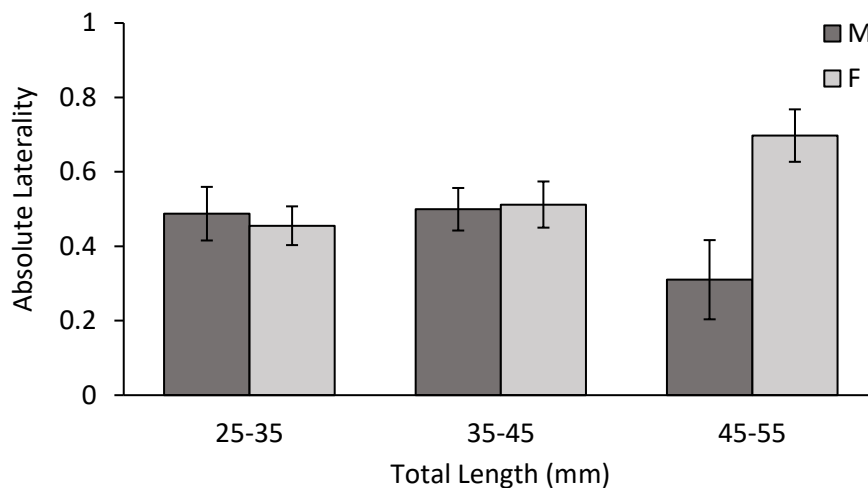
**Figure 6.3:** Mean ( $\pm$ S.E.) lateralinity index for male and female *B. cocosensis*. Positive scores indicate a preference for right-eye use and negative scores for left-eye use.

Analyses of absolute laterality revealed no effect of population ( $F_{1,46} = 0.231$ ,  $p > 0.05$ ) or TL ( $F_{1,46} = 0.038$ ,  $p > 0.05$ ). Sex was only marginally non-significant ( $F_{1,46} = 3.641$ ,  $p = 0.06$ ; Fig 6.4) with females being slightly more strongly lateralised than males, and there was a significant sex\*TL interaction ( $F_{1,46} = 4.918$ ,  $p = 0.03$ ). Pairwise post-hoc comparisons showed there were no differences between sexes in the smaller size classes (25-35mm and 35-45mm Fisher's PLSD  $p > 0.05$ ) but females were significantly more strongly lateralised than males in the largest size class (45-55mm: Fisher's PLSD  $p = 0.015$ ; Fig 6.5). Overall, proportion of strongly lateralised individuals (i.e.  $> 80\%$  use of L or R eye) was similar between populations, with 13% strongly lateralised individuals in the captive-reared population and 20% in the wild population.





**Figure 6.4:** Absolute laterality scores and the corresponding percentage of male and female gobies from both populations.



**Figure 6.5:** Mean ( $\pm$ S.E.) absolute laterality index in male and female *B. cocosensis*, in three size classes. Higher values indicate more strongly lateralised responses.

## Discussion

We examined eye-use preference in wild and captive-reared intertidal gobies when viewing their reflection in a mirror as a measure of cerebral lateralisation. As predicted, we found no evidence of population-level laterality in either wild or captive-reared populations and analysis of the frequency distribution suggests it did not differ from normal, thus, there was no evidence of directional or disruptive selection. While there were no differences

between the sexes in the small size classes, females in the largest size class showed a left-eye preference while larger males tended to use their right eye to view their mirror image. This pattern was also evident in the absolute laterality scores which varied significantly between sexes only in the largest size class, with females being more strongly lateralised than males. The increased laterality strength in larger size classes in both populations demonstrates that lateralisation remains plastic throughout ontogeny, likely shifting with changes in the emotive value of seeing an unfamiliar conspecific in the mirror.

The lack of significant eye-use bias in *B. cocosensis* conforms with the hypothesis that population-level lateralisation in fishes is an evolutionary product of sociality, driven by schooling behaviour. Rogers (1989) suggested that population lateralisation may have evolved in gregarious species in response to social behaviours and the required coordination, particularly in the context of schooling fish (Brown, 2005). This hypothesis is supported by several subsequent studies on shoaling species. Bisazza et al. (2000a), for example, investigated lateralisation in several fish species and reported that all shoaling species demonstrated population-level lateralised behaviour compared to only 40% of the non-gregarious species investigated. Likewise, our results agree with those of Moscicki et al. (2011), who found no population-level bias in the non-social convict cichlid *Amatitlania nigrofasciata* when evaluating a perceived social environment. Although larger individuals showed some L/R eye-use preference, the extent of lateralisation in *B. cocosensis* likely reaches an equilibrium at the population level because there is no selective pressure to coordinate behaviour with conspecifics as observed in shoaling species. Whether this reflects the nature of our methodology (i.e. a perceived social context when viewing mirror images rather than real conspecifics), or the motivation for each individual, remains equivocal. A recent study reported slight right-turn bias in populations of the temperate goby *Gobiusculus flavescens* in a detour test, which then switched to a left-turn bias when fish were reared in a

high CO<sub>2</sub> environment (Sundin and Jutfelt, 2018). It is important to note that this study likely tested motor bias since there were no objects or scenes to view in the test arena (Brown et al. 2004). Of all the research carried out on lateralisation, very few have looked at solitary benthic species, making some behaviours difficult to interpret owing to the lack of comparable literature. Nevertheless, existing evidence suggests that non-social fish are rarely strongly lateralised in any context.

It is possible that the lack of population-level laterality in wild and captive-reared populations of *B. cocosensis* may not be due to the extent of predation risk (Brown et al. 2004; 2007a), but rather the nature of that risk. It has been suggested that strongly lateralised individuals are more successful at escaping predation than non-lateralised individuals in species such as shiner perch (*Cymatogaster aggregate*; Dadda et al. 2010), but not in minnows (*Girardinus falcatus*; Agrillo et al. 2009). The authors of the latter study suggest that an individual's escape response may benefit from a non-lateralised mechanism which makes escape trajectories less predictable, in contrast to less risky situations such as foraging and conspecific confrontation which may benefit lateralised individuals (Agrillo et al. 2009). This suggestion is applicable to *B. cocosensis*, given that rockpool fishes experience considerable predation threats from aerial predators (Stevens et al. 2014) and attacks do not occur in a social context. Turesson et al. (2009) reported higher escape responses in black gobies (*Gobius niger*) when they were threatened from above rather than the side, suggesting aerial predation is a risk commonly experienced. Despite the link between c-start body curvature and locomotion, gobies demonstrated escape responses that were not biased by their body curve (Weihs, 1973). Thus, in aerial predation contexts where risk is from above, L/R asymmetrical bias is likely redundant. In the case of lateral attacks, there is evidence linking mouth asymmetry in predators to escape response laterality in gobies (*Rhinogobius* sp.), where predation success is higher when a left-biased predator approaches a right-biased

goby (Yasugi and Hori, 2012). Without knowing a predator's attack direction bias, non-lateralised individuals may have a greater chance of escaping predation by random escape direction compared to lateralised individuals.

It has been suggested that strongly lateralised individuals have superior cognitive function than weakly or non-lateralised individuals (Sovrano et al. 2005). This proposed correlation makes the outcome of this study difficult to interpret as rockpool gobies such as *B. cocosensis* demonstrate incredible feats of spatial memory and homing, using a combination of landmarks and egocentric information to navigate towards a goal (White and Brown, 2015a; c). Sovrano et al. (2005) showed lateralised minnows (*Girardinus falcatus*) are better able to spatially orientate themselves than non-lateralised individuals, which suggests cerebral asymmetry is involved in discriminating left from right (Chiandetti and Vallortigara, 2008). In contrast, lateralised poeciliids take longer to solve a maze task than weakly lateralised individuals, possibly due to inhibited exploratory behaviour influenced by asymmetrical bias (Brown et al. 2004). It is worth noting, however, that we do not yet know if there is a link between individual-level laterality and cognitive performance in this species, but it does not appear to be borne out at the population level. Thus, the proximate link between lateralisation and fitness remains equivocal in this context (Bibost and Brown, 2014).

Sexually dimorphic lateralisation is not uncommon in fishes (e.g. Bisazza et al. 1998a; Bianki and Filippova, 2001). In non-social cichlids, females demonstrate slight left-eye bias when viewing their mirror image, while males show no preference (Moscicki et al. 2011). In contrast, males of the highly social cichlid *Neolamprologus pulcher* show a right-eye bias when viewing their mirror image, while females showed no eye-use bias (Reddon and Balshine, 2010). Here, females tended to display a left-eye bias while males showed a slight right-eye bias, but this was only evident in the larger individuals. Males demonstrated

298 higher strength of laterality in smaller size classes, but this trend was switched in the largest  
299 size class where females were more strongly lateralised than males, which may indicate that  
300 eye-use bias in social contexts is related to reproductive status. Our results clearly  
301 demonstrate that laterality remains plastic throughout ontogeny, and the observed shifts as  
302 individuals age, combined with variation between sexes, suggest that this is related to  
303 maturation and associated breeding behaviour.

304         In conclusion, we have shown that *B. cocosensis* demonstrates no population-level  
305 lateralisation when viewing a mirror image, which is likely related to the largely solitary  
306 lifestyle in this species. Individual-level laterality was observed only in larger individuals, but  
307 varied between sexes, indicating it is a plastic aspect of eye-use bias and may be linked to  
308 reproductive status. Our results agree with the notion that sociality (schooling) plays a critical  
309 role in the expression of lateralisation and suggest that aerial predation plays a strong  
310 selective force for the lack of population-level lateralisation in intertidal gobies.

## **GENERAL DISCUSSION**

Phenotypic plasticity is the ability of the genotype to produce one or more phenotypes in response to environmental variability and is advantageous for organisms inhabiting dynamic environments (Pigliucci, 2001). Cognitive ability underpins the neural and behavioural processes involved in everyday ecological challenges and hence, plays a critical role in an individual's survival. This thesis explored the extent of phenotypic plasticity in the intertidal goby *Bathygobius cocosensis* through two mechanisms; the first was evaluating behavioural and cognitive plasticity in a natural setting, where individuals would benefit from managing trade-off costs and benefits to flexible foraging (Chapter 2) and cognitive plasticity (Chapter 3). The second approach was in a comparative framework of wild versus captive-rearing, where environmental enrichment reshapes innate patterns of spatial learning ability (Chapter 4), lack of predation influences the development of behavioural syndromes and anti-predator behaviour (Chapter 5), and social patterns impact cerebral lateralisation (Chapter 6).

### **Trophic niche plasticity**

We investigated the trophic niche width of three intertidal goby species and found seasonal shifts between the two sympatric species, whereby niche overlap increased during winter. In comparison, the allopatric species *B. cocosensis* showed a stable trophic niche in both seasons which is indicative of low interspecific competition due to their high abundance and aggressive nature (Griffiths et al. 2003a; White et al. 2015). Diet in *B. cocosensis* varied ontogenetically, which is likely related to specialised micro-habitat use as they grow (Malard et al. 2016). Grossman (1986) initially suggested that the dynamic and unstable nature of the intertidal zone would lead to flexible diet and niche plasticity in fish species found there. Our findings, together with the few recent studies from intertidal zones worldwide (Compaire et al. 2016; Barrett et al. 2018; Vinagre et al. 2018), agree with this suggestion and contribute to

the understanding of the multifaceted nature of foraging plasticity as influenced by competition in a dynamic environment.

### **Spatial learning in *Bathygobius cocosensis***

Sexually dimorphic cognitive ability has been demonstrated in various mammalian taxa (Kavaliers et al. 1996; 1998), and related to mating/parenting strategy, where the dispersing males have a greater need for cognitive map formation than the site-attached females weaning their offspring (Galea et al. 1994; Gaulin, 1995). Sexually dimorphic cognitive ability in fishes has previously received little attention, although some recent studies have reported preliminary evidence of differing cognitive abilities between sexes (Fabre et al. 2014; Lucon-Xiccato and Bisazza, 2017; Roy and Bhat, 2017). Here, I investigated whether male and female gobies differ in their ability to solve a spatial task and evaluated changes in performance between seasons. I found both sexes solved the task in a similar amount of time in all seasons except spring, where females solved the task significantly faster than males. As *B. cocosensis* breeds between September and November and exhibits a female-choice/male nest-guarding reproductive strategy, these findings suggest that spatial learning ability decreases in males when they are nest-bound. While guarding their nests, males may forgo foraging excursions, suggesting a reduced need for navigation and thus the maintenance of cognitive processes. Other studies on sexually dimorphic spatial learning in fish have investigated species where males disperse further than females and, unsurprisingly, demonstrate greater spatial learning ability (Sovrano et al. 2003; Lucon-Xiccato and Bisazza, 2017; Roy and Bhat, 2017). However, our study species is an intertidal resident with high site fidelity and little, if any, dispersal following settlement (White and Brown, 2013; Thia et al. 2018), thus, the inhibited spatial cognition in males during spring appears to be related to reproductive strategy.



Fishes inhabiting structurally complex environments demonstrate impressive spatial learning abilities and rely on a number of cues to navigate between important resources efficiently (e.g. Odling-Smee et al. 2008). White and Brown (2015a) found that rockpool specialist *B. cocosensis* solves spatial tasks and retains cue information longer than sand species, which is an advantageous ability in a structurally complex but relatively stable habitat. Moreover, their spatial learning ability is an evolutionary critical part of their success in homing to optimal rockpools where they remain during low tide (White and Brown, 2013). The question remained whether this ability is innately hardwired or altered by experience. I collected juveniles of this species and reared them in captivity with different regimes of structural complexity to investigate how it impacted their spatial learning ability. All individuals learned to solve the maze, but those from the structurally simple treatments took twice as long as those from the complex treatments. Under conditions that mimicked their natural environment, rockpool gobies maintained the neural capacity to solve a spatial task that led toward an important resource. On the other hand, gobies reared in the structurally simple treatments had no reliable physical cues to navigate by and so the cognitive processes for spatial learning were reduced. Our results strongly implicate experience during ontogeny as the primary driver of commonly observed population differences in cognitive ability.

## **Behavioural syndrome development**

Almost all animals will encounter predators at some point in their life, and their responses to these high-risk situations can mean the difference between survival and death. Predation risk varies in space and time, so prey species are challenged with balancing their everyday behaviours (e.g. foraging) by assessing the level of risk and responding accordingly through a learning curve based on previous experiences (Lima and Dill, 1990; Kats and Dill, 1998). However, the development of an individual's response to risk is a multifaceted process

that includes correlations between personality traits such as boldness and activity, which suggests limitations are placed on plasticity (Bell, 2007). Previous studies suggest that exposure to predators may generate behavioural syndromes which are otherwise lacking in populations from low-risk environments (Bell and Sih, 2007; Dingemanse et al. 2007). Wild gobies showed evidence of a syndrome between activity and boldness, but only in high risk contexts (olfactory cues emanating from a predator). Captive-reared gobies, in contrast, showed no correlation between boldness and activity, suggesting that combinations of optimal traits are less likely to develop or are otherwise lost in low predation environments. In addition, I found that size plays an important role for gobies in high-risk situations, presumably because older individuals are wearier of threats.

## **Cerebral lateralisation**

In fishes, the preferential use of one brain hemisphere over the other to process specific sources of information is demonstrated through eye-use ratio when evaluating visual stimuli (Balzarini et al. 2014). In schooling species, it is critical to watch and respond to shoal mates and thereby maintain cohesion, whilst simultaneously looking out for food or predators. This socially coordinated, dual processing favours consistency in eye-use preferences at the population level (Bibost and Brown, 2013). Wild and captive-reared gobies demonstrated no population-level bias in eye preference, suggesting that coordinated lateralised eye use is obsolete in a solitary species that demonstrates no cohesive behaviour. Higher absolute laterality in larger individuals, and the fact that direction preference differed between males and females, suggests that lateralised eye use is not only ontogenetically plastic but also that it may be influenced by courting or agonistic interactions once sexual maturity is reached.

## Concluding remarks

The interest in phenotypic plasticity has increased in recent years, providing valuable insight into the fitness benefits associated with flexible behaviour. Preceding work demonstrated that goby species from mundane intertidal habitats show poor cognitive ability in the context of spatial navigation relative to species from rockpools (White and Brown, 2014a; b). This thesis is an extension of that topic and shows that a shift in selective pressures during early ontogeny alters the extent of cognitive and behavioural plasticity in individuals of the same species and that this plasticity extends to various everyday challenges including foraging, navigation and predator avoidance. It also highlights the usefulness of a comparative approach using a wild/captive framework to investigate how an environment devoid of natural variation results in reduced cognitive ability. The themes developed in this thesis have important implications for rearing fish in hatcheries either for fisheries restocking or conservation management. There are also considerable implications for rearing conditions in captive fish populations from an animal welfare perspective, as rearing conditions can have dramatic impacts on the development of brains and behaviour.

Several possible research pathways may be expanded upon following this research. The comparison of trophic niches in native and introduced goby species, such as the Yellowfin goby (*Acanthogobius flavimanus*), may provide valuable insight into the competitive strategies of invasive fish species (Cohen and Bollens, 2008). Invasive species may also vary in their personality traits which may be a driving factor in competitive interactions with native species (Fogarty et al. 2011).

I have shown that shifts in environmental enrichment from early ontogeny reshapes cognitive ability, however, many gaps exist in the understanding of flexible laterality, particularly in non-social fishes. Interesting questions in the topic of cerebral lateralisation and plasticity include: how do laterality and behavioural syndromes develop in gobies reared

125 in isolation? Are lateralised individuals better at solving spatial tasks than non-lateralised  
126 individuals? Is individual-level lateralisation linked to boldness? Future studies should  
127 consider environmental constraints on the development of laterality and personality in non-  
128 social fishes, and whether these factors aid or inhibit their success in ecologically relevant  
129 cognitive tasks.

## APPENDIX 1.1 – Chapter 2

Table 2.2. Results of gut content analyses. \*=prey taxa

Species	<i>B. cocosensis</i>		<i>B. krefftii</i>		<i>F. lentiginosus</i>	
Prey items	%F	%V	%F	%V	%F	%V
<b>CNIDARIA</b>						
<b>Actiniaria*</b>					<b>2.6</b>	<b>0.8</b>
Larvae					2.6	0.8
<b>ANNELIDA</b>						
<b>Oligochaeta*</b>	<b>2.2</b>	<b>0.2</b>			<b>5.3</b>	<b>0.6</b>
Unidentified	2.2	0.2			5.3	0.6
<b>Polychaeta*</b>	<b>6.5</b>	<b>6.6</b>	<b>12.5</b>	<b>9.8</b>	<b>31.6</b>	<b>16.9</b>
Cirratulidae			2.1	0.3		
Nereidae	2.2	3.9	4.2	4.7	5.3	5.4
Pectinariidae					15.8	8.5
Terebellidae					2.6	0.6
Unidentified	4.3	2.7	6.3	4.8	2.6	1.8
<b>MOLLUSCA</b>						
<b>Polyplacophora*</b>	<b>2.2</b>	<b>4.4</b>				
Unidentified	2.2	4.4				
<b>Gastropoda*</b>	<b>45.7</b>	<b>27.4</b>	<b>8.3</b>	<b>3.7</b>	<b>23.7</b>	<b>2.8</b>
<i>Cellana tramoserica</i>	10.9	7.2	6.3	3.7		
<i>Cinnalepeta cinnamomea</i>	10.9	3.7			2.6	0.4
Littorinidae	8.7	0.3			15.8	0.5
Nacellidae	26.1	13.1			5.3	1.5
Nudibranchia	2.2	2.9				
Skeneopsidae	4.3	0.1	2.1	0.0	7.9	0.4
Unidentified	2.2	0.1				
<b>Bivalve*</b>	<b>4.3</b>	<b>0.6</b>	<b>8.3</b>	<b>1.2</b>	<b>13.2</b>	<b>0.9</b>
Mytilidae					2.6	0.2
Unidentified	4.3	0.6	8.3	1.2	10.5	0.6
<b>INSECTA</b>						
<b>Chironomidae*</b>	<b>54.3</b>	<b>9.7</b>	<b>39.6</b>	<b>24.7</b>	<b>13.2</b>	<b>2.3</b>
Chironomidae larvae	54.3	9.7	39.6	24.7	13.2	2.3
<b>CRUSTACEA</b>						
<b>Copepoda*</b>	<b>56.5</b>	<b>4.8</b>	<b>58.3</b>	<b>12.9</b>	<b>26.3</b>	<b>3.7</b>
Calanoid Copepoda			27.1	6.5		
<i>Calanus</i> spp.	2.2	0.3	25.0	4.3	2.6	0.1
Harpacticoid	47.8	2.9	20.8	0.8	21.1	2.1
Copepoda						
<i>Oncaea</i> spp.	34.8	1.6	25.0	1.1	13.2	1.4
<b>Cirripedia*</b>			<b>2.1</b>	<b>0.0</b>		
Larvae			2.1	0.0		
<b>Ostracoda*</b>	<b>41.3</b>	<b>2.0</b>	<b>33.3</b>	<b>2.0</b>	<b>5.3</b>	<b>0.2</b>
Unidentified	41.3	2.0	33.3	2.0	5.3	0.2

<b>Isopoda*</b>			<b>4.2</b>	<b>1.2</b>		
Unidentified			4.2	1.2		
<b>Leptotraca*</b>	<b>8.7</b>	<b>1.5</b>				
Unidentified	8.7	1.5				
<b>Amphipoda*</b>	<b>15.2</b>	<b>2.2</b>	<b>12.5</b>	<b>2.2</b>	<b>63.2</b>	<b>68.9</b>
Ampeliscidae sp.					2.6	1.1
<i>Corophium</i> sp.	4.3	0.7			2.6	0.2
Gammaridea sp.A					42.1	55.3
Gammaridea sp.B					5.3	7.2
Gammaridea sp.C	4.3	0.5			15.8	5.2
Unidentified	6.5	1.0	12.5	2.2		
<b>Tanaidacea*</b>	<b>76.1</b>	<b>37.1</b>	<b>4.2</b>	<b>0.5</b>	<b>5.3</b>	<b>1.1</b>
Tanaididae	76.1	37.1	4.2	0.5	5.3	1.1
<b>Caridea*</b>	<b>2.2</b>	<b>0.5</b>				
Unidentified	2.2	0.5				
<b>Brachyura*</b>	<b>6.5</b>	<b>2.7</b>	<b>2.1</b>	<b>0.1</b>		
Grapsidae	4.3	1.8				
Megalopa larvae			4.2	0.2		
Unidentified	2.2	1.0				
<b>Other Crustacea*</b>			<b>4.2</b>	<b>1.4</b>		
Unidentified			4.2	1.4		
<b>ECHINODERMATA</b>						
<b>Ophiuroidea*</b>			<b>2.1</b>	<b>1.2</b>		
Unidentified			2.1	1.2		
<b>CHORDATA</b>						
<b>Asciacea*</b>			<b>2.1</b>	<b>2.3</b>		
Unidentified			2.1	2.3		
<b>Algae*</b>	<b>2.2</b>	<b>0.2</b>	<b>33.3</b>	<b>33.1</b>	<b>7.9</b>	<b>1.8</b>
<i>Myelophycus simplex</i>			2.1	5.9		
Unidentified	2.2	0.2	31.3	27.2	7.9	1.8
<b>Other materials</b>			<b>10.4</b>	<b>3.7</b>	<b>2.6</b>	<b>0.8</b>
Unidentified prey item			10.4	3.7	2.6	0.8

## APPENDIX 1.2

### Communication of Research During Candidature

- **P. Carbia** and C. Brown. (2015). *Spatial Learning and Memory Retention in Intertidal Gobies*. Australian and New Guinea Fishes Association (ANGFA), Macquarie University.
- **P. Carbia**. *Captivity Influences Cognition in Benthic Fish*. ConCienca Azul Podcast, Episode 23, with Melissa Cristina Márquez. August 2018. Available from: [https://www.ivoox.com/episodio-23-penelope-carbia-australia-audios-mp3\\_rf\\_27879150\\_1.html?autoplay=true](https://www.ivoox.com/episodio-23-penelope-carbia-australia-audios-mp3_rf_27879150_1.html?autoplay=true)
- **P. Carbia** and C. Brown. (2018). *Where I'm from or how I got there? Environmental enrichment influences spatial learning ability in captive-reared intertidal goby Bathygobius cocosensis*. 6<sup>th</sup> ASFB Conference, Melbourne, Australia.

## **APPENDIX 1.3**

### **Ethics and Fisheries**

The work in this thesis, including husbandry and experimental conditions, was carried out following the rules and regulations of Macquarie University Ethics Committee for works on vertebrate species (ARA 2014/003). Subjects were collected according to the permitted practices set out by Fisheries, N.S.W, under permit no. P08/0010-4.6 (see following pages).



AEC Reference No.: 2014/003-28

Date of Expiry: 21 February 2019

Full Approval Duration: 21 February 2014 to 21 February 2020

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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Catarina Vila Pouca	0403 161 454
Sherrie Chambers	0407 796 410
Laura Ryan	0402 690 468

**Others Participating:**

Connor Gervais	0413 550 910
Dennis Heinrich	0498 360 635
Catherine Downs	0421 983 251
Rianne Vogelmeier	0418 402 404
Alyssa Luongo	0435 758 764
Joshua Reed	0432 69 8515
Daniel Johnson	0435 503 977
Stephanie Bagala	0435 832 337
Sanjana Sriram	0466 967 983
Jack Clarke	0490 021 438
Amelia Armstrong	0407 373 551
Semonn Oleksyn	0413 532 284
Katherine-Lynn Benson	0432 316 614
Sophie-Dorothea Lieke	0467 015 005
Ben Carter	0415 803 323
Hayley Middleton	0430 077 885
Kaitlin McCloghry	0426 883 177

**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	500	Wild
23 - Fish	Port Jackson Sharks	Any	250	Wild
23 - Fish	Fiddler Rays (Trygonorrhina fasciata)	Any	3	Manly Sealife Sanctuary
23 - Fish	Crested Horn Shark (Heterodontus lesteus)	Any	3	Manly Sealife Sanctuary
23 - Fish	Small Non predatory fish TBA (e.g. rough leather jacket)	Any	3	Manly Sealife Sanctuary
		<b>TOTAL</b>	<b>759</b>	

**Location of research:**

Location	Full street address
SIMS	Building 19, Chowder Bay Road, Mosman NSW 2088
Marine Facility/Pauna Park	209 Culloden Rd, North Ryde, 2109
Manly Sealife Sanctuary	Manly NSW

**Amendments approved by the AEC since initial approval:**

- Amendment #1 - Change transport and capture methods (Exec approved 8 May 2014, ratified by AEC 15 May 2014).
- Amendment #2 - Add a new species - Port Jackson Sharks (Approved at AEC meeting 14 August 2014/Ratified 12 April 2017).
- Amendment #3 - Addition of Evan Byrnes as a Masters Student (Executive approved, ratified by AEC 11 December 2014).
- Amendment #4 - Addition of JennaLee Clark as an Associate Investigator (Executive approved, ratified by AEC 11 December 2014).
- Amendment #5 - Addition of 100 Port Jackson Eggs (Executive approved, ratified by AEC 11 December 2014). Add embryonic sharks so we can a) study learning during embryogenesis and b) manipulate the environment they are reared in to examine the effects on learning (Ratified 12 April 2017).
- Amendment #6 - Addition of Louise Toretto as Researcher (Executive approved, ratified by AEC 11 December 2014).
- Amendment #7 - Amend the way to feeding the gobies (AEC approved 19 February 2015).
- Amendment #8 - Administration of substances and amend experimental design (AEC approved 16 April 2015). To administer cortisol so we can describe the stress response in these animals. We also planned to measure the cortisol in the water around the sharks, but the test kits do not recognise shark cortisol. We abandoned this approach now get at this using respirometry and taking a small blood sample instead (#14). (Ratified 12 April 2017).
- Amendment #9 - Addition of Catarina Vila Pouca as PhD Student (Executive approved, ratified by AEC 16 July 2015).
- Amendment #10 - Amendment to Experimental Design - Item withdrawn by A/Prof. Brown.
- Amendment #11 - Addition of Sherrie Chambers as PhD Student (Executive approved. Ratified by AEC 10 December 2015).
- Amendment #12 - Amendment to length of time sharks can be held in captivity. (AEC approved 10 December 2015. To increase the length of time the baby sharks are held in captivity (allows us to test slightly older sharks). (Ratified 12 April 2017).
- Amendment #13 - Addition of Connor Gervais as PhD Student (Executive approved. Ratified by AEC 19 May 2016).
- Amendment #14 - Addition of a change of technique, equipment and experimental protocols (Executive approved. Ratified by AEC 06 July 2016). Replace the approach listed in #8 with respirometry and a blood sample. Baby sharks and embryos are placed in a respirometer (which measures oxygen consumption) and exposed to the smell of predators, non-predatory fish or other PIs. We also take a very small blood sample to examine changes in blood chemistry. It's a much less invasive way of looking at stress and its effects on learning. (Ratified 12 April 2017).
- Amendment #15 - Addition of Dennis Heinrich as PhD Student (Executive approved. Ratified by AEC 20 October 2016).
- Amendment #16 - Addition of Catherine Downs as Student Intern (Executive approved. Ratified by AEC 07 December 2016).
- Amendment #17(a) - Extend protocol from 20/02/2017 to 20/02/2019 (Executive approved for 3 months only - 16/02/2017). (AEC approved 3 year extension on the 12th of April 2017. New end date 21 February 2020).
- Amendment #17(b) - Additional 260 Gobies and additional 150 Port Jacksons (Approved by AEC 12 April 2017).

Adapted from Form C (issued under part IV of the Animal Research Act, 1985)

19. Amendment #18 - Addition to experimental design including administration of substances. Investigate the use of magnetic and olfactory (smell) cues in spatial learning, using a total of 48 animals. (Approved by AEC 16 February 2017). This enables us to manipulate the senses the sharks are using to orientate (smell and magnetic cues) (Ratified 12 April 2017).
20. Amendment #19 - Add Rianne Vogelnest as Research Assistant (Executive approved. Ratified by AEC 12 April 2017).
21. Amendment #20 - Add Alyssa Luongo as Volunteer (Executive approved. Ratified by AEC 18 May 2017).
22. Amendment #21 - Add Joshua Reed as Volunteer (Executive approved. Ratified by AEC 18 May 2017).
23. Amendment #22 - Add Daniel Johnson as Volunteer (Executive approved. Ratified by AEC 18 May 2017).
24. Amendment #23 - Add Stephanie Bagala as Volunteer (Executive approved. Ratified by AEC 18 May 2017).
25. Amendment #24 - Add Sanjana Sriram as Volunteer (Executive approved. Ratified by AEC 22 June 2017).
26. Amendment #25 - Add Jack Clarke as Volunteer (Executive approved. Ratified by AEC 20 July 2017).
27. Amendment #26 - Add extra-cellular electrophysiology to the approved protocol for a total of 4 animals (Executive approved. Ratified by AEC 15 February 2018).
28. Amendment #27 - Add Laura Ryan as Associate Investigator (Executive approved. Ratified by AEC 15 February 2018).
29. Amendments - 04/05/2018 - Additional experiments for the habituation of baby Port Jackson sharks and addition of Amelia Armstrong, Semonn Oleksyn, Katherine-Lynn Benson, Sophie-Dorothea Lieke and Ben Carter to protocol (Executive approved. Ratified by AEC 21/06/2018).
30. Amendments- Add Hayley Middleton and Kaitlin McCloghry to project (Executive approved. Ratified by AEC 18 October 2018).

#### Conditions of Approval:

1. Amendment #14- The Animal Welfare Officer is to observe the first couple of procedures and assist The Principal Investigator in the development of a monitoring checklist. A report and copy of the monitoring checklist is to be provided to the Animal Ethics Committee.
2. Approved Amendments #17(a) & 17(b). That the past amendments stipulated in the supplementary document (highlighted in bold above); as currently required protocols on this ARA, be the approved experimental protocols from the date of April 12, 2017.
3. Amendment - 04/05/2018 - The Port Jackson sharks are to be released back to the capture site within 3 days AFTER finishing the experiment.

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.



A/Professor Simon McMullan (Deputy Chair, Animal Ethics Committee)

Approval Date: 18 October 2018





Department of  
Primary Industries

Dr Culum Brown  
Macquarie University  
Department of Biological Sciences  
MACQUARIE UNIVERSITY NSW 2109

Our Reference: P08/0010-4.6 & OUT17/31343

18 September 2017

Dear Dr Culum Brown

I refer to your request for amendments to your scientific collection permit.

I am pleased to advise that your amendments have been approved. The permit is enclosed and is subject to the conditions as specified therein. Please note that the permit will expire on 15 September 2019.

The permit authorises activities under the *Fisheries Management Act 1994* and *Marine Estate Management Act 2014* only and does not in any way affect your obligations under the *Animal Research Act 1985*. You are advised that if you are using animals for research then you may require accreditation and/or licensing. It is your responsibility to ensure that you are not in breach of the *Animal Research Act 1985*, and this permit is conditional on that requirement. For further information you should contact the Licensing Clerk, Animal Welfare Unit, NSW DPI on 02 6391 3725 or fax 02 6391 3570.

This permit does not authorise you to collect from within Sanctuary Zones or Special Purpose Zones of Marine Parks, unless otherwise stated within this permit. You should refer to the Marine Park User Guide on the Marine Parks website (<http://www.dpi.nsw.gov.au/fishing/marine-protected-areas>) for details.

The Director Fisheries Research reserves the right to request information of the activities conducted under this permit either during or at the expiry of the permit.

Please be advised that if you wish to amend your permit again, an amendment request must be submitted in writing or via email. Also note that any amendments will incur an amendment fee.

Should you wish to renew an existing permit or apply for a new permit, please submit a completed application, REF, maps and relevant fee, as well as any required information listed above. Please allow approximately 4-6 weeks for assessment. The latest application forms and fee schedule are available on the department's website [www.dpi.nsw.gov.au](http://www.dpi.nsw.gov.au)

Should you have any further enquiries regarding this matter please do not hesitate to contact me on (02) 4982 1232.

Yours sincerely

Sheree Livingstone  
Fisheries Manager, Aquaculture

Fisheries NSW  
Port Stephens Fisheries Institute  
Locked Bag 1, NELSON BAY NSW 2315  
Tel: 02 4982 1232 Fax: 02 4982 1107  
ABN 72 189 919 072 [www.dpi.nsw.gov.au](http://www.dpi.nsw.gov.au)



**SCIENTIFIC COLLECTION PERMIT**  
**Section 37 Fisheries Management Act 1994**  
**Marine Estate Management (Management Rules) Regulation 1999**  
**Part II of the Marine Estate Management Regulation 2009**

**PERMIT HOLDER DETAILS**

Dr Culum Brown  
Macquarie University

Department of Biological Sciences  
MACQUARIE UNIVERSITY NSW 2109

**Permit No: P08/0010-4.6**  
**Issued Date: 15/09/2014**  
**Amended: 18/09/2017**  
**Expiry Date: 15/09/2019**

**Additional People:**

Any personnel under the direct authority of the permit holder.

**In accordance with the *Fisheries Management Act 1994* the permit holder listed on this permit and additional people are hereby authorised to:**

- Collect small fish species using nets and traps in specified waters of New South Wales.
- Catch and release (tagging) of Port Jackson Sharks and the collection of Port Jackson Shark eggs, both inside and outside of Jervis Bay Marine Park.
- Catch, handle and release (tagging/tissue sampling) of Smooth Stingrays inside and outside Jervis Bay Marine Park (all NSW marine waters).
- Collection of Smooth Stingrays inside and outside Jervis Bay Marine Park (all NSW marine waters).
- Catch, handle and release (tagging/tissue sampling) of Port Jackson Sharks inside and outside Jervis Bay Marine Park (all NSW marine waters).
- Collection of Blue-lined Goatfish, Southern Maori Wrasse, Australian Mado, Blacksaddle Goatfish and Yellowfin Bream in all NSW marine waters.
- Tag and release 40 Smooth Stingrays within Jervis Bay Marine Park.

**The specimens must only be taken from waters at the following locations:**

- All freshwater and marine waters within New South Wales (excluding Marine Parks and Aquatic Reserves).
- Jervis Bay Marine Park – only Shark and Stingray tagging and collection of Port Jackson Shark eggs is permitted (Habitat Protection and General Use Zones only).
- Port Stephens Great Lakes Marine Park - north side of Fingal Island and west side of Cabbage Tree Island (Habitat Protection Zones) - only Shark tagging is permitted.

**\*Note:** Sampling may be undertaken within the marine parks listed above, excluding (unless specified above) Sanctuary Zones, Special Purpose Zones or areas where





such authorised entry and/or use of a zone or location is contrary to the zoning/management plan.

### Methods

- Blood and tissue sampling from Smooth Stingrays
- Blood and tissue sampling from Port Jackson sharks
- Collection from Trawlers Smooth Stingrays and Port Jackson sharks from the bycatch of appropriately endorsed Commercial Fishing Trawlers
- Hand Collection (including whilst scuba diving or snorkelling)
- Hand Hauled Net (1m drop; 6m length; 13mm mesh)
- Hand-held net and small seine nets to catch Blue-lined Goatfish, Southern Maori Wrasse, Australian Mado, Black-saddle Goatfish and Yellowfin Bream whilst scuba diving
- Indian (Scissor) Net 2mm mesh
- Intra-muscular stainless steel shark tags (for tagging Smooth Stingrays)
- Minnow Traps - Set for 3 hours unbaited (0.5m x 0.02m diam; 7mm mesh, 28mm entrance)
- Pit Tags & Acoustic tags for tagging of Port Jackson Sharks and Smooth Stingrays
- Seine Nets
- Shrimp Nets - Set for 3 hours (0.6m x 0.5m x 0.5m; 10mm mesh, 35mm entrance)

**\*Note:** Sampling in marine parks must only be undertaken in accordance with the requirements of the relevant marine park zoning/management plan and must be approved in writing by the local Marine Park Manager prior to sampling.

**Species** (total numbers/amounts to be collected over the term of the permit unless otherwise specified)

- |                         |                                    |           |
|-------------------------|------------------------------------|-----------|
| • Australian Mado       | <i>Atypichthys strigatus</i>       | 100 total |
|                         | maximum 25 per location            |           |
| • Blacksaddle Goatfish  | <i>Parupeneus spilurus</i>         | 100 total |
|                         | maximum of 25 per location         |           |
| • Blue-striped Goatfish | <i>Upeneichthys lineatus</i>       | 350 total |
|                         | maximum of 50 per location         |           |
| • Port Jackson Sharks   | <i>Heterodontus portusjacksoni</i> |           |
| • Smooth Stingray       | <i>Dasyatis brevicaudata</i>       | 250 total |
| • Southern Maori Wrasse | <i>Ophthalmolepis lineolate</i>    | 100 total |
|                         | maximum of 25 per location         |           |
| • Yellowfin Bream       | <i>Acanthopagus australis</i>      | 100 total |
|                         | maximum of 25 per location         |           |



**Subject to the following conditions:**

1. Specimens must only be taken in accordance with research being undertaken by the permit holder, the information provided by the permit holder in their application, and the conditions of this permit. In the event of an inconsistency between the conditions of this permit and any information provided by the permit holder, the conditions of this permit shall prevail to the extent of the inconsistency.
2. Unless otherwise specified within this permit, all restrictions (collecting, fishing and otherwise) under the *Fisheries Management Act 1994* apply and must be complied with.
3. No activity other than the collection activity specified in this permit, may be carried out by the permit holder or other nominated person/s when engaged in any collection activity authorised by this permit.
4. The permit holder or any additional person/s operating under this permit must display a clearly visible sign, in letters at least 15cm high, at the location of the activity during all collecting and field activities, identifying the name of the organisation conducting the research the permit number and the word 'Research'.
5. The permit number must be displayed on any boat or vehicle used for the purposes of activities associated with this permit.
6. The Marine Park Manager and District Fisheries Officer in the areas of the activity must both be notified at least 72 hours prior to the commencement of any collecting activities (unless the Marine Park Manager and District Fisheries Officer agrees to a local arrangement). You must obtain written permission from the relevant Marine Park Manager prior to sampling within a Marine Park. Refer to the relevant zoning plan for each park: <http://www.mpa.nsw.gov.au> The DFO should also be contacted when an activity relating to this permit (even if not physically collecting) is conducted in an area of public concern (i.e. Aquatic Reserve or Intertidal Protected Area). The following information must be provided: details of the permit number; collecting methods; specific locations and accurate times for any proposed activities; where vessels are to be utilised, registration details and descriptions, including boat ramps being used. Details of the person coordinating the field work, including contact details in the field, must also be provided. This person will be responsible for supervising all collecting activities.  
For contacts see: <http://www.dpi.nsw.gov.au/fisheries/recreational/contact>.
7. The permit holder must if required by a Fisheries Officer or Marine Park Officer to do so, immediately provide the name, address, date of birth and contact number for any or all additional people that have been nominated by the permit holder to carry out collection activities associated with this permit.





8. The permit holder and any additional person/s listed under this permit must carry this permit (or a copy thereof) at all times during collecting activities and must be produced to a Fisheries Officer or Marine Park Ranger on demand.
9. The permit holder must ensure that any additional person/s operating under the authority of this permit reads and understands all conditions relating to the permit.
10. Nets used in connection with this permit must be clearly identified with a float/s attached to the net not less than 100 mm in diameter with the permit holders name and permit number clearly visible in a colour which contrasts to that of the float. The float/s must be set so that it is clearly visible above the surface of the water. Setlines must have a 100mmx50mm tag with the permit holders name and permit number in a colour which contrasts to that of the tag. The tag must be set so that it is clearly visible above the surface of the water. Traps may be identified with either a float or tag providing it is set consistently with the parameters listed above.
11. All nets and traps (other than bait/shrimp traps) that are used in any non-tidal (inland) waters are to be set in such a way that enables; captured, non-target, protected, air breathing animals to access the surface of the water to breathe, without exception. Nets are to be set to minimize entanglement. All nets must be set in such a way that the cod end is floated sufficiently on the surface of the water to allow any entrapped air breathing animals to breath. Nets and traps must not be set in a way that totally obstructs the fish passage in a waterway.
12. Nets and/or traps and setlines must be cleared regularly to release unwanted fish and animals back to the waters from which they were taken without undue injury. Gill nets or setlines must be checked at a minimum of every three hours and traps and fyke nets must be checked at a minimum of every 12 hours. Nets, traps and set lines must be lifted fully above the surface of the water when being checked.
13. Nets used in estuarine water may be weighted monofilament nets and must be used with a 'splash' technique.
14. This permit does not authorise collection activities from Marine Parks declared under Section 6 of the *Marine Estate Management Act 2014*, Aquatic Reserves declared under the Section 57(2)(b) of the *Marine Estate Management Act 2014* or the taking of fish from the waters of Lord Howe Island, unless otherwise specified within this permit.
15. Collecting within Marine Parks and Aquatic Reserves is not permitted on weekends or public holidays unless approved by the relevant Marine Park Manager or for Aquatic Reserves, the Manager Marine Planning.
16. This permit does not authorise the permit holder or any personnel operating under this permit to collect from sanctuary zones of Marine Parks.





17. The permit holder must confirm with the Marine Park Manager whether there are any culturally significant sites within the study area and if they are required to notify the local Indigenous community prior to undertaking collecting activities.
18. Collecting in Commonwealth waters will require prior approval from the Commonwealth Government.
19. Any scientific papers or reports developed as part of research undertaken within any Marine Park or Aquatic Reserve must be forwarded to [Lesley.Diver@dpi.nsw.gov.au](mailto:Lesley.Diver@dpi.nsw.gov.au).
20. No fish or invertebrate taken under this permit may be used for personal consumption, sale or public exhibition by any person, unless approved in writing by the Minister or a person who has the delegated authority of the Minister to issue this permit.
21. The permit holder and any additional person/s operating under this permit must behave in a professional, reasonable and lawful manner that is not likely to generate conflict and criticism with other waterway users.
22. The permit holder must implement one of the following actions to prevent the translocation of aquatic pests and diseases between waterways, either:
  - clean and disinfect all aquatic fieldwork equipment and vessels at the conclusion of field operations in each waterway **and** dry any equipment that has biofouling (such as oyster trays, moorings, other infrastructure) for at least 30 days; **or**
  - use separate equipment in each waterway.
23. The permit holder must destroy any noxious fish, noxious marine vegetation or pests [including Carp (*Cyprinus carpio*), Goldfish (*Carassius auratus*), Eastern Gambusia (*Gambusia holbrooki*) and Redfin Perch (*Perca fluviatilis*)] or parasites listed as Class B diseases (under Schedule 6B of the *Fisheries Management Act 1994*) collected, sampled or taken as by-catch in a humane manner and not return it to any waterway unless authorised in this permit. See Schedule 6B and 6C of the *Fisheries Management Act 1994* and [www.dpi.nsw.gov.au/biosecurity/aquatic](http://www.dpi.nsw.gov.au/biosecurity/aquatic).
24. The permit holder must avoid disturbing or interfering with any aquatic pest fish, pest marine vegetation or other pests or parasites listed as Prohibited Matter or Aquatic pests and diseases (under Schedule 2 of the *Biosecurity Act 2015* and Schedule 1 of the *Biosecurity Regulation 2017*) during research and collection activities unless authorised in this permit. See Schedule 2 of the *Biosecurity Act 2015*, Schedule 1 of the *Biosecurity Regulation 2017* and [www.dpi.nsw.gov.au/biosecurity/aquatic](http://www.dpi.nsw.gov.au/biosecurity/aquatic).





25. The permit holder must not intentionally or inadvertently translocate any biological material and/or water between waterways unless authorised in this permit.
26. The permit holder must notify a Fisheries Officer, Authorised Officer or the Aquatic Biosecurity and Risk Management unit (02 4982 1232) if they become aware of or reasonably suspect the presence of an aquatic notifiable pest or disease (within the meaning of Part 4, Division 3 of the *Biosecurity Act 2015* and Part 6, of the *Biosecurity Regulation 2017*) or new location of an aquatic notifiable matter (pest or disease. See <http://www.legislation.nsw.gov.au/#/view/act/2015/24> and <http://www.legislation.nsw.gov.au/#/view/regulation/2017/232/full>.
27. Fish taken and not required for purposes stated in this permit must be returned to the water with the least possible injury.
28. Operations involving the collection of specimens must be carried out in such a way so as to minimise impacts on the surrounding habitat.
29. This permit does not authorise the taking of protected fish or threatened species, populations or ecological communities listed under the *Fisheries Management Act 1994* unless otherwise specified within this permit. For information see <http://www.dpi.nsw.gov.au/fisheries/species-protection>.
30. This permit does not authorise the taking or harming of any threatened species. For any activity that is planned to be carried out where there are likely to be any impacts on threatened species, populations or ecological communities, then a "7 Part Test" (as prescribed in section 5a of the *Environmental Planning and Assessment Act 1979*) report must be submitted. If impacts are considered likely to be significant, further assessment will be required.
31. This permit extends to National Parks but does not give a right of access to these Parks. Authority must be obtained from the Office of Environment & Heritage (43 Bridge Street, Hurstville 2220) and any conditions attached to such authority and any Act, Regulation or By-Laws relating to the Park, are to be fully observed. Note: the waters of Cowan Creek within the Kuringai Chase National Park are managed by the Office of Environment & Heritage.
32. This permit does not authorise the trapping of any mammals, birds or reptiles (e.g. platypus or turtles/tortoises). If any collection activities authorised by this permit have the potential to impact on any mammals, birds or reptiles, the permit holder must be in possession of a current National Parks and Wildlife Service licence which permits the trapping of mammals, birds or reptiles.
33. This permit does not give the right of entry to private property. Permission to enter private property must be obtained from the owner or occupier.



34. The Director Aquaculture & Aquatic Environment reserves the right to request information of the activities conducted under this permit during the life of the permit, at or after the expiry of the permit.
35. This permit is valid only if the permit holder has complied with the requirements of the *Animal Research Act 1985* in respect of the activities proposed. This permit only authorises activities under the *Fisheries Management Act 1994* and does not in any way negate your obligations under the *Animal Research Act 1985*.
36. This permit may be cancelled or suspended at any time prior to the expiry date for any reason.

Dr Natalie Moltschaniwskyj  
Director Fisheries Research

Matt Carr  
Manager, Jervis Bay Marine Park  
6.9.2017

Giving consent pursuant to Clauses 1.16(1)(a), 1.19(1)(a) and 1.31 of the  
*Marine Estate Management (Management Rules) Regulation 1999*

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