Exploring elasmobranch cognition using juvenile Port Jackson sharks

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BSc, MSc

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Mar, metade da minha alma é feita de maresia

Sea, half of my soul is made of sea breeze

Mar sonoro, mar sem fundo, mar sem fim,
A tua beleza aumenta quando estamos sós
E tão fundo intimamente a tua voz
Segue o mais secreto bailar do meu sonho,
Que momentos há em que eu suponho
Seres um milagre criado só para mim.

Sonorous sea, bottomless sea, endless sea,
Your beauty increases when we are alone
And so deep intimately your voice
Follows the most secret dance of my dream,
That there are times when I suppose
You are a miracle created just for me.

Sophia de Mello Breyner Andresen

In *Dia do mar*, 1947

"I can hardly doubt they [hive-bees] were profiting by the workmanship and the example of the humble-bees: should this be verified, it will, I think, be a very instructive case of acquired knowledge in insects. We should be astonished did one genus of monkeys adopt from another a particular manner of opening hard-shelled fruit; how much more so ought we to be in a tribe of insects so pre-eminent for their instinctive faculties, which are generally supposed to be in inverse ratio to the intellectual!"

Charles Darwin

In *Letter no. 607 to the Gardeners' Chronicle*, 1841

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STATEMENT OF ORIGINALITY

I hereby certify that the work included in this thesis entitled “Exploring elasmobranch cognition using juvenile Port Jackson sharks” 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 help and assistance that I have received in my research work and in the preparation of the thesis itself have been appropriately acknowledged.

In addition, I certify that all information sources and literature used are indicated in the thesis.

The research done in this thesis was conducted in accordance with Macquarie University Animal Ethics Committee under protocols number 2014/003 & 2016/027 and a NSW Fisheries permit P08/0010-4.2.

Catarina Vila Pouca,
20th April 2018

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by Roman numerals.

I. Food approach conditioning and discrimination learning using sound cues in benthic sharks

II. Lack of social preference in juvenile sharks

III. Social facilitation of foraging behaviour in non-social juvenile sharks

IV. Incubation under climate warming affects behavioural lateralisation in Port Jackson sharks

V. Quantity discrimination in sharks incubated at different temperatures

CONTRIBUTION OF OTHERS

Contributor's initials and full name are listed alphabetically by last name, following mine: CVP – Catarina Vila Pouca; CB – Culum Brown; CG – Connor Gervais; DH – Dennis Heinrich; CH – Charlie Huveneers; JM – Jade Michard; JR – Joshua Reed. Author contributions per chapter are given in the table below.

	Chapter				
	II	**III**	**IV**	**V**	**VI**
Conception & design	CVP, CB	CVP, CB	CVP, CB, DH, CH	CVP, CB	CVP, CB
Animal husbandry & data collection	CVP	CVP	CVP, DH	CVP, CG, JR	CVP, CG, JM, JR
Statistical analyses	CVP, CB	CVP, CB	CVP, CB	CVP, CB	CVP, CB
Writing	CVP, CB	CVP, CB	CVP, CB, DH, CH	CVP, CB	CVP, CG, CB
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ABSTRACT

Learning plays a vital role in the behavioural development of all animals. Fish are not an exception, and teleosts have become a typical model in the study of animal cognition and behaviour. Among elasmobranchs, however, research in this field is very scarce. As one of the oldest extant jawed vertebrates, elasmobranchs hold a key phylogenetic position to understanding the evolutionary origins of the vertebrate cognitive toolbox. In addition, elasmobranchs have a wide range of life-history traits and occupy very diverse ecological niches, providing good models for an ecological or functional approach to cognition. This is especially relevant in an era where rapid climatic change is affecting the physiology, behaviour, and cognitive skills of many species. Despite an upsurge of cognitive research in elasmobranchs over the last decade, the number of species and cognitive abilities assessed are still hardly representative of the group, and the effects of near-future ocean warming on elasmobranch learning ability have not been assessed. In this thesis, I used juvenile Port Jackson sharks, *Heterodontus portusjacksoni*, as a model species to address some of these gaps in elasmobranch cognitive research, exploring three research areas that have been little investigated – sound discrimination learning, social cognition, and numerical competency. I also evaluated the effect of incubation under climate warming in behavioural lateralisation and numerical learning skills, and tested for a mechanistic link between individual personality and/or laterality traits and cognitive ability in each of the three areas.

The marine environment is filled with biotic and abiotic sounds. Some of these sounds predict important events that influence fitness while others are unimportant. Animals can learn specific sound cues and use them for vital activities such as

foraging, predator avoidance, and orientation. Chapter I investigated whether Port Jackson sharks could learn to associate a sound stimulus with a food reward, and discriminate between two distinct sound stimuli. I found that some sharks learned to associate a sound stimulus with a food reward. None were successful in discriminating between two sound cues and all developed strong side biases. Individual differences in activity and boldness were not linked to the learning performance assays we examined.

Sociality is a widespread phenomenon across multiple taxa, including elasmobranchs. However, species and populations vary in their degree of sociality depending on the trade-off between fitness benefits and costs of living in a group. While adult Port Jackson sharks are known to form large social groups during the breeding season, very little is known about juveniles. Chapter II investigated sociality in captive-reared juvenile Port Jackson sharks, by testing their preference to associate with other sharks in a controlled laboratory binary choice experiment. I found that sharks did not actively choose to associate with one or three conspecifics, and that sex, size, swimming activity, or foraging motivation had no effect on the results. This suggests that sociality in Port Jackson sharks is not stable during ontogeny, and that predation pressure might not play an important role in shaping the social behaviour of juveniles, unlike other shark species.

Animals often share similar needs and challenges with other individuals, such as finding food, the best habitat, or suitable mates. The ability to learn from another animal can, therefore, be beneficial as it can save them the costs of learning by trial-and-error. This should apply equally to social and non-social individuals, as all animals are regularly exposed to other individuals, conspecifics or heterospecifics. Chapter III investigated social information use and social learning in juvenile Port Jackson sharks. Naïve ‘observer’ sharks observed and interacted with either ‘demonstrator’ sharks, trained to gain access to food through one of two arbitrary routes, or ‘sham demonstrators’, with no previous experience in the task. After 10 days of social exposure, observer sharks were tested in isolation. I found that a similar proportion of observer sharks from the demonstrator group and the sham demonstrator group learnt the task, and took approximately the same number of days to reach learning criterion, suggesting that social facilitation enhanced learning abilities in both group conditions. Only a small proportion of sharks in both groups performed well when tested without demonstration, suggesting release from conformity to the demonstrator’s behaviour. The training intensity and quality of demonstration also influenced learning ability, while behavioural traits and laterality were not linked to any performance measurements.

As many other elasmobranch species, Port Jackson sharks are oviparous and have a very long incubation period. Together with multiple other factors, these two life history traits render them vulnerable to the changing climatic conditions we currently face, such as rising sea water temperatures. With the known effects on development and physiology, is it anticipated that behavioural and cognitive abilities might also be hampered by climate warming. Chapter IV examined the effect of elevated incubation temperature in behavioural lateralisation. I found that hatchlings reared at forecasted end-of-century temperature showed stronger absolute laterality and a rightward bias compared to sharks reared in current conditions, suggesting elevated temperature altered brain development.

The capacity to make relative quantity judgements is one among the many learning abilities animals evolved to deal with the ecological and social challenges they face. This ability has been extensively studied in all vertebrate groups except elasmobranchs. Chapter V investigated if Port Jackson sharks can discriminate between two quantities, and tested the effect of incubation temperature and laterality levels in learning ability. Here I show, for the first time, that sharks can discriminate between two quantities, and found that individuals incubated at elevated temperature performed better compared to sharks incubated at current conditions. I found some indication that individuals with stronger rightward bias reached learning criterion faster, yet further research is required in this topic. The findings of Chapters IV and V provide further evidence that elasmobranchs are susceptible to the effects of future ocean warming. Our results suggest that behavioural mechanisms might allow animals to adjust and/or counter some of the changes imposed by climate warming, potentially bringing beneficial effects to the individuals that are able to survive.

In summary, this thesis extends the known cognitive abilities of elasmobranchs to research areas poorly, or never, addressed in the group and provides additional experimental support to the view that elasmobranchs share most of the cognitive toolbox of teleosts and other vertebrates.

INTRODUCTION

Early philosophers such as Plato, Aristotle, and later Descartes contemplated about human and animal minds. In 1646, Descartes wrote to William Cavendish, the Marquess of Newcastle:

> *I cannot share the opinion of … others who attribute understanding or thought to animals. … The reason why animals do not speak as we do is not that they lack the organs but that they have no thoughts.*

This view of animals as ‘automata’ was widely accepted and would only be reformed much later. Around mid to late 1800s, the works of influential names such as Charles Darwin and his colleague Romanes, particularly the publications *The Descent of Man and Selection in Relation to Sex* (Darwin, 1871) and *Animal intelligence* (Romanes, 1878), began acknowledging that animals possess some cognitive and emotional traits. Despite being heavily based on anecdotal observations of behaviour, the first theories of animal cognition were proposed.

Animal cognition: brief history of the field

The most important influences and developments in the field emerged with the growth of comparative psychology as an experimental science. Researchers such as Morgan, Thorndike and Watson, among others, moved from anecdotal observations to a systematic analysis and pushed the field towards objective, quantifiable, and replicable measures of behaviour, a legacy that is apparent in the work we continue to develop today (Shettleworth, 2010; Olmstead and Kuhlmeier, 2015). This period marked the development of experimental devices designed to study behaviour in controlled laboratory settings, such as Thorndike’s ingenious puzzle-box and B. F. Skinner’s operant conditioning chamber. The notions of response-reinforcer association and reinforcement theory were also developed during this time (Thorndike, 1898; Skinner, 1948; Ferster and Skinner, 1957) and laid the foundation for modern experimental psychology. Even today, many researchers attempt to use operant conditioning principles and provide ‘simpler’ explanations of behaviour prior to attributing higher cognitive functions to their subjects (e.g. Heyes and Ray, 2000; Giurfa, 2013). While the 1950s were a prime time for experimental psychology and learning theory, there was a major disinterest in many of the larger ecological and evolutionary implications for animal cognition. The field remained largely influenced by Thorndike’s perspective for almost half a century, which slowed its development and limited the ‘breath, nature and sophistication of the psychological issues studied in animals’ (Wasserman, 1997).

Simultaneously in the nineteenth and twentieth centuries, many European naturalists and biologists were documenting the behaviour of animals in their natural environment. Ethology emerged as a discipline that considered behaviour within an ecological and evolutionary context, as an adaptation to the local environment in which species evolved. As the field expanded Nikolaas Tinbergen, one of its pioneers, saw the need to clarify the main questions in Ethology and provide a unifying framework to the research being developed. He outlined four fundamentally different types of questions that had been asked about behaviour – Tinbergen’s ‘four whys’: what is it for? (survival value); how did it develop during an animal’s lifetime? (ontogeny); how did it evolve over the history of the species? (evolution); and, how does it work? (causation) – which highlighted the value of a comprehensive understanding of behaviour (Tinbergen, 1963). Notably, in 1973 N. Tinbergen, K. Lorenz, and K. von Frisch were awarded the Nobel Prize in Physiology and Medicine, a sign that ethology had gained considerable impact and significance as a discipline. Tinbergen’s ‘four whys’ stood the test of time and the framework remains valuable today (Bateson and Laland, 2013). In the late 1900s, ethology began integrating theoretical perspectives from fields such as sociobiology, ecology, economics, and genetics, which lead to the emergence of several subfields: cognitive ethology (Griffin, 1978), behavioural ecology (Krebs and Davies, 1987), cognitive ecology (Real, 1993), evolutionary psychology (Daly and Wilson, 1999), and comparative cognition (Wasserman, 1993). All of them are interdisciplinary endeavours with the goal of understanding how and why humans and animals process information the way they do, looking at ultimate and proximate mechanisms of behaviour (Shettleworth, 2010; Olmstead and Kuhlmeier, 2015). The rise of these subfields, alongside the cognitive revolution in experimental psychology, meant an overall distancing of animal cognition research from studies aiming to understand the human perspective. Nonetheless, the hierarchical view of cognition as a linear progression from fishes through reptiles and birds to mammals and humans remained an engrained bias in the field until very recent years (Hodos and Campbell, 1969; Brown *et al.*, 2011).

Cognitive skills in fish

Fish are one of the groups that suffered the most through the misconception of a continuous ‘phylogenetic scale’. Whilst they are undoubtedly the most ancient lineage of vertebrates, they have continued to evolve until today and contemporary species’ traits, including behaviour and cognition, reflect multiple adaptive radiations to cope with particular niches (Brown *et al.*, 2011; Patton and Braithwaite, 2015). Over the last decades, however, research on the cognitive abilities of teleost fish has risen dramatically and teleosts have now become a typical model in the study of comparative cognition (Brown *et al.*, 2011; Vila Pouca and Brown, 2017). Recent reviews have highlighted the wide and sophisticated array of behaviours and skills of teleost fish, which include long-term memory, numerical competency, cultural traditions, individual and self-recognition, cooperative hunting, and tool use to name a few (Brown *et al.*, 2011; Bshary and Brown, 2014; Brown, 2015; Patton and Braithwaite, 2015; Vila Pouca and Brown, 2017). For example, documented cases of social learning, where an animal learns by observation or interaction with another animal, are now commonplace in teleost fish in the context of foraging, migration and orientation, and antipredator behaviour, among others (Brown and Laland, 2011). A classic demonstration of social learning in a wild population of fish was carried out by Gene Helfman & Eric Shultz in 1984. French grunts *Haemulon flavolineatum* from particular resting sites in coral reefs make daily migrations along the same route to their feeding grounds (Helfman *et al.*, 1982), and seemed to be joined occasionally by newly recruited juveniles. When a control group of juveniles was transplanted to new, experimentally emptied resting sites, they swam in the same direction as if they were at home and failed to find food; however, the group of juveniles transplanted into resting sites with resident grunts learnt the same foraging route of the resident adults (Helfman and Schultz, 1984). Social learning and shoaling behaviour can lead to the establishment of cultural traditions within a population through conformity and cross-generational transmission of information (Laland and Williams, 1997; Rendell *et al.*, 2011), which means that populations may lose some flexibility to directly respond and adjust their behaviour to changes in the environment. This lower behavioural flexibility is particularly relevant in a rapidly changing world as we experience today, with increasing environmental pressures including overfishing and climate change. For example, in species with cultural migratory or foraging routes, the systematic removal of older, more knowledgeable individuals by commercial fishing might cause a detrimental shift in their migration or spawning/foraging grounds, as recently observed in cod *Gadus morhua* (Fernö *et al.*, 2011).

Over the last few decades, extensive research on the neural functions and mechanisms of cognition in fish has allowed us to identify many fish brain regions that are involved in specific cognitive tasks (see Broglio *et al.*, 2011 for a review). Despite the lack of a typical mammalian neocortex organization in fish, their neural architecture has both analogous and homologous components with mammals and has a similar processing power (Broglio *et al.*, 2005). This is unsurprising, as natural selection often provides solutions to similar problems presented across species using different neural apparatus and mechanisms (Chittka and Skorupski, 2011; Patton and Braithwaite, 2015). Teleost fish also show asymmetry of functions between the two hemispheres of the brain (cerebral lateralisation), a phenomenon that is widespread in vertebrates and invertebrates (Rogers and Andrew, 2002; Bisazza and Brown, 2011; Frasnelli, 2013). As most fish have no overlap in visual fields, lateralisation can be easily assessed by observing asymmetries in eye preferences (Bisazza *et al.*, 1998). The examined teleosts show an overall left eye/right hemisphere dominance in processing biologically relevant stimuli such as predators or opposite sex conspecifics and emotional responses such as fear and aggression, while the right eye/left hemisphere is generally linked to shoaling, stimuli categorisation and object manipulation (Bisazza *et al.*, 2000; Bisazza and de Santi, 2003; Dadda and Bisazza, 2006b; Bibost and Brown, 2013). The existence of lateral biases in behaviour is puzzling from a biological point of view, as it leaves the animals expressing such asymmetries with disadvantages, for example becoming more vulnerable to attack on one side or having a reduced ability to attack prey or competitors appearing on one side (Rogers *et al.*, 2004; Vallortigara and Rogers, 2005). For cerebral lateralisation to be ubiquitous in the animal kingdom, it is likely that it offers significant advantages over potential ecological pitfalls. One hypothesis is that lateralisation enables separate and parallel information processing in each hemisphere, which might increase the brain's capacity to carry out simultaneous processing and thus enhance cognitive efficiency (Rogers *et al.*, 2004; Bisazza and Brown, 2011). Several studies, including in teleost fish, have provided evidence in support of this hypothesis (Rogers *et al.*, 2004; Magat and Brown, 2009; Dadda *et al.*, 2015). For example, strongly lateralised topminnows *Girardinus falcatus* were faster at capturing live prey when a predator was in sight and performed better in spatial reorientation tasks compared to non-lateralised fish (Sovrano *et al.*, 2005; Dadda and Bisazza, 2006a). The direction and strength of laterality, however, seems to be highly variable among species, populations, and individuals (Bisazza *et al.*, 1998; Bisazza *et al.*, 2000; Brown *et al.*, 2004). These differences can be a result of the trade-off between the costs and benefits of a lateralised brain (Dadda *et al.*, 2009), but might also be maintained through additional behavioural mechanisms, such as consistent inter-individual differences in behaviour (Reddon and Hurd, 2009; Irving and Brown, 2013). Such differences are commonly referred to as personality, or behavioural syndromes when multiple traits are correlated across situations (Sih *et al.*, 2004; Réale *et al.*, 2007). In some species, personality traits have been linked to cognitive skills and seem to be lateralised, but the relationships remain equivocal (Budaev and Brown, 2011). For example, two populations of the poeciliid *Brachyraphis episcopi* that differ in predation pressure show variation in both lateralisation (Brown *et al.*, 2004) and personality (Brown *et al.*, 2005). Strongly lateralised convict cichlids *Archocentrus nigrofasciatus* had higher boldness levels when exploring a familiar environment (Reddon and Hurd, 2009), and the direction of lateralisation in a novel environment was linked to sex and aggression (Reddon and Hurd, 2008). A weak link was found between laterality strength and boldness in female, but not male, guppies *Poecilia reticulata* (Irving and Brown, 2013), while no relationship was found between laterality and aggression or coping style in multiple anabantoid fishes and poeciliids (Clotfelter and Kuperberg, 2007; Dadda *et al.*, 2007).

Elasmobranchs, the missing piece of the puzzle

Despite the growing interest in fish cognition, research in learning and cognition among elasmobranchs (sharks and rays) is comparatively scarce. Guttridge *et al.* (2009b) wrote the first comprehensive review on learning abilities in elasmobranchs, which included about 30 studies spanning approximately 60 years of research. This number pales in comparison with the 500+ studies included in the first edition of *Fish Cognition and Behaviour* in 2006, with significantly more added in its second edition (Brown *et al.*, 2011).

Most of the early research in elasmobranch learning tested associative learning skills and habituation (Guttridge *et al.*, 2009b). In one of the first operant conditioning studies with sharks, Eugenie Clark trained adult lemon sharks *Negaprion brevirostris* to press against a target with their snout to receive a food reward (Clark, 1959), and later to discriminate targets differing in shape and pattern (Clark, 1963). Further operant and classical conditioning studies were conducted in subsequent years, but in many cases learning was a by-product of the research, usually aimed at inferring visual or auditory abilities (Aronson *et al.*, 1967; Nelson, 1967; Banner, 1972; Graeber and Ebbesson, 1972; Gruber and Schneiderman, 1975). For example, Kritzler and Wood (1961) conditioned a bull shark *Carcharhinus leucas* to respond to auditory stimuli and tested its response at different frequencies and amplitudes in order to obtain an audiogram for the species. Interestingly, a few of these studies provided some evidence that learning abilities in sharks were comparable to those of teleosts and mammals. In a light-dark discrimination task, one nurse shark *Ginglymostoma cirratum* showed a learning curve very similar to mice (Aronson *et al.*, 1967), and the stable and highly reliable conditioned responses of the nictitating membrane of lemon sharks were very similar to those of rabbits (Gruber and Schneiderman, 1975). Despite providing valuable and insightful knowledge, many of these experiments tested a very small number of individuals and in some cases subjects were not isolated in the tank, making the results difficult to assess (Guttridge *et al.*, 2009b).

Following a gap in research over the 1980s and 1990s, likely due to the difficulty of using sharks as experimental subjects paired with a hostile public perception of the group, in the last decades we have seen an upsurge of cognitive research in elasmobranchs (Guttridge *et al.*, 2009b; Schluessel, 2015). The majority of these studies, reviewed in Schluessel (2015), comprise research on higher cognitive abilities both on a behavioural and neuroanatomical level.

Spatial orientation and memory is arguably the field that has attracted most attention in elasmobranchs. Extensive research has reported large scale seasonal migrations, philopatry, and homing behaviour of several species in the wild (Meyer *et al.*, 2005; Papastamatiou *et al.*, 2011; Chapman *et al.*, 2015; Lea *et al.*, 2015; Bass *et al.*, 2016). Displaced Port Jackson sharks *Heterodontus portusjacksoni* and lemon sharks returned to the specific locations they were removed from in their home reef (O'Gower, 1995; Edrén and Gruber, 2005) and tiger and thresher sharks (*Galeocerdo cuvier* and *Alopias vulpinus*, respectively), species with wide home ranges, showed goal-directed movements at large spatial scales (Meyer *et al.*, 2010; Papastamatiou *et al.*, 2011). Remarkably, adult thresher sharks displayed more oriented movements at larger scales compared to juveniles (Papastamatiou *et al.*, 2011). These results suggest that the sharks might learn detailed ‘spatial mental maps’ of their home ranges or foraging areas (O'Gower, 1995; Meyer *et al.*, 2010; Papastamatiou *et al.*, 2011). The idea of a cognitive map assumes an animal learns and integrates spatial environmental cues, and is able to compute short-cuts and even novel routes if released in unfamiliar terrains (Tolman, 1948; O'Keefe and Nadel, 1978). While in many situations animals might be using environmental gradients as sensory cues (Kalmijn, 1978; Montgomery and Walker, 2001; Meyer *et al.*, 2005; Gardiner *et al.*, 2012; Nosal *et al.*, 2016), some laboratory studies provide detailed knowledge on cue use and spatial learning strategies in two sharks and one ray species (Schluessel and Bleckmann, 2005; 2012; Fuss *et al.*, 2014c; b). Freshwater stingrays *Potamotrygon motoro* were trained to navigate a plus maze for a food reward using either visual cues (allocentric group) or a combination of visual cues and body-centred turns (ego-allocentric group). Rays from the allocentric group, which were encoding information about reward location with respect to visual cues, excelled at the task and were able to reach the goal via novel routes, suggesting they can orient by means of a visual cognitive map of the environment. However, individuals from the ego-allocentric group favoured egocentric cues, i.e. encoding the location of the reward in space relative to body axes of the self, indicating their choice of spatial orientation strategy might be governed by the type of situation at hand (Schluessel and Bleckmann, 2005). Similar results were found in bamboo and coral cat sharks (*Chiloscyllium griseum* and *Atelomycterus marmoratus*, respectively) – sharks were capable of learning a spatial task using either visual cues (allocentric) or body-centred turns (egocentric), but relied more on egocentric cues when both were available (Fuss *et al.*, 2014c; b). Regarding the types of cues used, stingrays preferentially used geometric information, together with the overall spatial arrangement of landmarks, over individual landmark cues (Schluessel *et al.*, 2015). Some of these studies also included impairment of specific brain regions to investigate the neuroanatomical correlates of spatial orientation. Elasmobranchs don't have a neocortex, like teleost fish, so cognitive skills must also be based in different neural substrates. In teleosts the telencephalon, in particular the pallium, is one of the most likely brain areas involved in the processing of cognitive information (see Broglio *et al.*, 2011 for a review). Despite a differential brain morphogenesis process between the two groups – typical craniate evagination process in elasmobranchs but eversion in teleosts (Striedter and Northcutt, 2006; Smeets *et al.*, 2012), the pallium is also one of the key neural correlates linked to cognitive skills in elasmobranchs (Northcutt, 1978; 2011; Yopak, 2012a; Yopak, 2012b; Schluessel, 2015). Indeed, ablation of the dorso-medial pallium in the telencephalon of sharks compromised previously acquired allocentric orientation abilities, but did not affect egocentric navigation (Fuss *et al.*, 2014c; b). These results support the involvement of the telencephalon in cognitive abilities in elasmobranchs and indicate that, like with other vertebrates, multiple neural substrates are involved in spatial orientation and memory (Broglio *et al.*, 2005; Rodríguez *et al.*, 2006; Broglio *et al.*, 2011; Schluessel, 2015).

Social cognition is another area of interest to explore in elasmobranchs. Grouping behaviour is often observed in sharks and rays (reviewed in Jacoby *et al.*, 2011). While passive aggregations due to a shared benefit or resource (Economakis and Lobel, 1998; Sims, 2005; Wearmouth *et al.*, 2012) might not involve any type of social intelligence, active choice of individuals or groups, structured communities and dominance hierarchies (Allee and Dickinson Jr, 1954; Jacoby *et al.*, 2010; Guttridge *et al.*, 2011; Mourier *et al.*, 2012) likely involve some form of individual recognition and potentially transitive inference skills. Juvenile lemon sharks tested in a binary choice experiment preferred to associate with conspecifics over heterospecifics, and older sharks chose to join groups comprised of similar sized individuals (Guttridge *et al.*, 2009a). In addition, juvenile small-spotted catsharks *Scyliorhinus canicula* placed in a single-chamber laboratory arena used familiarity levels with other sharks to base their decision to join a group (Jacoby *et al.*, 2012). These results provide some evidence of individual recognition in elasmobranchs, an ability that has been reported in teleosts on par with many other vertebrates (Mehlis *et al.*, 2008; Thünken *et al.*, 2009). Two species of elasmobranchs have also been shown to learn by observation or interaction with other individuals (Guttridge *et al.*, 2013; Thonhauser *et al.*, 2013). Naïve juvenile lemon sharks learnt to approach an artificial target by interacting with experienced sharks taught to push their snout against the target for a food reward (Guttridge *et al.*, 2013) and freshwater stingrays *P. falkneri* learnt a food retrieval task faster when they had observed trained conspecifics extracting the reward before their own training commenced (Thonhauser *et al.*, 2013). Remarkably, in this food retrieval task stingrays had to use water as a tool to extract the food reward (Kuba *et al.*, 2010).

Impressive object and motion recognition abilities have recently been identified in sharks (see Schluessel, 2015 for a review). Grey bamboo sharks could distinguish different geometric shapes, were able to discriminate filled and empty shapes of the same type and generalise to Kanizsa figures, and learnt to categorise a series of stimuli in two general categories, ‘fish’ and ‘snail’, independently of stimulus features and type (Fuss *et al.*, 2014a; Fuss *et al.*, 2014d; Schluessel and Duengen, 2015). Additionally, bamboo sharks possess symmetry perception; sharks showed a spontaneous preference for symmetrical stimuli over non-symmetrical ones, and were able to distinguish between bilaterally and rotationally symmetrical stimuli (Schluessel *et al.*, 2014). Besides 2D object recognition, grey bamboo sharks are also capable of discriminating motion patterns and recognise an organism based on its biological motion with point-light displays (Fuss *et al.*, 2017). Some studies have also investigated electroreceptive abilities. For example, sandbar sharks *Carcharhinus plumbeus* (and one scalloped hammerhead shark *Sphyrna lewini*) were successfully conditioned to an artificial magnetic field (Meyer *et al.*, 2005), and small-spotted catsharks were conditioned to an electric field and could discriminate between two DC currents and DC and AC currents, but did not respond differently to a natural vs. artificial DC current of similar magnitude (Kimber *et al.*, 2011; 2014). Yellow stingrays *Urobatis jamaicensis* also discriminated between the anode and cathode of a DC dipole located on the floor of an experimental tank (Siciliano *et al.*, 2013). Some auditory discrimination abilities were also identified in early studies (Kritzler and Wood, 1961; Nelson, 1967; Kelly and Nelson, 1975). For example, Nelson (1967) trained one lemon shark to approach a speaker for a food reward with the presentation of a certain sound frequency, but avoid it by means of an electric shock with a different frequency.

A common trend in this brief review is that despite an exciting upsurge of cognitive research in elasmobranchs over the last decades, the number of species, sensory modalities, and cognitive skills assessed are still hardly representative of the group. Elasmobranchs comprise over 1,000 species with a wide range of life-history traits, very diverse ecological niches, and highly specialised sensory systems (Compagno, 1990; Hueter *et al.*, 2004; Carrier *et al.*, 2010; Gardiner *et al.*, 2014), thus providing good options for an ecological or functional approach to cognition. In addition, as one of the oldest extant jawed vertebrates, elasmobranchs hold a key phylogenetic position to understanding the evolutionary origins of the vertebrate cognitive toolbox (Bshary and Brown, 2014; Schluessel, 2015).

Cerebral lateralisation and personality traits in elasmobranchs

Behavioural lateralisation has only recently been described in two species of sharks. Individual levels of laterality bias in rotational swimming and T-maze turn preference were observed in juvenile Port Jackson sharks (Byrnes *et al.*, 2016a), and double T-maze turn preference in small-spotted catsharks (Green and Jutfelt, 2014). Left-right differences in behaviour are assumed to reflect an underlying functional asymmetry of the nervous system, and might be linked to other behaviours and cognitive functions (Bisazza and Brown, 2011). Indeed, Byrnes *et al.* (2016a) found a link between laterality strength and reactivity to a stressful situation in Port Jackson sharks. The way some animals consistently differ in how they react to stressful or novel circumstances, potential risks, or interaction with conspecifics is commonly referred to as personality, or behavioural syndromes if multiple traits are correlated across situations (Sih *et al.*, 2004; Réale *et al.*, 2007). The concept of personality traits and behavioural syndromes implies that animals do not always display the optimum behaviour in all contexts, which might have fitness implications. For example, animals that are bolder, more active, and/or proactive might obtain more food or mates, but take more risks along the way (Réale *et al.*, 2000; Brown and Braithwaite, 2004; Brown *et al.*, 2005; Bierbach *et al.*, 2015). This risk-reward trade-off seems to be linked to differences in decision making and learning ability; bold, more active animals might be favoured in tasks that are stable and routine forming, while shyer, less active individuals take the time to assess the environment and tend to adapt more accurately to situational demands (Carere and Locurto, 2011; Sih and Del Giudice, 2012; White *et al.*, 2016; Raoult *et al.*, 2017). The few personality studies undertaken in elasmobranchs in captive, semi-captive and wild individuals have recently been reviewed in Finger *et al.* (2017). To date, consistent individual differences in boldness, stress reactivity, docility, rate of movement in a novel arena, and sociability have been described in a small number of shark species (Jacoby *et al.*, 2014; Byrnes *et al.*, 2016a; Byrnes *et al.*, 2016b; Finger *et al.*, 2016; Finger *et al.*, 2018).

While most studies attempted to go beyond a descriptive investigation of laterality and personality in elasmobranchs, for example considering a mechanistic approach between the two or exploring links between personality and foraging or habitat exploration in a wild context, we currently have no knowledge of whether behavioural lateralisation and/or personality influence cognitive abilities in elasmobranchs.

Cognition in a changing world

In the modern world, the environment that animals experience is becoming increasingly impacted by human disturbances, in some cases on a global scale (Parmesan, 2006; Wong and Candolin, 2015). Climate change has been identified as one of the major human-induced environmental changes to ecosystems worldwide (Collins *et al.*, 2013; Pörtner *et al.*, 2014). Rising atmospheric greenhouse gas concentrations have increased global average air temperatures at a rapid rate of ~0.2°C per decade over the past 30 years (Hansen *et al.*, 2006), with most of this energy being absorbed by the world’s oceans. The average temperature of the upper layers of the ocean has increased by 1.0°C over the past 120 years, and is predicted to increase by 1–3ºC in the next century if the current trajectory of greenhouse gas emissions is maintained (Collins *et al.*, 2013; Pörtner *et al.*, 2014). In addition to absorbing the planet’s heat, the oceans have absorbed approximately one-third of the carbon dioxide (CO_2) produced by human activities and oceanic CO_2 levels have now reached historically high levels (Doney, 2010; Stocker *et al.*, 2013). Although environmental changes have been occurring long before humans, anthropogenic changes often take place too rapidly for evolutionary processes to respond. For many species, behaviour and learning may play a leading role in allowing individuals to adapt and keep track of environmental variation (Brown, 2012; Wong and Candolin, 2015). Nonetheless, a substantial body of research in teleosts is showing that elevated temperature and CO_2 levels in the ocean significantly impair sensory functions and alter critical behaviours. For example, fish exposed to elevated CO_2 levels show impaired olfactory and auditory responses, which can dramatically alter their responses to prey or predator cues or affect homing behaviour (Dixson *et al.*, 2010; Cripps *et al.*, 2011; Simpson *et al.*, 2011; Allan *et al.*, 2013; Munday *et al.*, 2014). Increased CO_2 levels also impacted behavioural lateralisation in some teleost species, which might affect schooling, predator evasion or multitasking, among other behaviours (Domenici *et al.*, 2011; Jutfelt *et al.*, 2013; Domenici *et al.*, 2014; Lopes *et al.*, 2016; Sundin and Jutfelt, 2018). Elevated temperatures also affected the physiology and behaviour of teleost fish, restricting growth, aerobic scope, reproductive output, and foraging (Munday *et al.*, 2008; Nilsson *et al.*, 2009; Donelson *et al.*, 2010; Nowicki *et al.*, 2012). Sharks and rays reared in future warming conditions also showed lower survival rates, impaired condition, and decreased escape responses compared to control individuals (Rosa *et al.*, 2014; Di Santo, 2016; Gervais *et al.*, 2016). Olfaction and hunting behaviour in Port Jackson sharks did not seem to be affected by temperature, but were severely impaired by elevated CO_2 levels (Pistevos *et al.*, 2015). In addition, exposure to increased CO_2 impacted cerebral lateralisation in small-spotted catsharks (Green and Jutfelt, 2014), while the effect of elevated temperature has not been assessed yet. With so many consequences on development, perception and behaviour, it is likely that rapid climate change might also impact cognitive skills in elasmobranchs.

The study species

The Port Jackson shark, *Heterodontus portusjacksoni* (Meyer 1793), is a nocturnal epibenthic species endemic to southern Australia (Last and Stevens, 2009). On the east coast of Australia, Port Jackson sharks undertake a long-distance migration every year from potential foraging areas to their breeding reef grounds (Powter and Gladstone, 2009; Bass *et al.*, 2016). Interestingly, both males and females have bisexual philopatry and very high site fidelity during the breeding season, with sporadic displacements between reefs (Bass *et al.*, 2016). Port Jackson sharks are an important mesopredator and play a key role in regulating coastal reef environments (Powter *et al.*, 2010). During the breeding season, adults often rest in single-sex groups, and show consistent social networks assorted by sex and size, but not kinship (Powter and Gladstone, 2009; Bass *et al.*, 2016; Mourier *et al.*, 2017).

Port Jackson sharks are oviparous, and eggs are deposited in large numbers on shallow rocky crevices. Embryos have a long incubation period of 10 to 11 months (Rodda and Seymour, 2008). Neonates likely hatch in close proximity to each other (in time and space), and juveniles are usually found in exposed soft-sediment areas, solitary, in dyads, or in loose aggregations of dozens of individuals in deeper water (Powter and Gladstone, 2009). Unlike other juvenile sharks (Guttridge *et al.*, 2009a; Jacoby *et al.*, 2012), these field observations suggest that juvenile Port Jackson sharks may not be as social as the adults.

We currently know very little about the cognitive abilities of Port Jackson sharks. A single experiment in a laboratory context has shown that the species can be conditioned to a bubble stream and a LED light with a food reward and is able to remember the association for at least 24 h, and possibly up to 40 days (Guttridge and Brown, 2014). Port Jackson sharks are colour blind (Hart et al., 2011) but have very high sensitivity to contrast and light, likely an adaptation to their benthic and nocturnal life-style and lower visibility in temperate waters (Ryan et al., 2016). The species' hearing threshold hasn't been investigated yet, but data from the horn shark Heterodontus francisci, a sister species, suggests they are most sensitive to lower frequencies up to 300 Hz, and that lateral line stimulation is also involved below approximately 100 Hz (Kelly and Nelson, 1975; Casper and Mann, 2007).

Port Jackson sharks have distinct personality traits, similar to many fish species (Budaev and Brown, 2011). Byrnes and Brown (2016) found highly repeatable individual differences in boldness and stress reactivity in juvenile Port Jackson sharks and a correlation between the two personality traits, with bolder sharks showing higher reactivity to handling stress. Wild adults also showed consistent individual differences in docility scores (Byrnes et al., 2016b). On par with most vertebrates, Port Jackson sharks have functional asymmetries between the two hemispheres of the brain (Bisazza and Brown, 2011; Byrnes et al., 2016a). Juvenile Port Jackson sharks showed individual levels of laterality bias in rotational swimming and T-maze turn preference, and females were more strongly lateralised than males (Byrnes et al., 2016a). In addition, laterality strength was linked to reactivity to a stressful situation (Byrnes et al., 2016a).

Port Jackson sharks are reasonably small and do well in captivity, thus they are a suitable species to further investigate cognitive abilities in elasmobranchs and to expand our understanding of individual personality traits and laterality, along with its ecological and cognitive relevance.

Outline and aims

In this thesis I set out to address some of the existing gaps in elasmobranch cognitive research, exploring three research areas that have been little investigated – sound discrimination learning, social cognition, and numerical competency, using juvenile Port Jackson sharks, *Heterodontus portusjacksoni* (Meyer 1793), as my model species. I also investigated potential mechanistic links between individual personality and/or laterality traits and cognitive ability in sharks. A final goal of this thesis was to evaluate the effect of projected end-of-century temperature during embryo incubation in hatchlings' learning skills.

Aim 1: The marine environment is filled with biotic and abiotic sounds. Some of these sounds predict important events that influence fitness while others are unimportant. Animals can learn specific sound cues and use them for vital activities such as foraging, predator avoidance, and orientation. In Chapter I, I investigated whether Port Jackson sharks could learn to associate a sound stimulus with a food reward, and discriminate between two distinct sound stimuli.

Aim 2: Sociality is a widespread phenomenon across multiple taxa, including elasmobranchs. However, species and populations vary in their degree of sociality depending on the trade-off between fitness benefits and costs of living in a group. While adult Port Jackson sharks are known to form large social groups during the breeding season, very little is known about juveniles. In Chapter II, I investigated sociality in captive-reared juvenile Port Jackson sharks by testing their preference to associate with other sharks in a controlled laboratory binary choice experiment.

Aim 3: Animals often share similar needs and challenges with other individuals, such as finding food, the best habitat, or suitable mates. The ability to learn from another animal can, therefore, be beneficial as it can save them the costs of learning by trial-and-error. This should apply equally to social and non-social individuals, as all animals are regularly exposed to other individuals, conspecifics or heterospecifics. In Chapter III, I examined social information use and social learning in juvenile Port Jackson sharks using a simple foraging task.

Aim 4: As many other elasmobranch species, Port Jackson sharks are oviparous and have a very long incubation period. They are therefore vulnerable to the changing climatic conditions we currently face, such as rising sea water temperatures. Given the known effects on development and physiology in sharks and rays, it is anticipated that behaviour and cognitive abilities might also be hampered by elevated temperatures. In Chapter IV I investigated if hatchlings' behavioural lateralisation was affected by incubation temperature. Since laterality is an expression of brain functional asymmetries, changes in strength and direction of lateralisation indicate that rapid climate warming might impact brain development and function.

Aim 5: The capacity to make relative quantity judgements is one among the many learning abilities animals evolved to deal with the ecological and social challenges they face. This ability has been extensively studied in many vertebrates including teleosts, and should be present in elasmobranchs as well. In Chapter V, I investigated whether Port Jackson sharks can discriminate between two quantities, and tested the effect of incubation temperature and individual behavioural lateralisation on solving this task.

CHAPTER I

Food approach conditioning and discrimination learning using sound cues in benthic sharks

Food approach conditioning and discrimination learning using sound cues in benthic sharks

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Abstract

The marine environment is filled with biotic and abiotic sounds. Some of these sounds predict important events that influence fitness while others are unimportant. Individuals can learn specific sound cues and ‘soundscapes’ and use them for vital activities such as foraging, predator avoidance, communication and orientation. Most research with sounds in elasmobranchs has focused on hearing thresholds and attractiveness to sound sources, but very little is known about their abilities to learn about sounds, especially in benthic species. Here we investigated if juvenile Port Jackson sharks could learn to associate a musical stimulus with a food reward, discriminate between two distinct musical stimuli, and whether individual personality traits were linked to cognitive performance. Five out of eight sharks were successfully conditioned to associate a jazz song with a food reward delivered in a specific corner of the tank. We observed repeatable individual differences in activity and boldness in all eight sharks, but these personality traits were not linked to the learning performance assays we examined. These sharks were later trained in a discrimination task, where they had to distinguish between the same jazz and a novel classical music song, and swim to opposite corners of the tank according to the stimulus played. The sharks’ performance to the jazz stimulus declined to chance levels in the discrimination task. Interestingly, some sharks developed a strong side-bias to the right, which in some cases was not the correct side for the jazz stimulus.

Keywords

Elasmobranch; Behaviour; Cognition; Associative learning; Personality; Hearing

Introduction

Sound is a reliable cue in aquatic environments. It is highly directional and propagates over large distances with little attenuation or impact from currents (Slabbekoorn *et al.*, 2010). Therefore, it is unsurprising that many aquatic species use acoustic cues for communication and orientation (reviewed in Tyack, 1998; Ladich, 2015).

Marine mammals are renowned for the use of sound as their primary method of communication, as well as a method to obtain information about the environment, for example using echolocation or the surf and ice noise to locate the shoreline (reviewed in Richardson *et al.*, 2013). There are also widespread examples of acoustic communication in many fish species across different behavioural contexts, namely during courtship, spawning, agonistic interactions or distressful situations (e.g. Crawford *et al.*, 1986; Myrberg Jr *et al.*, 1986; Ladich and Myrberg, 2006; Ladich, 2015). In addition, fish can use ambient 'soundscapes' as a means of orientation and navigation. Research in coral reef species demonstrated the importance of reef noise as a cue for larvae settling and navigation in juveniles (Simpson *et al.*, 2005; Radford *et al.*, 2011; Huijbers *et al.*, 2012). Interestingly, damselfish *Pomacentrus* sp. larvae responses to acoustic cues are flexible and cue-dependent (Simpson *et al.*, 2010). Settlement-stage larvae that had experienced either natural reef noise or an artificial tone for some hours moved towards a reef noise chamber in a choice experiment; however, when tested with the tone, the reef noise group responded adversely and moved away from the tone chamber, while the artificial tone group moved towards the tone chamber (Simpson *et al.*, 2010). These results suggest that fish larvae can discriminate different acoustic stimuli, and that recent acoustic experiences influence their behavioural plasticity in the selection of settlement sites.

In the wild, sound is likely associated with important biological events, such as prey and predators, and there are considerable fitness benefits in learning about these sounds (Mann *et al.*, 1997; Tyack, 1998; Remage-Healey *et al.*, 2006; Wilson *et al.*, 2008). For example, bottlenose dolphins *Tursiops truncatus* behaviourally orient toward vocalizations of Gulf toadfish *Opsanus beta* (Gannon et al., 2005). In turn, the toadfish dramatically reduce their vocalizations and have increased plasma cortisol levels in the presence of low-frequency dolphin sounds, suggesting potential coevolution of dolphins and their prey in a ‘soundscape’ context (Gannon *et al.*, 2005; Remage-Healey *et al.*, 2006). Teleost fish can also learn to associate and discriminate sounds in an artificial setting. Acoustic conditioning for guidance or ranching purposes in the context of fish aquaculture or fisheries has been extensively studied in several freshwater and marine species (Zion *et al.*, 2010; Zion and Barki, 2012). In a laboratory experiment, carp *Cyprinus carpio* were taught to associate a plain tone with a food reward, then to discriminate between the plain tone and a complex musical stimulus, and even to discriminate between two musical stimuli of different genres (Chase, 2001). Although music is an artificial auditory stimulus, several experiments have shown similar music perception and categorization between humans and non-human species, including birds, mammals and fish (D’Amato and Salmon, 1984; Porter and Neuringer, 1984; Hulse *et al.*, 1992; Watanabe and Sato, 1999; Chase, 2001).

While elasmobranchs are not known to make sounds, they have an inner ear and a lateral line system and their hearing ability has been investigated to some extent (Myrberg, 2001; Gardiner *et al.*, 2012; Hart and Collin, 2015). Sharks seem to be most sensitive to frequencies below 100 Hz and able to hear sounds up to around 1000 Hz, but so far only a relatively small number of species has been investigated (Gardiner *et al.*, 2012). A few classic field experiments tested whether acoustic signals acted as attractive stimuli to sharks, and pulsed, low-frequency sounds drew large coastal sharks to the speaker’s location (e.g. Myrberg Jr *et al.*, 1972; Nelson and Johnson, 1972). Most studies on elasmobranch hearing have focused on frequency range and threshold detection level (i.e. sensitivity) using classical or operant conditioning (e.g. Nelson, 1967; Kelly and Nelson, 1975) and, more recently, auditory evoked potential techniques (Casper and Mann, 2006; Casper and Mann, 2007; Casper and Mann, 2009). In the first audiogram obtained of a shark, Kritzler and Wood (1961) conditioned a bull shark *Carcharhinus leucas* to approach an underwater loudspeaker to obtain a food reward. A similar procedure was used by Nelson (1967) with lemon sharks *Negaprion breviostis*, including one individual that was trained in an approach-avoidance discrimination task, in which the shark had to approach the speaker following the presentation of a certain frequency, but avoid it following another frequency (by means of an electric shock). A reliable approach/avoidance response was obtained after 33 shock trials and 50 food trials (Nelson, 1967). Similar to teleost fish, elasmobranch hearing abilities have likely been shaped by the biotic and abiotic ambient noise in their environment, and many aspects of their behavioural ecology suggest potential to the use of sounds as reliable signals in the environment, namely in foraging and navigation contexts (Gardiner *et al.*, 2012). In fact, a playback experiment with young lemon sharks *Negaprion brevirostris* suggests that natural sounds of fish species, including the sounds of distressed prey or healthy prey fleeing after an encounter, induce investigatory behaviours and biting (Banner, 1972). In recent years, a growing body of studies have investigated the cognitive abilities of elasmobranchs in greater depth (reviewed in Schluessel, 2015), yet the majority used visual stimuli, and a substantial gap remains regarding our knowledge of sharks' behavioural flexibility to sounds.

This study investigated whether Port Jackson sharks *Heterodontus portusjacksoni* could learn to associate artificial sound stimuli with a food reward. The first experiment was a food conditioning task with a single artificial sound stimulus, and the second experiment was a dual stimulus discrimination task, retaining the previous sound as one of the stimuli. The Port Jackson shark (PJ) is a benthic, nocturnal species endemic to the southern half of Australia (Last and Stevens, 1994). In the east coast of NSW, PJs show a seasonal, long-distance migration from their breeding reef grounds to potential foraging areas (Powter and Gladstone, 2009; Bass *et al.*, 2016). Interestingly, these sharks have bisexual philopatry and very high site fidelity during the breeding season, with sporadic displacements between reefs (Bass *et al.*, 2016). We currently know very little about the cognitive abilities of Port Jackson sharks. A single experiment in a laboratory context has shown that PJs can be conditioned to a bubble stream and a LED light with a food reward, and that they are able to remember the association for at least 24 h, and possibly up to 40 days (Guttridge and Brown, 2014). Port Jackson sharks are colour blind (Hart *et al.*, 2011), but have very high sensitivity to contrast and light, likely an adaptation to their benthic and nocturnal life-style and lower visibility in temperate waters (McFarland, 1990; Ryan *et al.*, 2016). The species' hearing threshold hasn't been investigated yet, but data from the horn shark *Heterodontus francisci*, a sister species, suggests they are most sensitive to lower frequencies up to 300 Hz, and that lateral line stimulation is also involved below approximately 100 Hz (Kelly and Nelson, 1975; Casper and Mann, 2007; Hart and Collin, 2015). It seems reasonable that Port Jackson sharks might use sound cues in addition to other senses (e.g. olfaction, lateral line, electromagnetic reception and vision; Gardiner *et* *al.*, 2012) to navigate between reef areas and locate prey, especially during the night. Port Jackson sharks are also known to have distinct personality traits, similar to many fish species (Budaev and Brown, 2011). Byrnes and Brown (2016) found highly repeatable individual differences in boldness and stress reactivity in juvenile PJs, and a strong correlation between the two personality traits, indicative of a proactive-reactive coping style. Wild adults also showed consistent individual differences in docility scores (Byrnes *et al.*, 2016). Animal personality is likely an important source of behavioural variation that may affect cognitive performance (Carere and Locurto, 2011; Sih and Del Giudice, 2012; White *et al.*, 2016). For example, shy and less active individuals are generally reactive, less impulsive and sample more information from the environment, and seem to be linked to lower learning ability in associative tasks, but better performance in reversal learning tasks (Dugatkin and Alfieri, 2003; Sih and Del Giudice, 2012; Trompf and Brown, 2014). The study of individual personality traits in elasmobranchs and its ecological and cognitive relevance is in its infancy (Finger *et al.*, 2017), but can be valuable to understand the evolution of personality and of cognitive abilities due to elasmobranchs' basal position in the vertebrate tree.

In this study, we hypothesized that sharks could learn to associate a sound cue with a reward repeatedly presented in a specific location, and thus would approach the reward zone more quickly and retrieve the reward more often over time. If the sharks were successful in the single stimulus task, they were then exposed to a dual stimulus discrimination task, which retained the previous artificial sound as one of the stimuli. We hypothesized one of three scenarios could occur: 1) the sharks ignore the new stimulus all together, or 2) generalize that sound equals reward, and always choose the reward zone from the previous task; or 3) the sharks learn to respond correctly to each stimulus. We also expected that bolder and more active individuals would be faster in retrieving rewards and in achieving learning criterion in the approach conditioning task, but shyer sharks would perform better in the discrimination task.

Materials and methods

Subjects

Eight juvenile Port Jackson sharks (4 females, 4 males; Table 1), ranging between 35 and 42 cm in total length and hatched in captivity (eggs collected from Jervis Bay, NSW Australia), were used in the study. Sharks were housed at the Sydney Institute of Marine Science (SIMS), Australia, in three 1000-L seawater tanks at ambient temperature for 10 months prior to the experiment. Tanks had continuous circulation of fresh seawater, aeration, a thin layer of sand in the bottom and PVC structures and fake kelp to provide shelter and enrichment. Seawater was pumped directly from Sydney harbour at ambient temperature. Prior to the experiment, sharks were fed small pieces of squid, fish and prawns *ad libitum* three days per week. The experimental tank was adjacent to the housing tanks, and the room had a natural light/dark cycle.

Egg collection occurred under NSW Fisheries permit P08/0010-4.2. This work was approved by the Macquarie University Animal Ethics Committee under ARA 2014-003. At the end of the experiment, all sharks were released at their original site of capture.

Table 1. Summary information of the sharks used as subjects in the experiment. F: female; M: male; R: right-side; L: left-side.

Shark ID	Sex	TL (cm)	Correct choice in Exp. 1	Pre-training sessions	Exp. 1 sessions	Exp. 1 outcome	Exp. 2 outcome
388	F	42.0	R	3	12	Pass	Fail
359	F	40.0	L	4	14	Pass	Fail
375	M	36.0	R	4	12	Pass	Fail
374	F	41.5	L	3	16	Fail	NA
422	F	38.0	R	4	18	Fail	NA
415	M	38.5	L	3	18	Pass	Fail
363	M	40.0	R	3	12	Pass	Fail
419	M	35.0	L	4	16	Fail	NA

Apparatus and stimuli

The testing arena (180 × 50 × 50 cm) was placed within a 10-foot circular tank filled with 30 cm of seawater. The arena was divided into a starting compartment (SC; 30 × 50 × 50 cm) and the experimental arena by a sliding Perspex door (Fig. 1). The walls of the SC were blacked out, and a black Perspex door and lid were used to close the SC during acclimation and inter-trial intervals. On the wall opposite to the

SC, a small black divider (37.5 cm long) separated a left from a right choice zone. The experimental arena was split into five distinct zones for data analysis (0 – 4, indicating distance from correct choice zone; Fig. 1). White curtains visually isolated the main circular tank in the room, but there were no special provisions for visual or acoustic isolation in the testing arena. Auditory stimuli were comprised of two 20-s music clips from Oscar Peterson's Bossa Beguine and Philip Glass's Metamorphosis One. The stimuli were chosen based on peak frequency range and tempo (Fig. S1) and the known hearing range of heterodontid sharks (Kelly and Nelson, 1975; Gardiner *et al.*, 2012). We chose not to filter low frequencies in the stimuli (lateral-line stimulation) since we were not interested in a specific sensory system used in the learning process. Indeed, in a natural setting animals generally use multiple senses simultaneously to gain information and learn about the environment (Shettleworth, 2010). Auditory stimuli were fed to an air speaker facing down in a waterproof container partially submerged in the middle of the back wall and on top of the divider by a laptop running a custom Matlab (The MathWorks®, 2004) program using Psychtoolbox-3 (Brainard, 1997). Sound was broadcast at 160 dB re 1µPa. All experimental sessions described below were conducted individually for each shark, once a day in consecutive days during daylight hours and always at the same time. We changed 2/3 of the water in the circular tank between every individual session, and mixed the water in the arena between trials. In the experimental sessions, sharks were fed daily on squid (their preferred food) during the trials using aquarium tongs. Sessions were video recorded with a webcam mounted above the arena.

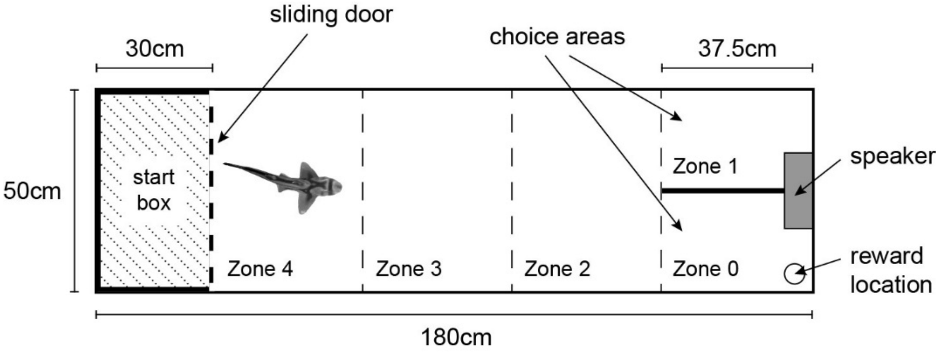

Figure 1. Experimental set up, showing the starting compartment (start box), the five zones of the experimental arena (0 – 4, indicating distance to the correct choice zone of Experiment 1), positioning of the speaker in the tank and location of the food

reward. Note that the correct choice zone was randomly assigned to be on the left- or right-side of the tank for different sharks, thus for some individuals the position of Zone 0 and Zone 1 is the opposite of this scheme.

Experimental procedure

Swimming activity

Swimming activity levels were assessed two months before the learning experiment. Sharks were moved from the housing tank to an experimental arena and tested individually. The experimental arena (90 × 50 × 30 cm) was placed within a 10-foot circular tank filled with 30 cm of seawater. The arena was considered to have three equal zones (each 30-cm wide), and we measured the number of times sharks crossed between zones (head and pectoral fins over the demarcation line) over five consecutive days. Subjects were given 15 min to acclimate before each trial began, and trials lasted 60 min.

Open-field emergence test and pre-training

Before starting the learning experiments, sharks had to get used to being moved to the experimental tank and fed from the aquarium tongs. We designed the pre-training sessions as open-field emergence trials in the SC to test for boldness (similar to Byrnes and Brown, 2016), followed by acclimation and training to feed from aquarium tongs.

At the start of the pre-training session, the shark was placed in the SC (in blackout) for 2 min and allowed to acclimate. Then a sliding door was lifted 20 cm above the floor, the individual was left undisturbed and time until emergence (boldness score) was recorded.

Once the emergence trial was over, the shark could swim freely for 10 min in the experimental tank to settle. After that, we allowed them to retrieve 8 free rewards from the aquarium tongs at random time intervals and in random locations in the tank (excluding SC). If the shark didn't approach the tongs within 8 min, the reward was removed.

The sharks were deemed ready to start the experiment when they retrieved all 8 rewards in less than 60 s each and did not show avoidance behaviour towards the aquarium tongs.

Experiment 1: Food approach conditioning

In this experiment, sharks had to learn to associate a jazz sound stimulus with a food reward in a specific location. One group (n = 4) was randomly assigned to associate the stimulus with the left side of the choice zone, and another (n = 4) with the right side of the choice zone (Table 1).

The first three sessions consisted of 6 trials with a correction procedure, i.e., each trial was repeated up to three times if the shark missed the reward (thus sessions could have up to 24 trials), to maximize exposure to training contingencies in the initial days. Sessions 4 onwards comprised 10 regular trials, without the correction procedure.

In all sessions, sharks were given 2 min of acclimation in the SC before starting the trials. The general structure of each training trial was as follows. Once the SC lid was removed and sliding door was opened, the shark was given 30 s to emerge. If 30 s elapsed, the shark was gently ushered into the experimental arena and the SC was closed. The sound stimulus was presented after a random delay (20 – 40 s), and a food reward was introduced in the choice zone (left or right side) 5 s after stimulus offset. Sharks were given 180 s to consume the reward or the trial was terminated. The shark was then ushered back to the SC, the door and lid were closed and an inter-trial interval of 30s preceded the next trial.

For each trial, we recorded the latency to enter the correct choice zone and the latency to eat the reward. We also recorded the position of the shark in the tank 5 s prior to stimulus onset, 5 s after stimulus onset and at reward onset, since we expected that sharks would be conditioned to the sound stimulus if they showed anticipatory behaviour induced by the stimulus, namely changing position in the tank and moving towards the correct choice zone (Guttridge and Brown, 2014). The sharks were considered to have entered an area if their head and pectoral fins were over the demarcation line.

To test if the sharks were conditioned to the sound, we ran a probe trial on day 6 and 11 (after 5 and 10 training sessions), and then every second session until reaching criterion. The probe trial was unrewarded, and differed from training trials in that the stimulus was presented at least 40 s after opening of the SC (maximum delay in training trials) and when the shark was resting in the zone furthest away from the choice zone. We recorded the position of the shark at 5 s after stimulus onset and at 5 and 10 s following stimulus offset (corresponding to reward onset and 5s within reward in training trials). Sharks were considered to have learnt the association between stimulus and reward if they showed directed swimming towards Zone 0 induced by the sound stimulus and were in Zone 0 at 10 s following stimulus offset in two consecutive probe trials.

Experiment 2: Discrimination task

After successful training with a single sound stimulus, sharks were moved to a discrimination task. In this experiment, sharks were presented with either the same jazz stimulus as in Experiment 1, where the correct choice zone was also the same as in the previous task (e.g. left side), or a new classical music stimulus, where the correct response was to enter the opposite choice zone (e.g. right side; Fig. S1).

The first three sessions consisted of 8 trials with a correction procedure (thus sessions could have up to 32 trials) and sessions 4 onwards comprised 10 regular trials, without correction procedure, all with 2 min of acclimation in the SC before starting the trials. In half of the trials, the stimulus was the jazz music clip, and in the other half the classical music clip. Trials were pseudo-randomized in blocks of two to prevent more than two consecutive trials of the same stimulus.

The general structure of each trial was slightly different to the previous task. The trial began with removal of the lid and black barrier of the SC, but a transparent barrier kept the shark inside the SC. The stimulus was presented after a random delay (20 – 40 s) and the transparent barrier was removed at stimulus offset, allowing the shark to make a response by swimming to the choice zone. If the shark made a correct choice, a food reward was introduced in the choice zone and the shark was given 60 s to consume it. After eating the reward, or if the shark entered the wrong choice zone, it was gently ushered back to the SC, both doors and lid were closed and an ITI of 30s preceded the next trial.

For each trial, we recorded the latency to make a response, if the choice was correct or not and the latency to eat the reward in correct response trials. The learning criterion was set to 80% correct choice in both stimuli in two consecutive sessions.

Data analysis

All statistical analyses were conducted in R v. 3.1.3 (R Core Team, 2016). Where applicable, model residuals were inspected and assumptions were met in all cases.

Swimming activity

We tested for agreement repeatability (R) of swimming activity over the five trials using a generalised linear mixed-effects model fitted by PQL (penalized-quasi likelihood) estimation for count data, with individual shark ID as random effect (package rptR, Schielzeth & Nakagawa, 2013). We used PQL estimation since it is recommended that repeatability with count data (here number of area crossings) be estimated using multiplicative GLM models (Nakagawa & Schielzeth, 2010).

Open-field emergence test

Emergence times for the open-field assay were *ln* transformed for normality due to heteroscedasticity. Agreement repeatability (R) of emergence times was estimated using a linear mixed-effects model (LMM) with REML estimation, with individual shark ID as random effect (packages lme4 and rptR, Schielzeth and Nakagawa, 2013; Bates *et al.*, 2015). An information-theoretic approach was used to build candidate models to examine for other factors that influenced emergence time. Potential fixed effects included in candidate models were total length, sex, housing tank and session number. Models were ranked based on corrected Akaike information criteria (AICc), and differences in AICc and in Akaike model weight were used to choose the best-fit model. The coefficient of determination (R^2) was used to assess goodness-of-fit and estimate the amount of variance explained by the model following Nakagawa and Schielzeth (2013), using the package piecewiseSEM (Lefcheck, 2016). Inclusion of the random effect in the model was tested by comparing the best-fit model with a null model using parametric bootstrap analysis utilizing exact likelihood ratio tests (5000 simulations; package RLRsim, Scheipl *et al.*, 2008; Bolker *et al.*, 2009). Adjusted repeatability (R_A) was then estimated using the best-fit model. Confidence intervals (CI), standard errors (SE) and *P* values for both R and R_A were calculated from parametric bootstrapping of likelihood ratios (1000 simulations; Nakagawa and Schielzeth, 2010).

Experiment 1: Food approach conditioning

Lack of motivation was apparent in some trials throughout the single stimulus task. Port Jackson sharks are a benthic species, and in most trials sharks would typically show directed swimming in the bottom of the tank towards the choice zones; however, on occasion we observed an odd vertical swimming at the water surface in a circular pattern, without approaching the choice zone. These null trials were excluded from the analysis (ranging from 5.2 to 25.9 % of trials per individual shark; Fig. S2). A Mann–Whitney U test was used to test if the overall proportion of null trials of the sharks that did not learn the task was different from those who learnt.

The information-theoretic approach described for the open-field emergence test was also used to examine the latency to enter the choice zone and latency to eat the reward in the Food approach conditioning task. The average emergence time per individual was taken as a boldness score and used as a fixed effect. We first explored the optimal structure of the random components (comparing random intercept models with random intercept and slope models) before testing the fixed effects (Zuur *et al.*, 2009). Wald tests were used to test the significance of fixed effects (Bolker *et al.*, 2009; Zuur *et al.*, 2009).

To test for anticipatory behaviour induced by the sound stimulus, we assigned a binary response for presence/absence of each shark in Zone 0 and used generalized linear mixed-effects models (GLMM) to compare presence in Zone 0 in different time periods of the trial: (1) 5 s prior to stimulus onset and 5 s during stimulus onset; and (2) 5 s prior to stimulus onset and reward onset.

Experiment 2: Discrimination task

The latency to make a choice and eat the reward, the percentage of correct choices and the percentage of left and right choices were recorded for each individual. The learning criterion consisted of eight correct responses out of 10 consecutive trials for each of the stimuli presented (Binomial test: P = 0.044). Individual results are provided to show intraspecific variation. Discrete Time Markov Chain (DTMC) transition probability matrices between trials (t - 1) and t were estimated for each individual shark to investigate if choice/stimulus and outcome in one trial would influence choice on the following trial (package markovchain, Spedicato *et al.*, 2016). We computed transition matrices for sessions 1-5 (initial days of the task) and for the overall task to explore rule formation over time. Confidence intervals of individual transition matrices should be considered cautiously due to low raw counts of transition steps.

Results

Swimming activity

Swimming activity was highly variable between individuals, ranging between 0.02 and 23.4 zone changes per minute (median = 3.38). The frequency of changes between zones was repeatable across sessions within individuals: sharks that were more active in the first session were generally more active in subsequent sessions (R = 0.690 (0.088), 95% CI 0.496 to 0.840, *P* = 0.001).

Open-field emergence test and pre-training

Four sharks ran three open-field emergence trials and pre-training sessions, and the remaining four sharks ran four open-field emergence trials and pre-training sessions (Table 1). On the last day of pre-training all sharks approached and aggressively bit the tongs.

Emergence time ranged from 1.54 to 180.23 s, with a median of 12.56 s. The best-fit model had only sex as fixed effect: males were shyer (took longer to emerge) than females. Sex accounted for 54.2 % of the variance in emergence times and shark ID for 6.8 % of the variance. Inclusion of shark ID didn’t significantly improve the model (LRT = 0, *P* = 0.267), but we chose to keep it since personality traits are inherently individually based. Boldness was repeatable across trials within individuals (R = 0.626 (0.196), 95% CI 0.092 to 0.847, *P* = 0.001): sharks that were faster to emerge in the first trial were generally faster to emerge in subsequent trials (Fig. 2). However, no repeatability was found when sex was included as fixed effect (R_A = 0.157 (0.170), 95% CI 0 to 0.542, *P* = 1).

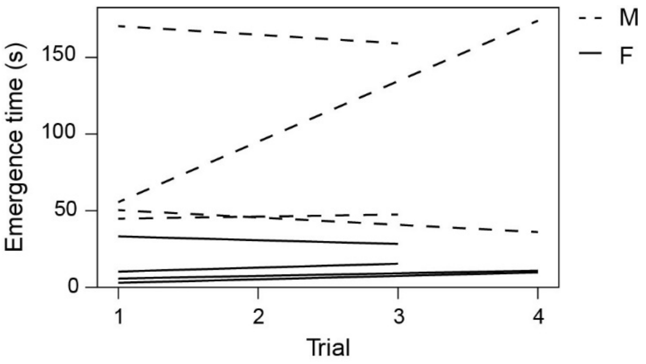

Figure 2. Individual boldness (emergence time) across open-field emergence trials. Lines represent individual sharks (M: males; F: females) and slopes represent individual changes in emergence times across all trials.

Experiment 1: Food approach conditioning

Latency to enter choice zone and eat reward

Sharks entered the choice zone on 86.5% of the trials, and consumed the reward 78.3 % of the times. The model that best explained changes in latency to enter the choice zone had only session number as significant fixed effect (latency decreased over sessions; F = 19.243, d.f. = 1, $P < 0.001$; Fig. 3a). The inclusion of shark ID as random intercept increased the strength of the model (LRT = 9.814, $P < 0.001$), and from observation of individual regressions and AICc scores we also included a random slope in the final model. Session accounted for 25.1 % of the variance in latency to enter the choice zone and random effects for 20.4 % of the variance. The model that better explained changes in latency to eat reward had both session and sex as fixed effects (latency decreased over sessions, and females were slower to eat the rewards compared to males; session: F = 29.984, d.f. = 1, $P = 0.0014$; sex: $\chi^2 = 7.380$, d.f. = 1, $P = 0.007$; Fig. 3b). The inclusion of shark ID as random intercept increased the strength of the model (LRT = 1.100, $P = 0.04$), and we also included a random slope in the final model. The fixed effects accounted for 41.5 % of the variance in latency to eat the reward and random effects for 11.7 % of the variance.

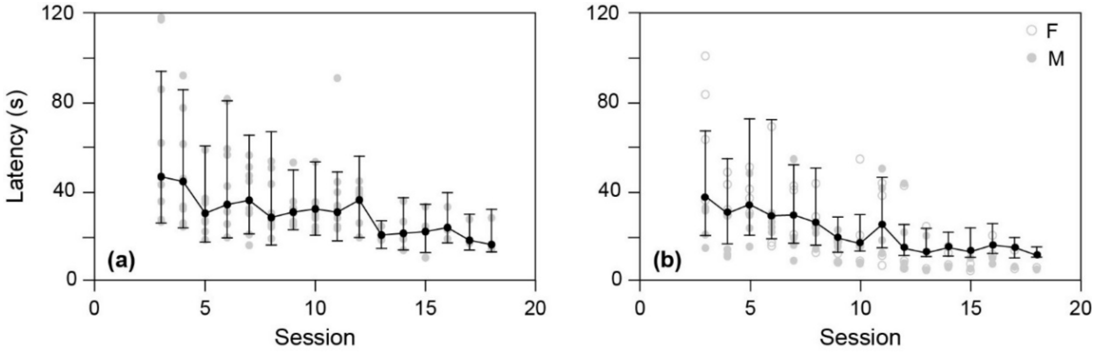

Figure 3. Median (±IQR) latency to **(a)** enter the choice zone and **(b)** eat reward across daily sessions, during Experiment 1 (n = 8). Individual data points are shown in grey.

Anticipatory behaviour

The best-fit GLMM models had trial time period (5 s before the song, 5 s during the song and reward onset) and session as fixed effects and individual shark ID as random effect. Sharks were observed significantly more times in Zone 0 at 5 s during the song (Fig. 4a) or at reward onset (Fig. 4b) compared to 5 s before the song, and presence in Zone 0 increased over sessions, suggesting the sharks formed an association between the song and food delivered in a specific location (before song/during song: timestamp, $\chi^2 = 31.406$, d.f. = 1, $P < 0.001$; session, z = 4.614, d.f. = 1, $P < 0.001$; before song/reward onset: timestamp, $\chi^2 = 49.724$, d.f. = 1, $P < 0.001$; session, z = 5.532, d.f. = 1, $P < 0.001$).

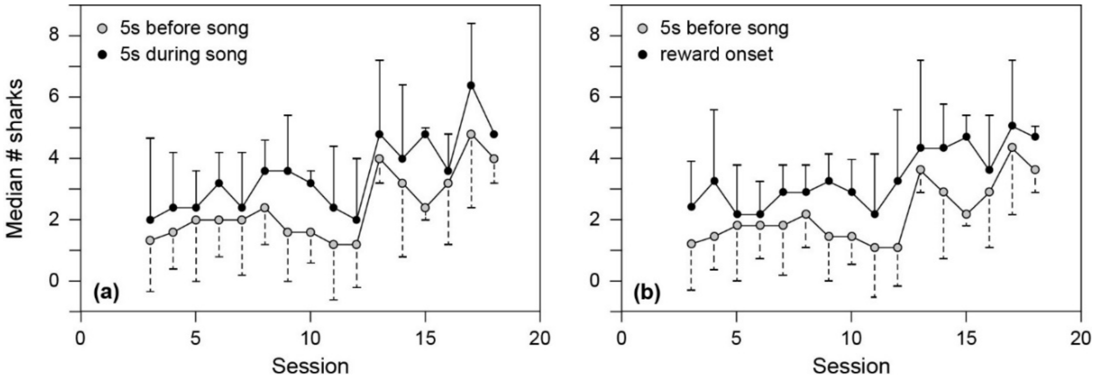

Figure 4. Median (±IQR) number of sharks observed in Zone 0 at 5 s before stimulus onset (grey circles) and **(a)** at 5 s during stimulus or **(b)** at reward onset (black circles) across daily sessions, during Experiment 1 (n = 8).

Probe trials

Five out of eight sharks reached learning criterion after an average of 13.6 training sessions, with small individual variation (median: 12; range: 12–18; Table 1). The remaining three sharks did not pass a single probe trial after 16 training sessions, and were excluded due to time constraints. Interestingly, all three showed quick, steady latencies to enter the correct choice zone, with an average of 26.19 ± 15.01 s (median 20.99 s) over the last two probe trials, but did it irrespective of the sound stimulus. In addition, we found that the three sharks that failed the probe trials were as motivated during training, showing a low proportion of null trials similar to the group of sharks that learnt the task (W = 9, $P = 0.764$).

Experiment 2: Discrimination task

Sharks were presented with the same jazz stimulus and a novel classical music stimulus. Task participation was high, with sharks showing a response in 98.8 % of the trials on average (individual participation from 96.6-99.7 %). Sharks were also fast in their response (median ± IQR: 12.44 ± 7.41 s), and in retrieving the reward if choice was correct (median ± IQR: 5.69 ± 2.93 s).

Learning curves are shown in Fig. 5 for each shark individually (a-e), and DTMC transition probabilities are depicted in Fig. 6. The sharks' performance to the previously learned jazz stimulus was low in the initial sessions of this task. After a mean of 31.4 sessions (median: 33; range: 26-34; Table 1), none of the five sharks learned to discriminate the jazz and the classical music stimulus.

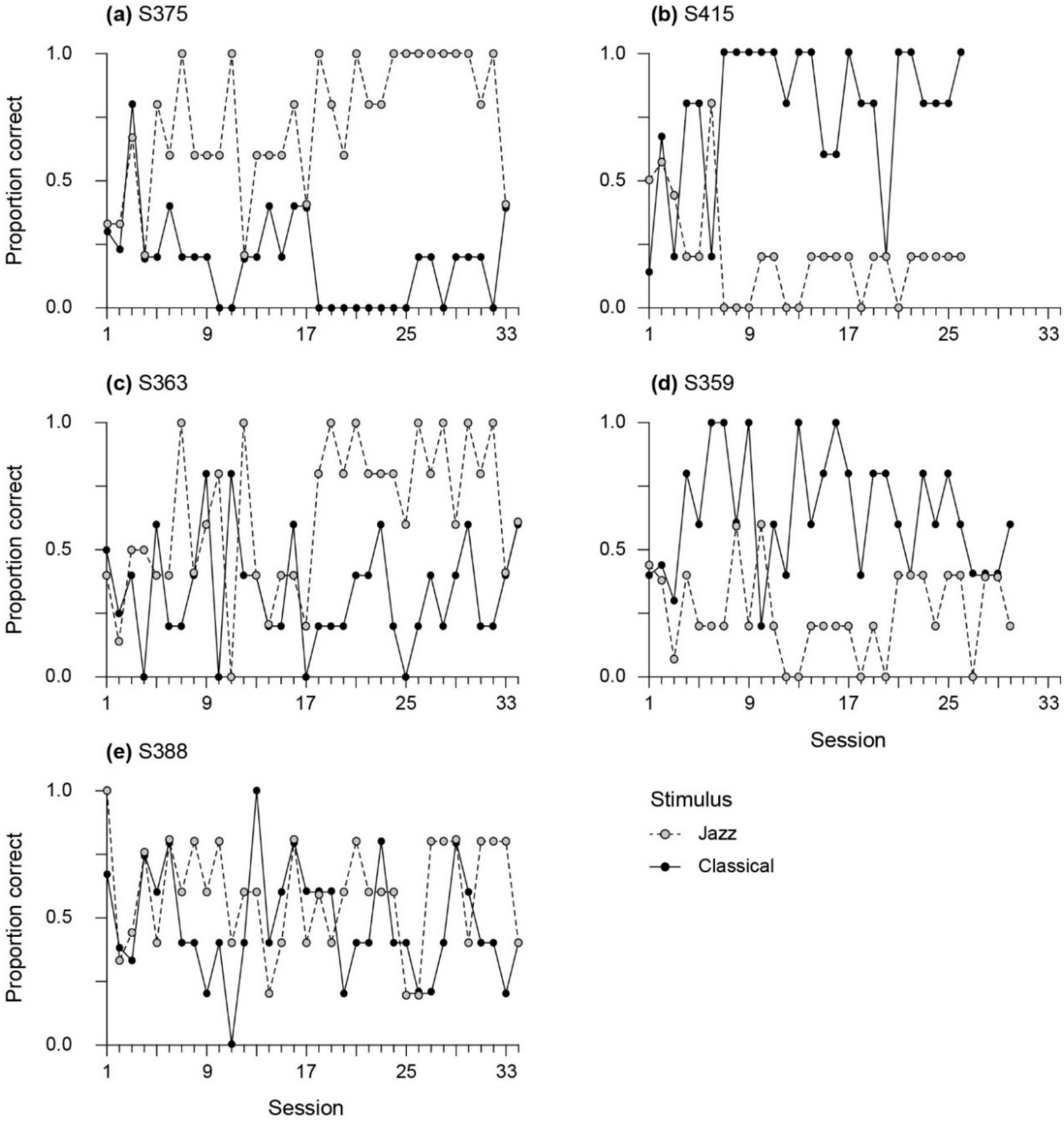

Figure 5. Performance of individual sharks **(a-e)** over training sessions during Experiment 2. The proportion of correct choices to the Jazz stimulus is given by the grey circles and dotted line, and for the Classical music stimulus by the black circles and full line (each session comprised five trials per stimulus). **Left panel:** sharks had to enter the right-side choice zone with Jazz; **Right panel:** sharks had to enter the right-side choice zone with Classical music. Note that none of the five sharks reached learning criterion.

Choice/outcome DTMC transition probabilities during the initial sessions suggest only one shark was choosing a zone randomly (S363; Fig. 6a), with all others showing an overall bias to the right-side zone – even those successfully trained to the left-side with the jazz stimulus in Experiment 1 (S359, S415, Fig. 5 b,d). Individual variation in choice strategy was also seen: S359 had a right-side bias but went left on half the trials following no reward on the left-side, and S375 and S415 predominantly choose the left-side after being rewarded on the right (Fig. 6a). Choice/outcome DTMC matrices over all experimental sessions suggest that S388's choice was random, while the remaining four sharks were predominantly biased to choose the right-side zone regardless of the stimulus and outcome of the previous choice (Fig 6b).

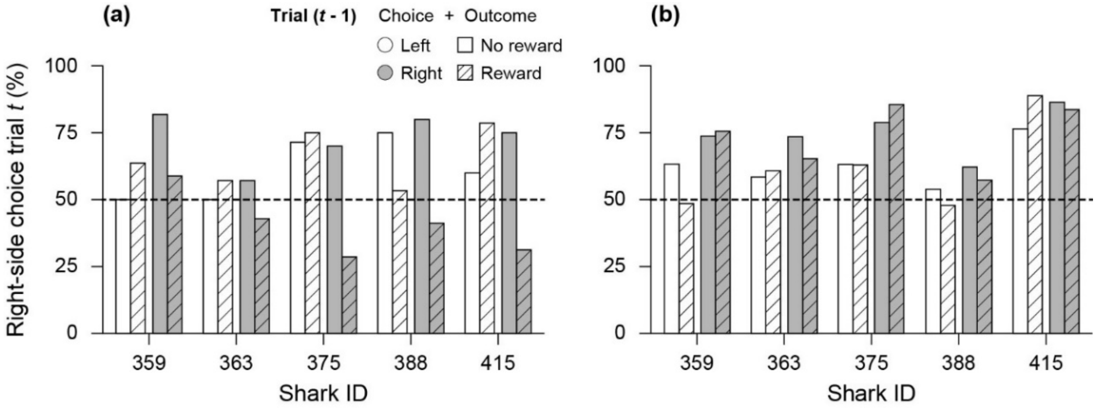

Figure 6. DTMC transition probability of choosing the right-side zone in trial *t* over **(a)** sessions 1-5 and **(b)** all sessions of Experiment 2, depending on choice and outcome of trial (*t* – 1): shark chose the left-side and was wrong (white bars); chose the left-side and was correct (white dashed bars); chose the right-side and was wrong (grey bars); or chose the right-side and was correct (grey dashed bars). Dashed horizontal lines mark chance levels. Note that S359 and S415 were originally trained to the left-side zone with the Jazz stimulus. Only one shark (S363) chose a zone randomly during sessions 1-5 (a) and another (S388) over all sessions, with all others showing an overall bias to the right-side zone.

Discussion

Our results show that juvenile Port Jackson sharks learnt to associate an artificial sound with a food reward. However, not all sharks were able to learn the association. None were successful when required to discriminate between two sound cues and all developed strong side biases. We observed repeatable individual differences in activity and boldness, yet these personality traits were not linked to the sharks' learning performance.

In this study, five sharks learned to associate a sound stimulus with a food reward. In agreement with our predictions, the sharks became faster in approaching the correct choice zone and in retrieving the reward, and showed anticipatory behaviour induced by the stimulus. Interestingly, the three sharks that failed to learn the association were consistent in entering the correct choice area early in the trial, but paid no attention to the sound stimulus. In this task, the food reward was always accessible in the same location after a short random interval. If we exclude the sound cue, the task resembles a place-learning task with a variable-interval (VI) schedule of reinforcement – the shark makes an operant response (enter the choice area), and a food reward is delivered at random time intervals. It is possible these sharks ignored the sound cue and were responding to the VI place-learning task, and indeed their steady rate of response is characteristic of VI schedules (Ferster and Skinner, 1957).

None of the five sharks that successfully associated the jazz sound with reward learnt the discrimination between jazz and classical music. The possibility that the sharks could not acoustically distinguish the two stimuli cannot be ruled out, but is unlikely given the many differences in the sonograms. In addition, if that were the case we would expect them to maintain the response previously learnt regardless of the stimulus. This was not observed, as all sharks showed a reduction in performance for the jazz stimulus in the first sessions of the discrimination task. Decreased performance to a previously learnt association during the initial stages of a new task is commonly observed in operant conditioning before individuals acquire the new discrimination, which suggests an attempt at rule formation in our sharks.

Interestingly, most individuals developed a bias to the right-side choice zone after a few sessions in the discrimination task. While we did not directly test for laterality and side bias in a choice scenario, we found no prior preference to spend time on the left- or right-side of a rectangular arena in any of the sharks used in this experiment (Chapter II of this thesis). Together with the fact that some of the sharks were successfully trained to the left-side choice zone in the food conditioning task, it seems that the side bias only developed when the task was too difficult to learn. Strong side-bias are often seen in animal learning experiments with two-choice responses, and perhaps arise from an animal's default-option when facing indecision, which yields a higher payoff compared to random choice.

In this experiment, sharks took a median of 12 sessions to learn the food approach conditioning task, which amounts to approximately 120 trials in total, before beginning the discrimination task. This overall number of trials ran before starting the discrimination task is very low compared to the training sessions of birds, rats and other fish (D'Amato and Salmon, 1984; Porter and Neuringer, 1984; Chase, 2001), and might explain their poor performance. For example, in a music discrimination experiment, koi carp were given 40 days of 50-trial sessions with one of the stimulus before they began the discrimination phase (Chase, 2001). In addition, these koi carp had been serving as experimental subjects for 5 years (as is the case for a great number of subjects in animal cognition experiments), while our sharks were naïve to learning experiments.

Sharks can respond to artificial magnetic fields (Meyer *et al.,* 2005), as well as weak electric fields (Jordan *et al.*, 2011), thus another option to consider is that the magnetic and/or electric field created by the speaker acted as a cue, which the sharks might have used to learn the task. We were unable to test this hypothesis; it would have been valuable to assess the sharks' response to a sound stimulus outside their hearing ability (thus to the speakers' electromagnetic field alone), or start the discrimination training with an easier discrimination, such as the jazz stimulus versus a plain tone. Another potential issue to consider is background noise in the tank, reverberation and signal distortion (however from visual comparison of the in-water sonograms and the original stimuli, these factors do not seem to have been extreme).

Associative learning has been widely investigated in many species of teleost fish, comprising a large range of tasks and multiple sensory modalities (e.g. visual, tactile or auditory; Brown *et al.*, 2011). Studies using auditory stimuli have shown teleost fish use acoustic cues for communication and orientation, and can learn both with natural or artificial sounds (Chase, 2001; Simpson *et al.*, 2010; Ladich, 2015). A few studies have investigated associative learning skills with auditory stimuli in large costal shark species, including a single lemon shark that was trained to approach a speaker following one frequency, but avoid it with another frequency (Nelson, 1967). Our study is the first to assess acoustic conditioning in a benthic elasmobranch, and to examine if boldness and swimming activity were linked to any learning performance assays in the food approach conditioning task. We found repeatable within-individual emergence times and activity levels which is consistent with previous studies (Byrnes and Brown, 2016; Byrnes et al. 2016b); however, neither of the two traits were correlated with latency to enter the choice zone, latency to eat the reward, or anticipatory behaviour in our eight sharks. Far more research in multiple species and with a greater sample size is needed to properly address this topic in elasmobranch fishes.

In conclusion, this study shows that benthic elasmobranchs can learn an association task with a sound stimulus. Underwater sounds are likely ecologically relevant cues to benthic species, especially nocturnal ones, to aid in locating prey and in navigating between reef areas. Further studies should investigate preferential behaviour and associative learning using natural reef sounds, including those made by fish and crustaceans living there.

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Compliance with Ethical Standards

Conflict of Interest: The authors declare they have no conflict of interest.
Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This work was approved by the Macquarie University Animal Ethics Committee under ARA 2014-003.

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Supplementary information

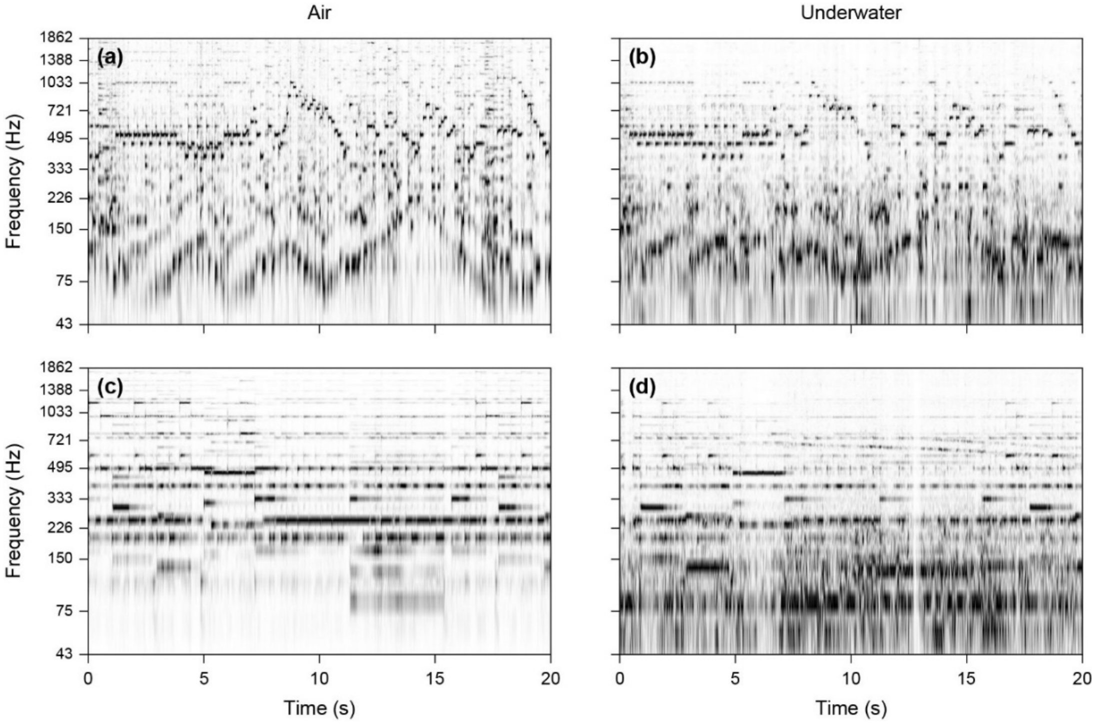

Figure S1. Peak frequency sonograms of the auditory stimuli used in the experiment. **(a, b)** 20-s clip from Oscar Peterson's Bossa Beguine; and **(c, d)** 20-s clip from Philip Glass's Metamorphosis One. Panels on the left were recorded in air and panels on the right were recorded underwater in the experimental tank.

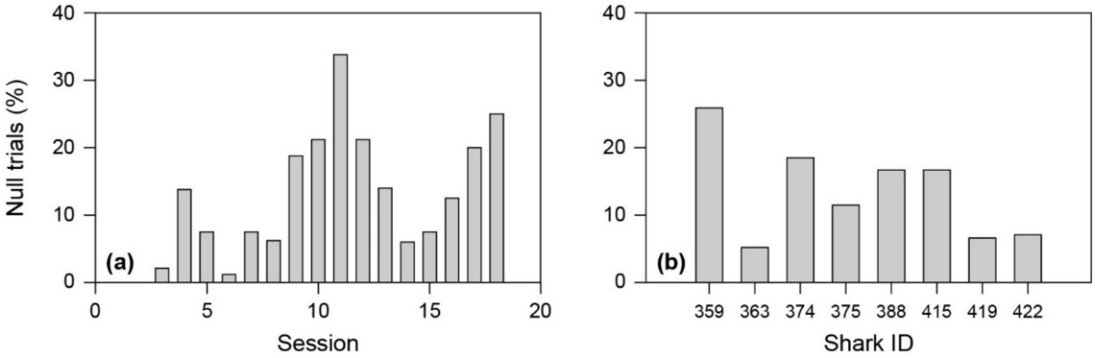

Figure S2. Percentage of trials without choice, classed as null, **(a)** across sessions and **(b)** individuals during Experiment 1.

CHAPTER II

Lack of social preference in juvenile sharks

Lack of social preference in juvenile sharks

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Abstract

Sociality is a widespread phenomenon across multiple taxa, including humans. However, species and populations vary in their degree of sociality, depending on the trade-off between fitness benefits and costs of living in a group. Aggregations and social behaviour are commonplace, but its drivers have only recently started to be investigated in elasmobranchs. Characteristics such as size, hunger levels and personality traits seem to play a role in social choice. However, the range of life-history traits and ecological niche of sharks is such that more species need to be investigated to have a broad view of elasmobranch aggregation and social behaviour. Here we investigated sociality in captive-reared juvenile Port Jackson sharks. We tested their preference to associate with other sharks in a controlled laboratory binary choice experiment, and assessed if individual activity and hunger levels contributed to explain the preference patterns observed. All sharks underwent a control phase with two empty compartments to ensure there were no side preferences, and a second phase with a food cue to ensure they could detect the gradient of olfactory cues in the apparatus. We observed repeatable individual differences in activity over time. Sociality was then tested by giving the sharks an option to associate with conspecifics or not. We found that juvenile Port Jackson sharks did not actively choose to associate with conspecifics, and that treatment (1 or 3 shoal mates), sex, size, swimming activity or foraging motivation had no effect on the results observed. These findings suggest that sociality in the species is not stable during ontogeny.

Keywords:

Grouping behaviour; sociality; predation; personality; elasmobranchs;

Introduction

Animal aggregations and social groups are widespread in the animal kingdom, including freshwater and marine taxa. Aggregations are considered a passive process, often resulting from attraction to a specific habitat or resource, or from collective avoidance of predation or harassment by conspecifics (Johnson et al. 2002; Jacoby et al. 2011). In contrast, social groups are formed through active social attraction between individuals (Krause & Ruxton, 2002; Wilson et al. 2014). Passive and active grouping behaviour depends on a trade-off of benefits and costs of living in a group. For example, while group living might reduce the probability of predation, it increases competition for food. Thus, animals may trade-off predation risk and hunger when deciding to join a group (Alexander, 1974; Krause & Ruxton, 2002). Indeed, while shoaling confers anti-predator benefits for many teleost fish, individuals with high hunger levels are less likely to shoal, potentially to reduce competition among shoal mates (Robinson & Pitcher, 1989; Krause, 1993; Krause & Ruxton, 2002). The decision to join a group may also be based on phenotypic characters such as body length, species and colour (Krause et al., 2000; Krause & Ruxton, 2002; Guttridge et al., 2009), and on behavioural differences, which include personality traits such as boldness and activity (Réale et al., 2007; Croft et al., 2009; Krause et al., 2010). For example, shy three-spined sticklebacks prefer to associate with a smaller number of individuals compared to bold sticklebacks (Pike et al., 2008), and individuals with many social contacts associate with each other more often than to other conspecifics (Croft et al., 2005).

Grouping behaviour is often observed in elasmobranchs in both juvenile and adult stages (reviewed by Jacoby et al., 2011). Elasmobranchs can actively form social groups, with structured communities and dominance hierarchies (Allee & Dickinson Jr, 1954; Jacoby et al., 2010; Guttridge et al., 2011; Mourier et al., 2012), and also passive aggregations for protection or avoidance of sexual harassment (Economakis & Lobel, 1998; Sims, 2005; Wearmouth et al., 2012). Juveniles, in particular, seem to enjoy a survival benefit from aggregation behaviour, perhaps due to their higher vulnerability to predation (Heupel & Simpfendorfer, 2005; Guttridge et al., 2011). Explicit hypothesis testing of the mechanisms and functions of social behaviour in sharks is not easy, even in juveniles, but some progress has been made. Using a binary choice experimental set-up, Guttridge et al. (2009) found that juvenile lemon sharks actively choose to associate with conspecifics, and that older sharks prefer to join groups comprised of similar sized individuals. Similarly, juvenile small-spotted catsharks formed active social groups in a single-chamber arena, and familiarity was a driver of group formation in older individuals (Jacoby et al., 2012). Nevertheless, the extent of variation in life-history traits and ecological niche of sharks is such that more information of elasmobranch aggregations and social behaviour is required.

The Port Jackson shark (PJ), *Heterodontus portusjacksoni*, is a small benthic elasmobranch endemic to the southern half of Australian waters (Last & Stevens, 1994). In the east coast of NSW, PJs show a seasonal, long-distance migration from potential foraging areas to their breeding reef grounds (Powter & Gladstone, 2009; Bass et al., 2016). During the breeding season, adults often rest in single-sex groups, and show consistent social networks assorted by sex and size, but not kinship (Bass et al., unpubl. data; Powter & Gladstone, 2009). In parallel with a few other shark species (Finger et al., 2017), juvenile PJs have consistent individual personality differences in captive tests of boldness and stress reactivity, and the two personality traits seem to be correlated, with bolder sharks showing higher reactivity to handling stress (Byrnes & Brown, 2016). PJ sharks are oviparous, and eggs are deposited in large numbers on shallow rocky crevices. Neonates likely hatch in close proximity to each other (in time and space), and juveniles are usually found in exposed soft-sediment areas, solitary, in dyads, or in loose aggregations of dozens of individuals in deeper water (pers. obs.; Powter & Gladstone, 2009). Unlike many other juvenile sharks (Guttridge et al., 2009; Jacoby et al., 2012), these field observations suggest that juvenile Port Jackson sharks may not be as social as the adults.

Elasmobranch aggregation and social behaviour is poorly documented compared to teleost fish, and research is particularly scarce in benthic elasmobranchs, which compose the majority of species in this vertebrate group. Thus, it seems important to examine the likelihood and potential mechanims of social attraction behaviour in benthic shark species. The aim in this study was to (1) quantify the social preferences of juvenile Port Jackson sharks, *Heterodontus portusjacksoni*, using a controlled binary choice experiment, and (2) assess if individual activity levels and foraging motivation shape social preference levels. We hypothesised that juvenile PJs would not show strong social attraction to conspecifics, and that foraging motivation would accentuate lack of social attraction, while swimming activity would promote it.

Methods

Subjects

A total of 24 young-of-the-year Port Jackson sharks (12 females; 12 males), ranging between 22 and 37 cm in total length and hatched in captivity, were used in the study. Eggs were collected from Jervis Bay, NSW Australia. Sharks were housed at the Sydney Institute of Marine Science (SIMS), Australia, in three 1000-L seawater tanks for 6 months prior to the experiment. Tanks had continuous circulation of fresh seawater, aeration, a thin layer of sand in the bottom and PVC structures and fake kelp to provide shelter and enrichment. Seawater was pumped directly from Sydney harbour at natural temperature. Sharks were fed squid, fish and prawns *ad libitum* every other day, following experimentation. The experimental tank was adjacent to the housing tanks, and the room had a natural light/dark cycle.

Experimental procedure

The testing arena (180 × 50 × 50 cm) was placed within a 10-foot circular tank filled with 30 cm of seawater. The arena was physically divided into three compartments: two outer areas (45 × 50 cm) and a central one (90 × 50 cm; Fig. 1). The compartments were separated by mesh, allowing visual and olfactory cues to be detected. For data analysis purposes, the central compartment was considered to have three equal zones (30 × 50 cm), and the two zones adjacent to the outer compartments were designated as preference zones (Fig. 1).

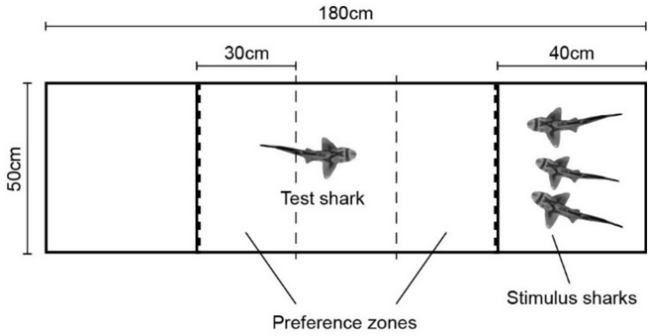

Figure 1. Binary choice experimental set-up, showing the central test arena and two outer compartments, separated by a mesh.

Sharks were moved from the housing tank to the experimental arena and tested individually. At the end of the trial, sharks were placed back into the housing tank and 2/3 of the water in the circular experimental tank (> 1000 L) was changed to dilute olfactory cues in the arena and remove any gradients. The order of testing of each subject was randomised every day. All subjects were given two 60-min acclimation sessions on consecutive days to become familiar with the experimental tank. For all the experiments detailed below, daily sessions comprised 15 min of acclimation after being moved to the experimental tank, and a 60-min trial.

Our first experiment investigated if any of the sharks showed a side bias in the tank, as individual-level laterality had been observed in the species (Byrnes et al., 2016a). Sharks ran two trials on consecutive days, in which they were given a choice between two empty outer compartments (Control 1; n = 24).

Secondly, to confirm that we had an olfactory gradient in the tank and that sharks were able to detect it, subjects were given a choice between an outer compartment with food (three 1 cm squid pieces) versus an empty compartment (Control 2; n = 22). Each shark ran three trials on consecutive days. The position of the empty and food compartment was counterbalanced across trials.

Our last experiment investigated juvenile Port Jackson shark sociality; we assessed if sharks have a preference to associate with size-matched, unfamiliar conspecifics (± 3 cm), and if individual activity levels or foraging motivation correlated with preference behaviour. In Treatment 1, ‘test’ sharks were given a choice between one shark versus no sharks (n = 12), and in Treatment 2 between three sharks versus no sharks (n = 12). Each ‘test’ shark was assigned to a single treatment and ran five trials on consecutive days. ‘Test’ sharks were also used as ‘stimulus’ sharks more than once but never in the same combination, and ‘stimulus’ and ‘test’ sharks were housed in separate tanks so had no opportunity to become familiar with each other. The position of the empty and social compartment was counterbalanced across trials.

We chose to describe and present results for Control 1, Control 2 and Social preference trials in this order for logical reasons; however, sharks first ran Control 1 trials, immediately followed by the Social preference trials; Control 2 trials were run three months after the Social preference experiment.

Data analysis

Data was collected in real time using BORIS v. 2.62 (Friard & Gamba, 2016). We measured the number of times sharks crossed between preference zones (head and pectoral fins over the demarcation line) as an estimate of swimming activity, and the amount of time they spent inside each zone as a measure of preference for a particular outer compartment. Trials in which the shark did not explore the whole extent of the tank (i.e. did not enter both preference zones at least once) were excluded from the analysis. For all experiments, exploratory analysis showed no differences in preference data over trials; thus, we calculated the cumulative time in each preference zone over all trials, and estimated the proportion of time the 'test' shark spent near the stimulus compartment relative to the total time spent in both preference zones. Statistical analysis was performed in R Studio v. 1.0.143 (R Core Team, 2016).

Swimming activity levels during social preference test days did not differ from swimming activity during side preference tests, when no stimulus sharks were in the experimental tank. For this reason, we used swimming activity data from five social preference trials. We tested for agreement repeatability (R) of swimming activity over the five trials using a generalised linear mixed-effects model fitted by PQL (penalized-quasi likelihood) estimation for count data (package rptR, Schielzeth & Nakagawa, 2013). We used PQL estimation since it is recommended that repeatability with count data (here number of area crossings) be estimated using multiplicative GLM models (Nakagawa & Schielzeth, 2010).

Side preference proportion data did not differ significantly from a normal distribution; thus, a one-sample t test was used to compare the observed proportion of time in the left compartment against a chance value of 0.5 (null hypothesis of no preference).

Food preference proportion data did not differ significantly from a normal distribution; thus, a one-sample t test was used to compare the observed proportion of time in the food compartment against a chance value of 0.5 (null hypothesis of no preference). To examine potential acclimatization or averaging effects over the trial, we calculated the proportion of time spent near the stimulus compartment on the first 5 min of the trial (0-5 min) and on the last 5 min (55-60 min) of the trial. We used a one-sample t test to compare the observed proportion of time in the food compartment against a chance value of 0.5 for each case; in the absence of acclimatization/averaging effects, the results of the three tests (full trial, initial 5min and last 5min) should be the same. Generalised linear models (GLM) were used to examine whether the proportion of time near the food compartment was influenced by total length, sex and swimming activity. Before fitting the models, we checked for collinearity between predictor variables. Although one statistically significant partial correlation was present (between sex and size), it was below 0.5; variance inflation factors (VIFs) were less than 1.4 in all cases, which further reassured us that collinearity was not a significant problem (Zuur et al. 2010). This makes biological sense, as our sharks were one-year-old juveniles and don't show sexual dimorphism yet. We first ran an exploratory information-theoretic approach to build candidate models. Models were ranked based on corrected Akaike information criteria (AICc), and differences in AICc and in Akaike model weight were used to choose the best-fit model. We then ran the best-fit model on our data and inspected diagnostic plots; residuals were roughly normally distributed and two cases had high Cook's distance scores. A closer look at the two cases suggested that the high variability in activity levels and in the response variable could be a problem. Activity data was log transformed and food preference data was logit-transformed (Warton & Hui, 2011). We refitted the model with transformed variables and found good indication of normality and heteroscedasticity of residuals, and of absence of non-linear relationships and influential cases. Wald tests were used to test if the inclusion of the fixed effects on the best-fit model significantly increased model strength compared to a null model (Bolker et al., 2009; Zuur et al., 2009).

Social preference proportion data did not differ significantly from a normal distribution; thus, a one-sample t test was used to compare the observed proportion of time in the social compartment against a chance value of 0.5 (null hypothesis of no preference). To examine potential acclimatization or averaging effects over the trial, we calculated the proportion of time spent near the stimulus compartment on the first 5 min of the trial (0-5 min) and on the last 5 min (55-60 min) of the trial. We used a one-sample t test to compare the observed proportion of time in the social compartment against a chance value of 0.5 for each case. Generalised linear models (GLM) were used to examine whether the proportion of time near the social compartment was influenced by total length, sex, swimming activity and feeding motivation (taken as the individual cumulative proportion of time near the food compartment in Control 2), following the same approach described above. Although one statistically significant partial correlation was present (between feeding motivation and activity), it was below 0.6 and VIFs were less than 1.4 in all cases, so collinearity was not an issue (Zuur et al. 2010). The best-fit model was chosen from the candidate model list based on differences in AICc and in Akaike model weight. We then ran the best-fit model on our data and inspected diagnostic plots; there was indication of a non-linear relationship between predictor and response variables, and two cases had high Cook’s distance scores. A closer look at the two cases suggested the high variability in activity levels and in the response variable could be a problem. We refitted the model with transformed variables, and again without the two influential cases. In both cases we found good indication of normality and heteroscedasticity of residuals, and while the relationship between residuals and fitted values didn’t improve much, model parameters remained similar and results didn’t change. We chose to report results for the model refitted with transformed variables, without removal of outliers.

Ethical note

Egg collection occurred under NSW Fisheries permit P08/0010-4.2. The experiments were approved by the Macquarie University Animal Ethics Committee (ARA 2014-003), and involved behavioural observations with as little intervention by the researcher as possible. Sharks were kept under optimal conditions and remained healthy during the experiments. All sharks were released at their original site of capture a few months after the experiment.

Results

Swimming activity

Swimming activity was highly variable between individuals, ranging between 0.02 and 23.4 zone changes per minute (median = 3.38). The frequency of changes between zones was repeatable across sessions within individuals: sharks that were more active in the first session were generally more active in subsequent sessions (R = 0.690 (0.088), 95% CI 0.496 to 0.840, *P* = 0.001; Fig. 2).

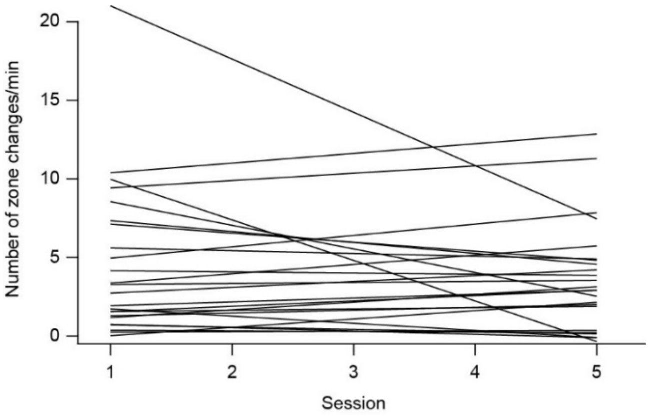

Figure 2. Individual swimming activity (frequency of preference zone changes) across daily sessions. Lines represent individual sharks and slopes represent individual changes in swimming activity across all sessions.

Control 1: Side preference

In all three binary choice tests, subjects entered both preference zones at least once. Juvenile Port Jackson sharks showed no preference for either side of the testing arena (one-sample t test: t_{23} = 0.417, P = 0.680; Fig. 3).

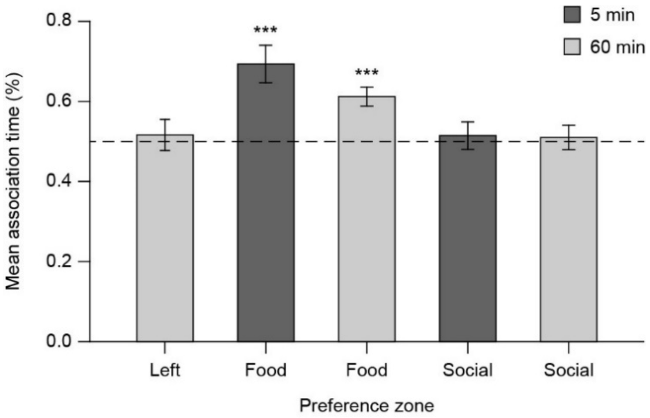

Figure 3. Mean proportion of time ± SEM spent by juvenile PJ sharks near the left outer compartment (n = 24), the compartment with food (n = 22) or the compartment with ‘stimulus’ sharks (n = 24). Dark grey shading represents proportion data during the initial 5 min of each session, and light grey shading represents proportion data over the whole session (60 min). Significant departure from the null hypothesis of no preference (0.5, dashed line) is indicated by asterisks: *** $P < 0.001$, one-sample *t* test.

Control 2: Food compartment preference

All sharks entered both preference zones at least once in all trials, hence none were excluded from the analysis.

Juvenile PJ sharks showed a significant preference for the compartment with food (mean ± 95% CI, 0.61 ± 0.049; one-sample t test: $t_{21} = 4.754$, $P < 0.001$; Fig. 3). This preference was maintained across the trial (0-5 min: $t_{21} = 7.479$, $P < 0.001$; 55-60 min: $t_{21} = 3.425$, $P < 0.001$); however, there was a significant decrease in preference from the initial 5 min (mean ± 95% CI, 0.76 ± 0.072) to the last 5 min of the trial (mean ± 95% CI, 0.61 ± 0.063), indicating some habituation occurred (repeated measures t-test: $t_{21} = 3.791$, $P < 0.001$). The best-fit model included only swimming activity levels as predictor variable (Table 1). The proportion of time spent near the food compartment was significantly influenced by swimming activity (Type II Wald test: $\chi^2 = 7.397$, d.f. = 1, $P < 0.01$): sharks with higher activity levels spent more time near the food compartment (Fig. 4).

Table 1. Model selection parameters from a global GLM model with proportion of time near the food compartment (logit transformed) as response variable and total length (TL), sex and swimming activity (log transformed) as predictor variables. Models with AICc differences (ΔAICc) > 4 were not considered. df: degrees of freedom; AICc: corrected Akaike Information Criterion.

Model	Residual df	Residual deviance	ΔAICc	AICc weight
Activity	20	3.646	0.00	0.554
Activity + TL	19	3.443	1.76	0.230
Activity + Sex	19	3.462	1.89	0.216

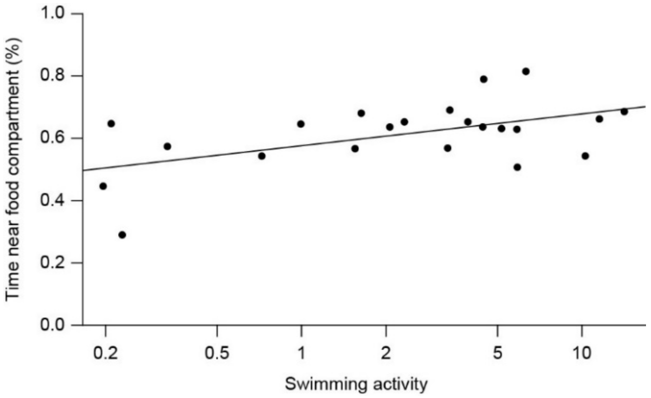

Figure 4. Relationship between the proportion of time spent near the food compartment and swimming activity levels (frequency of preference zone changes per minute). Note the x-axis is shown in log scale. Each point represents an individual shark, and the regression line was fitted using a linear model (y = 0.395x + 0.0444).

Social compartment preference

Nine sharks did not enter both preference zones in 16 trials (out of 120; Table 2), and those trials were excluded from the analysis.

We found no preference for the compartment with ‘stimulus’ sharks (one-sample *t* test: $t_{23} = 0.329$, $P = 0.745$; Fig. 3). Lack of preference occurred both in the beginning and end of the trial (0-5 min: $t_{23} = 0.425$, $P = 0.675$; 55-60 min: $t_{23} = -0.842$, $P = 0.409$), suggesting no average effects that might have diluted the results. No differences in preference were found between the initial and last 5 min of the trial (repeated measures *t*-test: $t_{23} = 0.975$, $P = 0.340$). The majority of sharks were randomly distributed between the social compartment and the empty compartment (mean proportion near social = 0.510, 95% CI 0.447 to 0.573), which is expected if the test sharks' movement is not influenced by the presence of the stimulus sharks. We found high individual variation: six out of the 24 sharks spent significantly less time near the social compartment, and two spent significantly more time near social companions (Fig. 5). The best-fit model was a null model with no fixed effects (Table 3), indicating that the proportion of time spent near the social compartment was not influenced by the number of ‘stimulus’ sharks in the outer compartment (Treatment 1 *vs.* Treatment 2), total length, sex, swimming activity or feeding motivation.

Table 2. Frequency of sharks that had *n* trials excluded from the analysis.

n trials excluded	Frequency of sharks
0	15
1	5
2	2
3	1
4	1

Table 3. Model selection parameters from a global GLM model with proportion of time near the social compartment (logit transformed) as response variable and total length (TL), sex, swimming activity (log transformed), treatment and feeding motivation as predictor variables. Models with AICc differences (ΔAICc) > 4 were not considered. df: degrees of freedom; AICc: corrected Akaike Information Criterion.

Model	Residual df	Residual deviance	ΔAICc	AICc weight
null	21	10.462	0.00	0.362
Sex	20	9.905	1.50	0.171
Treatment	20	10.173	2.09	0.128
Size	20	10.422	2.62	0.098
Activity	20	10.447	2.67	0.095
Feeding motivation	20	10.462	2.70	0.094
Sex + Treatment	19	9.616	3.87	0.052

Note: feeding motivation was defined as the individual cumulative proportion of time near the food compartment in Control 2.

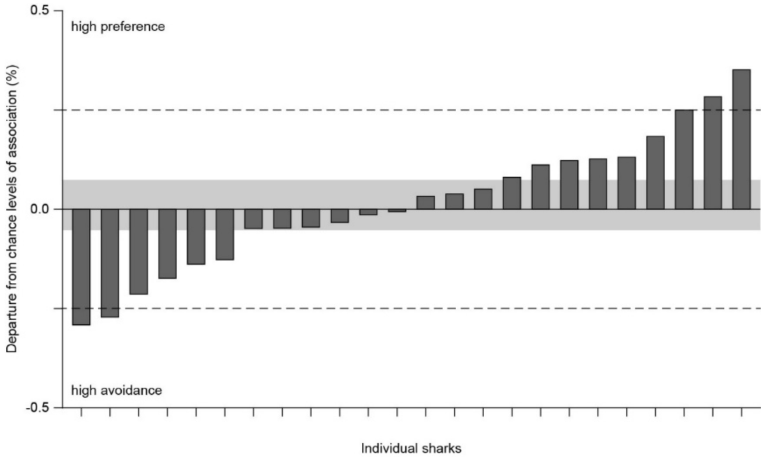

Figure 5. Cumulative proportion of time spent by individual juvenile PJ sharks near the compartment with ‘stimulus’ sharks over five test days, represented as difference

from chance levels of association (0.5; null hypothesis of no preference). Values over zero indicate that the proportion of time near the compartment with ‘stimulus’ sharks was greater than the time near the empty compartment, and the opposite is true for values below zero. The shaded area illustrates the 95% CI, and dashed lines mark proportions 0.25 and 0.75 near the social compartment.

Discussion

The results of this study indicate that juvenile Port Jackson sharks do not actively choose to be social. We found repeatable individual differences in swimming activity, which is consistent with the current evidence that elasmobranchs have personality traits (Jacoby et al., 2014; Byrnes & Brown, 2016; Byrnes et al., 2016b; Finger et al., 2017). Test sharks did not prefer to associate with a compartment containing stimulus sharks compared to an empty compartment, and we found no differences between sexes nor an effect of individual activity levels or feeding motivation.

Juvenile elasmobranch aggregations are common, even in large costal shark species, and predator avoidance has been advanced as a significant driver of this behaviour (Morrissey & Gruber, 1993; Sims et al., 1993; Heupel & Simpfendorfer, 2005; Duncan & Holland, 2006). Adults of several shark species are known to predate on juveniles, so grouping potentially confers antipredator benefits (Ward & Hart, 2003; Guttridge et al., 2009; Guttridge et al., 2011; Jacoby et al., 2012). Port Jackson sharks lay their eggs in coastal reefs, but juveniles are most often found in deeper, exposed soft-sediment areas, where vulnerability to predation is presumably high. It is intriguing, however, that PJs rarely show signs of apprehension and disturbance when divers or snorkelers come close to them (pers. obs.), and they are not consumed by humans anywhere in Australia despite being ubiquitous. Neonates and juveniles have a sharp spine on the leading edge of each dorsal fin, and a thick mucus layer covering their skin which likely play key roles in predator defence. PJs also have a combination of background matching and disruptive camouflage, a strategy that highly enhances concealment, especially in stationary objects (Fraser et al., 2007; Hall et al., 2013). Perhaps these potential defence mechanisms provide a good antipredator strategy that, together with the costs of increased conspicuousness and competition when in a group, outweighs the benefits of group living. Hunger levels have been identified as a factor influencing shoal choice in fish, with hungrier fish spending less time in a shoal (Krause, 1993), yet we did not observe a link between motivation to feed and time spent near the social compartment. Familiarity is another factor that has been linked to aggregation and social grouping behaviour in teleosts and sharks (Griffiths & Magurran, 1999; Jacoby et al., 2012). This experiment only assessed association preferences in unfamiliar treatments; further research is required to determine if familiarity would promote association preferences in juvenile Port Jackson sharks.

Interestingly, adult Port Jackson sharks form large aggregations during the breeding season, and actively associate with familiar sex- and size-matched individuals (Bass et al., unpubl. data; Powter & Gladstone, 2009; Mourier et al., 2017). Therefore, our results suggest the species undergoes an ontogenetic shift in social behaviour, in addition to the known shift in diet and habitat use (Powter et al., 2010). Upon reaching sexual maturity, social harassment of male sharks towards females and other males likely becomes a strong factor in the pay-off matrix, and aggregation becomes beneficial (Sims, 2005; Wearmouth et al., 2012). To our knowledge, ontogenetic shifts in social behaviour have not been previously described in elasmobranchs.

Repeatable individual behavioural traits such as boldness, activity or sociability, for example, have now been widely demonstrated in teleost fish (reviewed by Budaev & Brown, 2011) and, more recently, in sharks (Jacoby et al., 2014; Byrnes et al., 2016b; Finger et al., 2017). Personality traits have been linked to cognitive ability and to the way individuals make decisions (Sih & del Giudice 2012; Raoult et al. 2017), and this includes social behaviour decision-making. In addition, predation pressure in teleosts has been linked to traits such as shoal activity (Krause & Godin, 1995), which suggests that activity levels might also be relevant for group joining decisions in juvenile sharks. Here, we found repeatable within-individual activity levels; however, activity was not linked to the proportion of time spent near conspecifics. On the other hand, given the lack of attraction to conspecifics it is likely that some of the drivers of group joining decisions in shoaling teleost fish do not apply to juvenile PJs. Unfortunately, we did not record the activity levels of stimulus sharks during the trials to assess if the test sharks’ preferences were influenced by the activity of stimulus sharks.

Hatching and rearing elasmobranchs in captivity and conducting experiments in the lab poses a few potential issues, namely ensuring that fish are not stressed and that and that normal behaviour is recorded. Our sharks were housed in large tanks provided with fresh seawater and enrichment, in a room with natural light, and the experimental tank was placed near the housing tanks, in an attempt to minimise these issues. It is possible the olfactory gradient given off by conspecifics was not strong enough to elicit an association preference; however, this is unlikely given the small volume of water in the tank and the fact that visual cues were also available. Additionally, sharks showed a strong preference when tested with a small amount of food, and this preference albeit lower was still significant after 60 min when the cue was more diffuse in the arena.

Aggregation behaviour and sociality bring many advantages, yet there is a trade-off between costs and benefits that is particular to each species and even life history-stage. The lack of social preference found here contrasts with the results from juveniles of other shark species, and even from what is known of adults of this species, highlighting the variability and complexity of social behaviours in elasmobranchs, and how comparatively little knowledge we have on this vertebrate group.

Social behaviour has for long been deemed necessary for animals to acquire locally adaptive behaviour or information from conspecifics; however, there is now compelling evidence that sociality is not a defining feature of social learning (Reader & Lefebvre, 2001; Webster & Laland, 2017). Juvenile Port Jackson sharks might thus be an interesting species to further investigate the cognitive mechanisms underlying social learning, especially since social information transfer is still poorly understood in elasmobranchs.

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Conflicts of interest: none

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CHAPTER III

Social facilitation of foraging behaviour in non-social juvenile sharks

Social facilitation of foraging behaviour in non-social juvenile sharks

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Note: the sample size of the control group (referred to as 'sham observer-sham demonstrator' group in the manuscript) is low; we will be collecting more data on this group before submitting the chapter for publication – this was not possible before submission of this thesis as we did not have enough experimentally naïve individuals in the lab, and have to wait until new hatchlings reach a similar age as the sharks included here. The conclusions drawn in this chapter are therefore not final and should be considered with caution.

Abstract

Learning from the observation or interaction with another individual can be a shortcut for acquiring locally adaptive information. Social learning is taxonomically widespread, but has only been tested in two species among sharks and rays. Considering their basal position in the vertebrate tree and wide range of life-history traits and ecological niches, social learning studies in elasmobranchs can provide important insights into the cognitive mechanisms and evolutionary origins of social learning. Here we investigated social information use and social learning in non-grouping juveniles of a benthic shark species, the Port Jackson shark *(Heterodontus portusjacksoni),* and examined the relationship between intensity of training, personality traits, and laterality with social learning ability. Naïve 'observer' sharks could observe and interact with either 'demonstrator' sharks, trained to gain access to food through one of two arbitrary routes, or 'sham demonstrators', with no previous experience in the task. We found no differences in number of individuals and number of days to reach learning criterion between sharks paired with a knowledgeable demonstrator or a sham demonstrator; however, social facilitation seemed to enhance learning abilities in both group conditions. After 10 days of social exposure, observer sharks were tested in isolation. Only a small proportion of sharks in each group performed well, suggesting some form of release from conformity to the demonstrator's behaviour. Against expectations, sharks from the lower training intensity schedule took fewer trials to reach learning criterion. The quality of demonstration also influenced learning ability; observers paired with 'poor' demonstrators were faster in responding to the task, perhaps because 'good' demonstrators were too fast to be followed. Behavioural traits and laterality were not linked to any task performance measurements. Our results suggest that Port Jackson sharks can use social information to learn a foraging route, but the frequency and quality of demonstration are important factors.

Keywords

Social learning; local enhancement; conformity; behavioural syndrome; elasmobranchs

Introduction

Animals often share similar needs and challenges with other individuals, such as finding food, the best habitat, suitable mates, or avoiding predators. The ability to learn from another animal can therefore be beneficial, as it can save them the costs of learning by trial-and-error (Galef Jr, 1995; Laland *et al.*, 1996; Galef and Laland, 2005). Social learning has been documented across taxa, from mammals, birds, reptiles, and fish to insects (Leadbeater and Chittka, 2007; Brown and Laland, 2011; Hoppitt and Laland, 2013; Noble *et al.*, 2014). While the field initially attracted most interest from a descriptive viewpoint, as well as an evolutionary and adaptive perspective, we now see a considerable body of research examining processes, strategies, and mechanisms that allow social learning to occur (Heyes and Galef Jr, 1996; Rendell *et al.*, 2011). Animals may learn through social facilitation, when they are more likely to perform a behaviour in the company of others performing it; local enhancement occurs when an observer shows an increased chance of visiting a place from a demonstrator’s presence at that location; or observational learning, when observation of a demonstrator exposes an observer to a relationship between a response and a reinforcer that effects a change in their behaviour, among many others (Hoppitt and Laland, 2008).

In all forms of social learning, particular characteristics of demonstrator and observer individuals will affect the likelihood of social learning (Coussi-Korbel and Fragaszy, 1995; Laland, 2004; Rendell *et al.*, 2011). Sex, age, social rank, familiarity, and developmental stress are some of the factors known to influence social information use and social learning across taxa (Nicol and Pope, 1999; Swaney *et al.*, 2001; Benskin *et al.*, 2002; Noble *et al.*, 2014; Farine *et al.*, 2015). For instance, female zebra finches preferred to feed from the hopper used by male demonstrators rather than female ones (Benskin *et al.*, 2002), and observer guppies that have familiar bonds with demonstrators learnt a foraging task faster than observers grouped with unfamiliar guppies (Swaney *et al.*, 2001). The extent of social information and stimulation also influences learning performance. For example, group size can enhance learning skills in guppies and rainbowfish (Lachlan *et al.*, 1998; Brown and Warburton, 1999), but the proportion of knowledgeable demonstrators in the group plays a key role; observer Atlantic charr were only successfully conditioned when the demonstrators were the minority of the group

(Vilhunen *et al.*, 2005). In addition, the quality of demonstrator training is linked to observer performance. Observer guppies paired with well-trained demonstrators had lower performance compared to those paired with poorly-trained demonstrators, likely because the well-trained demonstrators were too fast to be followed (Swaney *et al.*, 2001). Overall, these results suggest a complex link between the extent of social exposure and observer learning performance.

Social learning research has traditionally focused on group-living species. While sociality is expected to facilitate access to social information, there is little experimental evidence supporting a link between social learning and sociality (Reader and Lefebvre, 2001). In fact, social learning seems to simply be linked to an animal's general learning ability. All animals, including solitary species, are regularly exposed to other individuals, conspecifics or heterospecifics, or to their products (e.g. scent marks, excreted waste), and therefore social cues should be one among many environmental cues that can lead to changes in behaviour (Heyes, 2012; Webster and Laland, 2017). It is therefore unsurprising that social learning has been demonstrated in non-social species, including reptiles (Wilkinson *et al.*, 2010; Noble *et al.*, 2014), fish (Brown and Laland, 2002a; Webster and Laland, 2017), cephalopods (Fiorito and Scotto, 1992; but see comments by Biederman and Davey, 1993), and a non-colonial insect (Coolen *et al.*, 2005). For example, even though juvenile Atlantic salmon are highly territorial, they can still use social learning to learn to identify novel prey items (Brown and Laland, 2002a) and where they are likely to appear (Brown *et al.*, 2003).

Social learning is considered to be linked to individual cognitive skills, so mechanisms known to enhance individual learning abilities might also be related to social learning. Animals differ in the way they react to stressful or novel situations, potential risks, or interaction with conspecifics, usually in a consistent way across time and context. Such differences are commonly referred to as personality, or behavioural syndromes if multiple traits are correlated across situations (Sih *et al.*, 2004; Réale *et al.*, 2007). The concept of personality traits and behavioural syndromes implies that animals do not always display the optimum behaviour in all contexts, and thus has clear fitness implications. For example, animals that are bolder, more active, and/or proactive might obtain more food or mates, but take more risks along the way (Réale *et al.*, 2000; Brown and Braithwaite, 2004; Brown *et al.*, 2005; Bierbach *et al.*, 2015). This risk-reward trade-off seems to be linked to differences in decision making and learning ability; bold, more active animals might be favoured in tasks that are stable and routine forming, while shyer, less active individuals take the time to assess the environment and tend to adapt more accurately to situational demands (Carere and Locurto, 2011; Sih and Del Giudice, 2012; White *et al.*, 2016; Raoult *et al.*, 2017). Cerebral lateralization (asymmetry of functions between the two brain hemispheres) is another mechanism linked to cognitive abilities in several species, with strongly lateralised individuals showing faster learning rates, greater cognitive loading, and enhanced cooperative behaviours (Rogers *et al.*, 2004; Magat and Brown, 2009; Bibost and Brown, 2014; Dadda *et al.*, 2015).

In the present study, we investigated social information use and social learning in non-social juveniles of a benthic shark species, the Port Jackson shark *(Heterodontus portusjacksoni)*, and examined the relationship between personality traits and laterality with social learning ability. Port Jackson sharks (PJs) are oviparous. Juveniles hatch after *ca*. 10 months of incubation and have no parental care. Results from a laboratory binary choice test indicate juveniles do not actively choose to associate with conspecifics (Ch. III of this thesis). Social learning may increase their learning rates and greatly improve their chances of survival. In the east coast of NSW, adult PJs form breeding aggregations during winter, and the same sites have been used for more than 50 years (O'Gower, 1995). After the breeding season, males and females undertake a migration of hundreds of kilometres, only to return the following year (Powter & Gladstone, 2009; Bass et al., 2016). The drivers or mechanisms of this large-scale migration are unknown, but social learning has been suggested to be one of the factors that facilitates it (O'Gower, 1995). Among sharks and rays (elasmobranchs), social learning has only been tested in two species (lemon sharks, Guttridge *et al.*, 2013; freshwater stingrays, Thonhauser *et al.*, 2013). Consistent individual differences in boldness, stress reactivity, docility, and individual lateralization has been described in a few species, including juvenile and adult PJs (Byrnes *et al.*, 2016a; Byrnes *et al.*, 2016b; Finger *et al.*, 2017). Considering their basal position in the vertebrate tree (Naylor *et al.*, 2005), and wide range of life-history traits and ecological niches (Compagno, 1990), social learning studies in elasmobranchs can provide important insights into the perceptive and cognitive mechanisms of social learning as well as its evolutionary origins among vertebrates (Schluessel, 2015). In addition, social behaviours and social information transfer are known to play a vital role in the establishment of migratory and foraging routes in some fish species, such as grunts and cod (Helfman and Schultz, 1984; Fernö *et al.*, 2011). These results have special importance when there is a conservation or commercial interest, as with several elasmobranch species, since the systematic removal of older, more knowledgeable individuals by commercial fishing might cause a detrimental shift in their migration or spawning/foraging grounds, as recently observed in cod (Fernö *et al.*, 2011).

Here, we tested the social processes involved in learning a new foraging route in juvenile Port Jackson sharks, adapting the task design of Laland and Williams (1997) to a benthic forager. Naïve 'observer' sharks were allowed to observe, and interact with, either sharks that were trained to gain access to food through one of two arbitrary routes ('demonstrators') or naïve sharks with no previous experience in the task ('sham demonstrators'). We assessed (1) repeatability of boldness and stress reactivity and (2) lateralisation levels of observer sharks, and (3) level of performance of demonstrator sharks. We then investigated (4) the ability of observer and sham observer sharks to succeed in the task; and the effect of (5) training contingencies and individual traits on social learning ability. We predicted that observer sharks paired with knowledgeable demonstrators would perform better than observers exposed to sham demonstrators; higher frequency of social demonstration would result in faster learning; bold, proactive observers learn the task faster than shy, reactive ones; and sharks with strong laterality preference would perform better than non-lateralised individuals.

Methods

Subjects and apparatus

Forty-four juvenile Port Jackson sharks ranging 27–39 cm total length were used in the study. Eggs were collected from Jervis Bay, NSW and hatched in captivity. Sharks were housed in groups of two to four at the Sydney Institute of Marine Science (SIMS), Australia, in 1000-L seawater tanks at ambient temperature for six months prior to the experiment. Tanks had continuous circulation of seawater, aeration, a thin layer of sand in the bottom, and PVC structures and fake kelp to provide shelter and enrichment. Seawater was pumped directly from Sydney harbour at ambient temperature (ranging 18.2–22.4 ºC, April to June 2016). Prior to the experiment and during the boldness and stress reactivity assays, sharks were fed small pieces of squid, fish, and prawns *ad libitum* three times per week. The experimental tank was adjacent to the housing tanks and the room had a natural light/dark cycle.

The experimental tank measured 180 × 100 × 40 cm and was divided lengthways by a black Perspex partition (Fig. 1a). The sharks could access both sides of the tank through two holes (22 × 10 cm) in the partition, located at the bottom and 5 cm from each end of the partition. Sliding doors would allow us to block access through the holes (Fig. 1b). The surrounding of the holes was marked to facilitate visual discrimination of the two holes, with the right side of the partition marked with a black-and-white chequered frame, and the left side marked with a plain black frame with white outline (Fig. 1b). At one end of the tank (‘Task side’, Fig. 1a), a sliding mesh wall was used to create a starting compartment. At the opposite end of the tank (‘Reward side’, Fig. 1a), a black divider (50 cm long) separated two reward locations visually marked with a black-and-white chequered band or a plain black band accordingly. Sharks were rewarded using long aquarium tongs and the daily food intake per individual during the experimental period was equivalent to 2% of their wet body weight in squid (*Loligo opalescens*) pieces. Water inflow was provided from both sides of the tank, with multiple small inflow points along the bottom of the tank, and water outflow was located on the reward end of the tank (Fig. 1a).

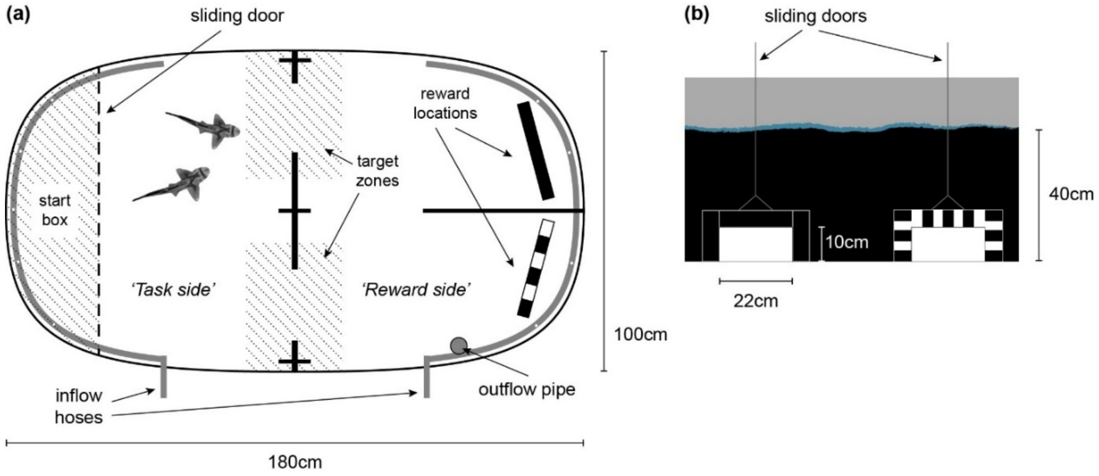

Figure 1. Schematic representation of the experimental tank. **(a)** Top view of the tank; and **(b)** front view of the partition (as seen from the ‘Task side’), with two holes through which the sharks could swim from one side of the tank to the other.

Procedure

Sharks were pseudo-randomly assigned to one of four groups, naïve observers (‘O’; n = 20), naïve sham observers (‘SO’; n = 5), trained demonstrators (‘D’; n = 8) and sham demonstrators (‘SD’; n = 5), and allocated to one of two treatment pairs: observer-demonstrator (O-D; n = 20) or sham observer-sham demonstrator (SO-SD; n = 5). Observer and demonstrator sharks were housed in separate tanks so had no opportunity to become familiar with each other. Half of the demonstrators were trained to take the hole on the right side of the partition, and half trained to take the hole on the left. In addition, pairs were trained in one of two training schedules: lower training intensity (3 exposure trials/day; ‘3T’) or higher training intensity (6 exposure trials/day; ‘6T’), following a balanced design. Demonstrator sharks were reused two to four times, but sham demonstrators were used only once. A timeline of each experimental phase is given in supplementary material (Table S1).

Boldness assay

Observer sharks were individually tested for their propensity to emerge from cover in a different, novel experimental arena, akin to an open-field emergence test, following Byrnes and Brown (2016). Each shark ran three trials with a three-day interval between them (the sharks were left undisturbed in the housing tanks during this period).

At the start of the trial, the shark was transported from the housing tank in an opaque bucket to a black refuge box (40 × 68 × 19 cm), and left to acclimate for 2 min. A sliding door in the front of the refuge box was then opened *via* a sting pull, leading to the centre of the experimental arena (120 × 68 × 19 cm). The individual was left undisturbed and time until emergence was recorded. To account for hesitancy, we recorded two stages of emergence: (1) initial emergence (IE); and (2) complete emergence (CE), as defined in Table 1. The hesitancy score was taken as the difference between CE and IE. If the shark did not emerge after a total trial time of 20 min, the trial was terminated and the shark was given a maximum score of 1200 s. The hesitancy score and complete emergence were combined using a principal component analysis (PCA; *princomp* function from the base package in R v. 3.3.2) using a correlation matrix. We only retained principal components with eigenvalues greater than one (Zuur *et al.*, 2009). The two behavioural traits were highly correlated and negatively loaded on the first principal component (PC1; supplementary material, table S1). PC1 explained 66.3 % of the variance in the data, and was used in further analyses as our ‘boldness score’: as the value increases, it reflects lower hesitancy and lower emergence times, and thus bolder behaviour.

Stress reactivity assay

Observer sharks were individually tested for changes in swimming activity after acute handling stress, following Byrnes & Brown (2016). The stress assay consisted of three trials with a three-day interval between them. Baseline activity levels were obtained from two trials with a five-day interval between them. To eliminate observer effects, a video camera was mounted above the test tank, and activity levels in all trials were scored from video recordings.

To induce stress, the shark was transported from the housing tank in an opaque bucket to the vicinity of the experimental arena and held out of water, with two hands underneath, for 1 min. Individuals were captured only when within 15 cm of the surface of the housing tank ensuring easy capture on first attempt and minimising individual pre-test variability. The shark was then placed in the centre of an oval, opaque experimental arena (180 × 100 × 40 cm), and activity level (tail beat frequency) was recorded for 30 min. Tail beat frequency was counted by complete oscillations of the caudal fin within 1 min timeframes at predetermined times (1, 2, 3, 4, 5, 10, 15, 20, 25, and 30 min following handling; Hunter and Zweifel, 1971), and stressed activity levels were calculated as the cumulative number of tail beats *per* minute over all timeframes.

Table 1. Ethogram of experimental behaviours measured from video recordings during open-field, stress reactivity and laterality tests and the experimental phase, for observer and/or demonstrator sharks.

Behaviour	Definition	Measure	Individual
Initial emergence	First instance the shark’s head appears outside the refuge box.	Latency (s)	Observer
Complete emergence	Shark fully emerges from the refuge box.	Latency (s)	Observer
Turn on Y-maze	The rear edge of at least one pectoral fin is past the imaginary line that comprises the start of the arm of the maze.	Direction of turn	Observer

Attempt to swim through partition	Shark is in the bottom of the tank, within 10 cm and facing the hole in the partition, but swims away.	Number of events & latency (s)	Observer
Swim through partition (choice)	Shark swims through either one of the holes in the partition with its head and at least one pectoral fin.	Latency (s) Number of events	Observer Demonstrator
Inside target zone	Shark enters the target zone (200-cm^2 area on both sides of the partition adjacent to the hole) with its entire head and at least one pectoral fin.	% of trial time	Demonstrator
Detect reward	Shark makes physical contact for the first time with the aquarium tongs using snout tip.	Latency (s)	Observer
Eat reward	Shark eats the food reward.	Latency (s)	Observer
Perceived reward value	Interval of time between detecting the reward and consuming it.	Latency (s)	Observer

For baseline activity trials, the shark was transported from the housing tank in an opaque bucket and gently placed in the centre of the experimental arena. It was left undisturbed to acclimate for 30 min in the experimental arena and tail beat frequency was recorded as per the stress trials on the subsequent 30 min. Individual baseline activity level was estimated by averaging the cumulative number of tail beats *per* minute of the two trials.

‘Stress reactivity’ was calculated by subtracting baseline activity levels from activity levels following the stressor, with negative values indicating a ‘freeze response’ (decrease in activity induced by the stressor), and positive values an ‘escape response’ (increase in activity after the stressor).

Laterality assay

A detour test using a Y-maze was used to assess individual lateralisation levels (Bisazza et al., 1997). The test consisted of 10 trials conducted individually on a single day.

The shark was ushered down a corridor and its turn direction at the end of the maze was recorded. Based on results from a pilot study, a small piece of food was placed behind a partition at the end of the corridor and individuals were fed at the end of the 5th trial to elicit directed swimming along the maze and maintain motivation in the task.

Training of demonstrators

Demonstrator sharks (n = 8) were moved from the housing tank to the experimental arena each day and trained individually. Before the start of the experiment, each shark was given three 45-min acclimation sessions in consecutive days, where they could swim freely in both sides of the experimental tank and through the holes of the partition. Sharks were fed on the last 15 min of acclimation sessions in random places in the experimental tank to become familiar with receiving food from aquarium tongs.

Training sessions started with 5 min of acclimation in the ‘task side’ of the experimental tank (both holes were blocked with a sliding transparent door) for the sharks to settle after being moved to the experimental tank, followed by six training trials. Trials would begin in the starting compartment. Once the starting compartment was open, the shark was given 90 s to swim through the partition and reach the ‘reward side’ of the tank, or the trial was terminated. On sessions 1 to 4, individuals were only allowed to swim through the target hole (the wrong option was blocked by a transparent sliding door) and the reward was placed near the target hole. On sessions 5 to 9, the reward was moved progressively towards the far end of tank, and on sessions 10 onwards the sharks could swim through any of the holes, and the reward was delivered at the far end of the tank upon correct choices only. After eating the reward, or if the shark swam through the wrong hole in the partition, it was gently ushered back to the starting compartment and a random inter-trial interval of 30–60 s preceded the next trial. After the last trial of the day, sharks were given a random interval of 3–6 min to settle, and fed the remainder food allocated for the day inside the starting compartment.

Individuals were considered sufficiently trained to act as demonstrators if they swam through the correct hole in 8 out of 10 trials, and given 5 additional training days to consolidate learning.

Social learning task

Naïve observer sharks (n = 20) and sham-observer sharks (n = 5) were pre-exposed to the experimental tank in pairs for three consecutive days to allow them to overcome any stress associated with moving tanks, sharing the space with an unfamiliar shark (different pairs were used each day) and swimming through the holes in the partition, as familiarity with the test environment facilitates learning (Brown, 2001; Paulissen, 2008). Each session lasted 45 min, and in the last 15 min sharks were fed with aquarium tongs in random locations in the experimental tank. For the following 10 days, each observer shark was introduced to the experimental tank with its assigned demonstrator (D or SD). Both sharks were given 5 min of acclimation on the ‘task side’ of the experimental tank (both holes were blocked with a Perspex sheet) to settle after being moved to the experimental tank, followed by either 3 or 6 exposure trials according to the training schedule assigned to it (‘3T’ or ‘6T’). Trials would begin in the starting compartment. Once the sliding mesh was removed, the observer was given 90 s to swim through any of the holes in the partition and reach the ‘reward side’ of the tank. During this time, the demonstrator shark could swim multiple times through the holes, with only the first correct choice being rewarded. A trial would end when the observer shark swam through the correct hole and consumed the reward, swam through the wrong hole or did not swim through the partition within 90 s. Both sharks were then gently ushered back to the starting compartment and a random inter-trial interval of 30–60 s preceded the next trial. Following the end of each session, sharks were given a random interval of 3–6 min to settle, and then given the remainder of the food individually allocated for the day inside the starting compartment. After 10 days of social foraging experience, observer sharks were tested in isolation over two consecutive days. Sharks from both treatment groups ran six trials each day.

Table 1 gives exact definitions of the variables and behaviours measured for each trial. During exposure and test trials, we recorded the observer shark’s choice and latency of the first attempt to swim through the partition, total of correct and wrong attempts, choice and latency to cross the partition, and the latency to eat the reward in correct response trials. We estimated a ratio of correct to wrong attempts = number of correct attempts / total number of attempts. In some trials, the sharks would attack the aquarium tongs and take a long time to consume the reward; we thus used the interval of time between detecting and consuming the reward as a measure of sharks' persistence and indicator of perceived ‘reward value’. The sharks never gave up on the reward and would bite the tongs until consuming it. We hypothesise that a reward will be more ‘salient’ or ‘valuable’ if the shark spent more time attacking the tongs.

During exposure trials, we also recorded the demonstrator shark's first choice and latency to cross the partition, the cumulative number of correct and wrong partition crossings and proportion of trial time spent in the target zones, to determine 'quality' of demonstration (Fig. 1a; Table 1).

Data analysis

Trials were video recorded and trial statistics were collected using BORIS v. 2.62 (Friard and Gamba, 2016). All statistical analyses were conducted in R v. 3.3.2 (R Core Team, 2016). Prior to conducting analyses, we explored the data following Zuur *et al.* (2009), and assumptions of normality of residuals and homogeneity of variance were always verified.

Lack of motivation was apparent in some trials, in which the sharks did not swim through the partition (hereafter referred to as null trials). For these trials, we assigned a ceiling value of 270 s to the choice latency (three times the maximum trial duration).

Are behavioural traits repeatable and correlated?

We separately performed two Gaussian linear mixed-effects models (LMM) with Restricted Maximum Likelihood (REML) estimation for each behavioural trait ('boldness score' and 'stress reactivity') to examine how they were affected by weight (continuous) and sex (categorical with two factors, 'M' and 'F'), and included individual ID as a random effect. We chose to exclude total length as it was highly correlated with weight and had a variance inflation factor score greater than 3 (Zuur *et al.*, 2009). We used a rank transformation to normalise both response variables (Riley *et al.*, 2017) and Wald tests to test if the inclusion of the fixed effects significantly increased model strength compared to a null model (Bolker *et al.*, 2009).

Agreement repeatability (R) and adjusted repeatability (R_A) of each behavioural trait were estimated using separate Gaussian LMMs, all with individual shark ID as random effect and the R_A models with the significant fixed effects of the previous model analysis (package rptR; Nakagawa and Schielzeth, 2010). Confidence intervals (CI) and standard errors (SE) for both R and R_A were calculated from parametric bootstrapping of likelihood ratios (1000 simulations), and *P* values derived from permutation tests (Schielzeth and Nakagawa, 2013).

We used PCA analysis to evaluate if there were correlations between the three behavioural variables (emergence time, hesitancy score, and stress reactivity), indicative of a behavioural syndrome. Our PCA used the correlation matrix because our variables were on different scales and this approach standardises the data.

Are individuals lateralised?

We estimated a ‘laterality index’ as L_I = (number of right turns – number of left turns)/(total number of turns). L_I is a continuous value from -1 to 1, in which a positive score indicates a preference for rightward turns and a negative score indicates a preference for leftward turns. ‘Laterality strength’ (L_S) was estimated by taking the absolute value of L_I.

How did demonstrators perform?

We separately performed Mann–Whitney U tests to compare the following trial statistics between ‘D’ and ‘SD’ sharks: (1) proportion of correct choice; (2) proportion of correct choice excluding null trials; (3) latency of choice; and (4) latency of correct choice.

Quality of demonstration was estimated from a ‘Correct hole crossing index’ (C_I) and a ‘Correct hole preference index’ (P_I) for each demonstrator, where C_I = number of correct crossings / total number of crossings, and P_I = trial time in the correct target zone / (trial time in the correct and wrong target zones). Demonstrator sharks were allocated to one of two classes (‘good’ or ‘poor’) according to these indices: ‘good’ demonstrators had both a C_I and a P_I greater than or equal to 2/3, indicating that the average number of correct crossings per trial, or trial time near the correct hole, respectively, was double compared to that of the wrong hole.

Do O-D pairs learn the task faster than SO-SD pairs?

Separate Mann–Whitney U tests were used to compare the overall proportion of null trials of ‘O’ and ‘SO’ sharks, ‘3T’ and ‘6T’ sharks, and of sharks that learnt or did not learn the task.

We considered that observer sharks were successful during exposure trials if they made a correct choice in 9 out of 12 consecutive trials. If a shark did not reach learning criterion during the exposure phase, we assigned a ceiling value of 60 or 120 trials to the number of trials taken to learn the task (double of total trials ran by individuals in training schedule ‘3T’ or ‘6T’, respectively). We used a Gaussian LMM with REML estimation to test if the number of exposure trials to reach learning criterion was linked with potential predictor variables, namely observer group (categorical with two levels, ‘O’ and ‘SO’), training schedule (‘3T’ or ‘6T’), correct side (‘R’ and ‘L’), demonstrator quality (‘poor’ and ‘good’), day (continuous), weight (continuous), sex (‘M’ and ‘F’), reward value (continuous), behavioural syndrome (continuous), L_I (continuous), and L_S (continuous). Individual ID was included as a random factor. Due to a small sample size within groups, we used an information-theoretic approach to select potential predictor variables that might have influenced each response variable. The coefficient of determination (R^2) was used to assess goodness-of-fit and estimate the amount of variance explained by the model following Nakagawa and Schielzeth (2013), using the package piecewiseSEM (Lefcheck, 2016).

Of the individuals that reached learning criterion during the exposure phase, we compared the proportion of null trials during test days (in the absence of demonstrators) between ‘O’ and ‘SO’ sharks, and between sharks that performed above or below learning criterion during the test phase, using Mann–Whitney U tests.

Do training contingencies and individual traits influence learning ability?

We separately performed Gaussian LMM with REML estimation for each of the following response variables: (1) proportion of correct choice; (2) proportion of correct choice excluding null trials; (3) latency of choice; (4) latency of correct choice; (5) latency of the first attempt to swim through the partition; and (6) ratio of correct to wrong attempts, using the approach described above. Potential predictor variables were the same listed above. To improve model fit response variables 1, 2, and 6 were logit transformed and variables 3 to 5 were log transformed.

Ethical note

Egg collection occurred under NSW Fisheries permit P08/0010-4.2. The experiments were approved by the Macquarie University Animal Ethics Committee (ARA 2014-003). Sharks were kept under optimal conditions and remained healthy during the experiments. Tanks had continuous supply of fresh seawater, aeration, and PVC structures and fake kelp to provide shelter and enrichment. Tanks were scrubbed clean at least once a week. Seawater was pumped directly from Sydney harbour at ambient temperature, and the room had a natural light/dark cycle. During non-experimentation periods, sharks were fed on a mixed diet of squid, fish, and prawns *ad libitum* three times per week, and given vitamin supplements (Elasmo tabs 150mg, Vetafarm) on a weekly basis. All sharks were released at their original site of capture a few days after the experiment.

Results

Are behavioural traits repeatable and correlated?

All sharks emerged from the refuge box within the maximum trial time during the boldness assay. Complete emergence times ranged 2–752 s (median = 11 s) and hesitation scores ranged 0–113 s (median = 3 s). Sex was significantly correlated with our 'boldness score' (males were bolder than females; $\chi^2 = 11.709$, d.f. = 1, $P < 0.001$). 'Boldness scores' were not repeatable across trials within individuals (R = 0.197 (0.131), 95% CI 0 to 0.456, $P = 0.079$), even when adjusted for sex (Fig. 2a; $R_A = 0.018$ (0.09), 95% CI 0 to 0.307, $P = 0.46$). From inspection of the data, two individuals had extremely high scores in one of the trials that seemed to drive this effect; after careful examination of trial contingencies we did not find a plausible explanation for outlier behaviour and decided not to exclude them from the analysis.

Our 'stress reactivity score' ranged -41.45–53.10 tail beats *per* min, indicating that some individuals reduced activity while others increased activity after the stressor, and was not significantly influenced by sex or weight. Stress reactivity was highly repeatable across trials within individuals (Fig. 2b; R = 0.797 (0.079), 95% CI 0.586 to 0.897, $P < 0.001$).

Our PCA analysis on emergence time, hesitation score, and stress reactivity combined the three variables into a single component that accounted for 54% of the variance in the data (supplementary material, table S2). The two 'boldness' traits loaded positively on PC1, while stress reactivity had a negative loading, indicating that sharks with higher emergence and hesitation times (shyer) had lower stress reactivity scores (reduced activity after the stressor). PC1 scores were used as a behavioural syndrome in the analysis of learning performance, to examine the hypothesis of speed-accuracy trade-off between behavioural types (bold, proactive; shy, reactive).

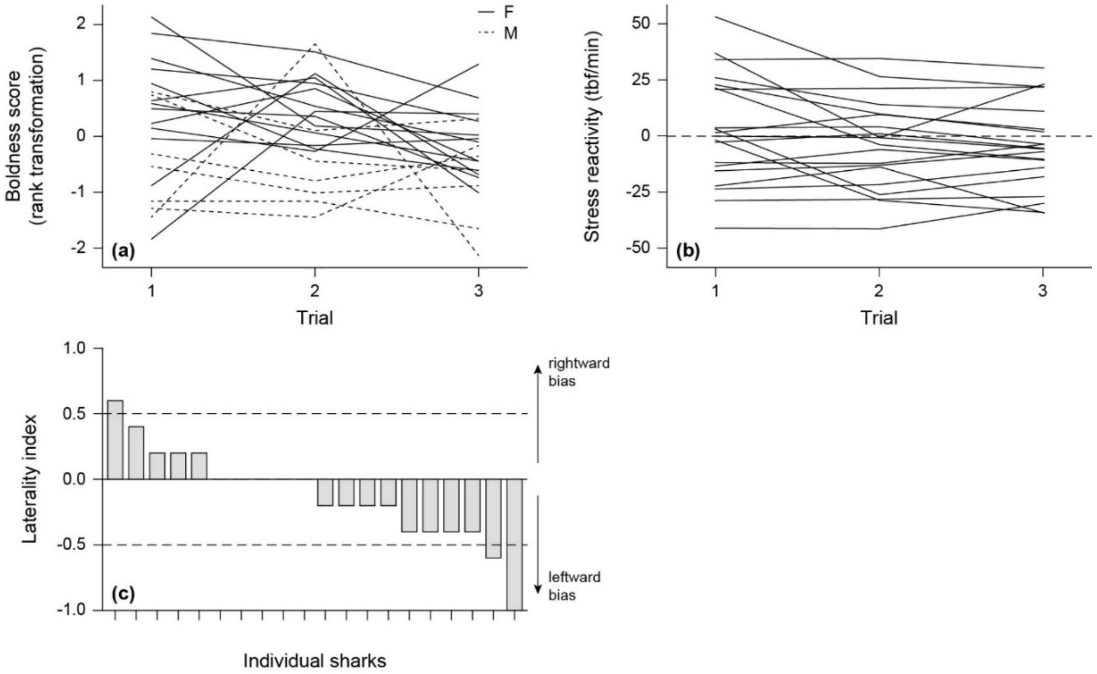

Figure 2. Behavioural traits assessed in observer sharks. **(a)** ‘Boldness score’ (rank transformed PC1; F: females; M: males) and **(b)** stress reactivity (tail beats per minute) across trials. The dashed line in (b) marks a stress reactivity score of zero; negative scores indicate that the shark decreased its activity after the stressor compared to baseline levels. **(c)** Laterality index, where positive scores indicate a preference for rightward turns and negative scores a preference for leftward turns.

Are individuals lateralised?

Of the 20 sharks, only three showed strong lateralisation of their turn preference, one of them to the right and two to the left (Fig. 2c). Individual laterality index (L_I) and laterality strength (L_S) scores were used in the analysis of learning performance.

How did demonstrators perform?

Demonstrator (‘D’) sharks showed a higher proportion of correct choice trials compared to sham demonstrators (‘SD’), even when excluding null trials (Fig. 3a; all trials: W = 6382, $P < 0.001$; excl. null trials: W = 4024.5, $P = 0.011$). ‘D’ sharks were also faster in crossing the partition compared to ‘SD’ sharks (Fig. 3b; W = 1116, $P < 0.001$). Five ‘D’ sharks were classed as ‘good’, and three ‘D’ and all five ‘SD’ sharks were classed as ‘poor’ (Fig. 4).

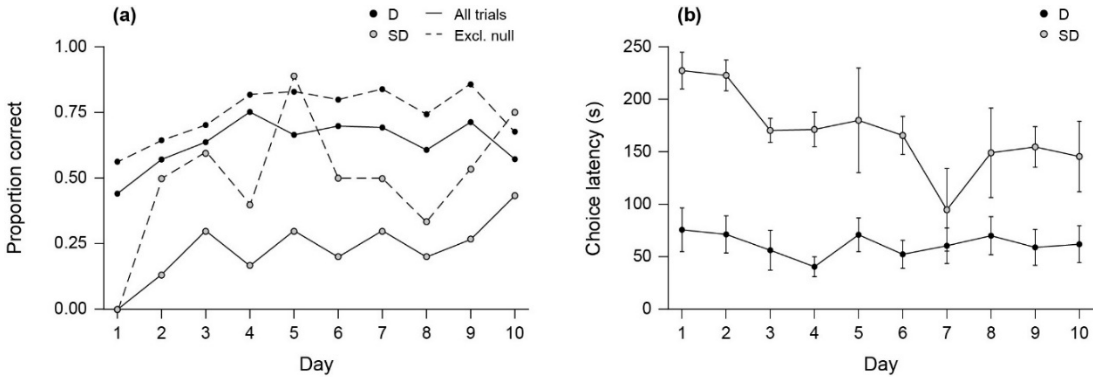

Figure 3. Performance of demonstrator ('D', black dots) and sham demonstrator ('SD', grey dots) sharks over exposure phase training days. **(a)** Proportion of correct choices considering all trials (solid lines), or excluding null trials (dashed lines); **(b)** Mean ± SEM latency to cross the partition for the first time.

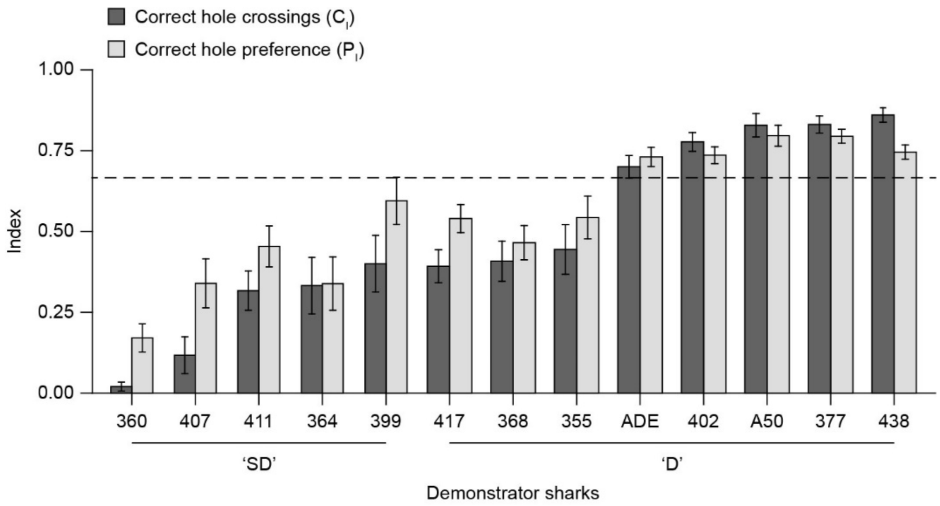

Figure 4. 'Correct hole crossing index' (C_I) and 'Correct hole preference index' (P_I) for each demonstrator shark ('SD': sham demonstrator; 'D': demonstrator). Values above 2/3 (dashed line) indicate that the average number of correct crossings per trial, or trial time near the correct hole, respectively, was double compared to that of the wrong hole. Demonstrators with both C_I and P_I above the dashed line were classed as 'good', and the ones with C_I and P_I below the dashed line as 'poor'.

Do O-D pairs learn the task faster than SO-SD pairs?

We found no differences in the proportion of null trials of observer ('O') and sham observer ('SO') sharks (W = 74.5, *P* = 0.103); however, sharks that did not learn the task had a higher number of null trials (43.1% of all trials) compared to those that learnt the task (24.5% of all trials; W = 30.5, $P = 0.02$).

Thirteen out of 20 (65%) ‘O’ sharks and three out of five (60%) ‘SO’ sharks reached learning criterion during the exposure phase, in similar proportions between training schedules (Table S4). When tested in isolation, only six out of 20 (30%) observer sharks and two out of five (40%) sham-observer sharks performed above learning criterion.

Observer group (‘O’ or ‘SO’ sharks) was not linked to the number of exposure trials to reach learning criterion (Fig. 5a). The final model included training schedule and sex as predictor variables, and individual ID as random effect. We found that sharks from training schedule ‘6T’ took longer to learn the task compared to ‘3T’ sharks (Fig. 5b; $\chi^2 = 10.991$, d.f. = 1, $P < 0.001$), and males were slower than females (Fig. 5b; $\chi^2 = 7.047$, d.f. = 1, $P = 0.008$). The predictor variables accounted for 41.7% of the variance in number of trials to reach criterion, and individual ID for 51.1% of the variance.

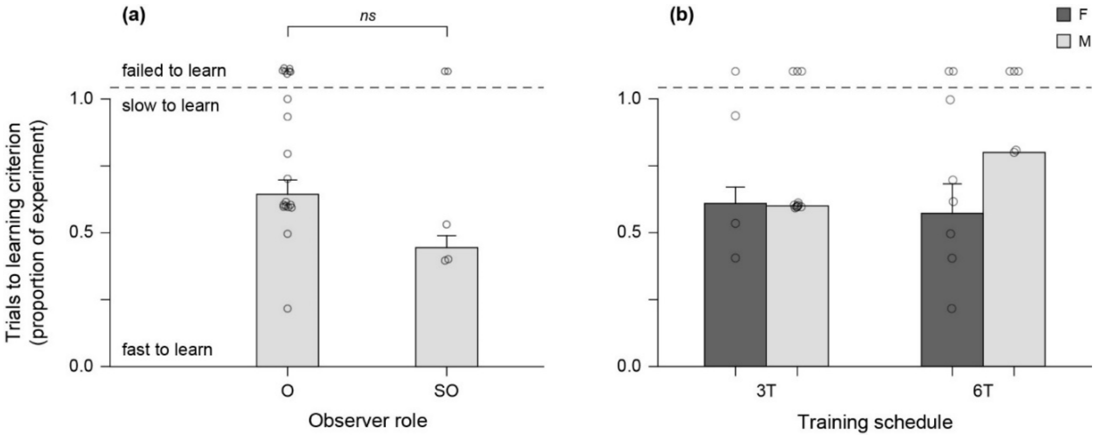

Figure 5. Number of exposure trials taken to reach learning criterion during the exposure phase (as a proportion of the total number of trials ran in the experiment), by **(a)** observer role (‘O’ or ‘SO’); and **(b)** training schedule (‘3T’ or ‘6T’; ‘F’, females, dark grey bars; ‘M’, males, light grey bars).

Considering only the sharks that reached learning criterion in the exposure phase, we found no differences in the proportion of null trials during the test phase of ‘O’ and ‘SO’ sharks (W = 48, $P = 0.918$). Sharks that failed the test phase showed high variation in proportion of null trials (Fig. 6), with a trend towards higher proportion of null trials (median = 33.5%) compared to those that learnt the task (median = 12.5%), though this difference was not significant (W = 48.5, *P* = 0.188). However, it is worth noting that some sharks that failed the test showed very low proportion of null trials, indicating they were responding to the task, but using both the ‘correct’ and the ‘wrong’ hole and thus failed learning criterion.

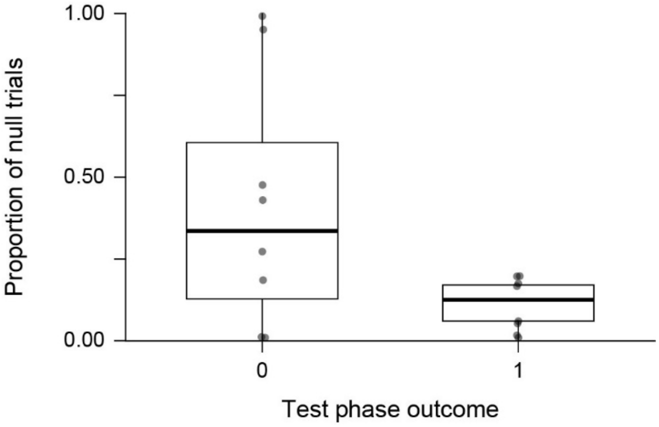

Figure 6. Proportion of test phase null trials of observer sharks that reached learning criterion during the exposure phase, grouped by their performance during the test phase (0: did not reach learning criterion during the test; 1: reached criterion). Each point represents an observer shark. Note that a low proportion of null trials means the shark was responding to the task most of the time, through either the correct or wrong hole.

Do training contingencies and behavioural traits influence learning ability?

While observer group (‘O’ or ‘SO’ sharks) did not help to explain differences in proportion of correct choice over all trials, we found that, when excluding null trials, observer sharks paired with trained demonstrators (‘O’) had a higher performance compared to ‘SO’ sharks (Fig. 7a; χ^2 = 5.098, d.f. = 1, *P* = 0.024). Day was also linked to proportion of correct choice, both including and excluding null trials, with sharks improving their choice performance over training days (all trials: t_{224} = 9.186, *P* < 0.001; excl. null trials: t_{197} = 2.146, *P* = 0.033).
Training schedule and sex also contributed to explain variation in proportion of correct choice over all trials. Sharks from training schedule ‘6T’ had lower performance than ‘3T’ sharks (Fig. 7b; χ^2 = 6.188, d.f. = 1, *P* = 0.013), and males

had slightly lower performance compared to females, but the difference was not statistically significant ($\chi^2 = 3.623$, d.f. = 1, $P = 0.057$).

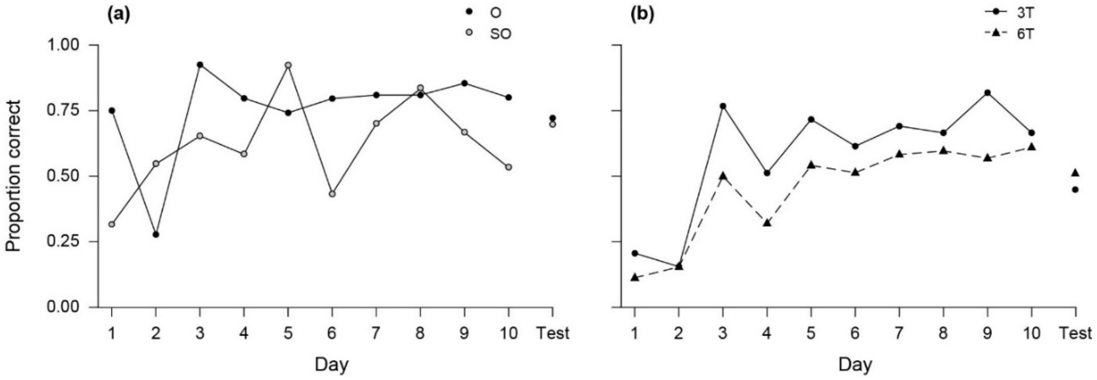

Figure 7. Proportion of correct choice of observer sharks during the exposure phase, and overall performance during the test phase (absence of demonstrator). Comparison between **(a)** observer (‘O’, black dots) and sham observer (‘SO’, grey dots) sharks, excluding null trials; and **(b)** training schedule ‘3T’ (dots, solid line) or ‘6T’ (triangles, dashed line), considering all trials.

The latency to swim through the partition (choice latency), as well as the latency of correct choice, was best explained by day and demonstrator class (Fig. 8a). The sharks showed faster responses over training days (all trials: $t_{224} = -12.073$, $P < 0.001$; correct trials: $t_{183} = -4.596$, $P < 0.001$), and observers paired with ‘poor’ demonstrators were faster in responding compared to observers paired with ‘good’ demonstrators (all trials: $\chi^2 = 5.423$, d.f. = 1, $P = 0.020$; correct trials: $\chi^2 = 4.122$, d.f. = 1, $P = 0.042$).

The latency of the first attempt to swim through the partition was also best explained by day and demonstrator class, with similar results (Fig. 8b). The sharks were faster to make an attempt over training days ($t_{138} = -3.606$, $P < 0.001$), and observers paired with ‘poor’ demonstrators made attempts earlier in the trial compared to observers paired with ‘good’ demonstrators ($\chi^2 = 4.561$, d.f. = 1, $P = 0.033$). Considering the ratio of correct to wrong attempts per trial, our final model had sex and reward value as predictor variables. We found that females and sharks with higher reward values had a higher ratio of correct to wrong attempts (Fig. 8c; sex: $\chi^2 = 4.223$, d.f. = 1, $P = 0.040$; reward value: $t_{22} = 2.871$, $P = 0.009$).

Behavioural syndrome, laterality scores, weight, and correct side were not linked to any of the trial statistics we assessed.

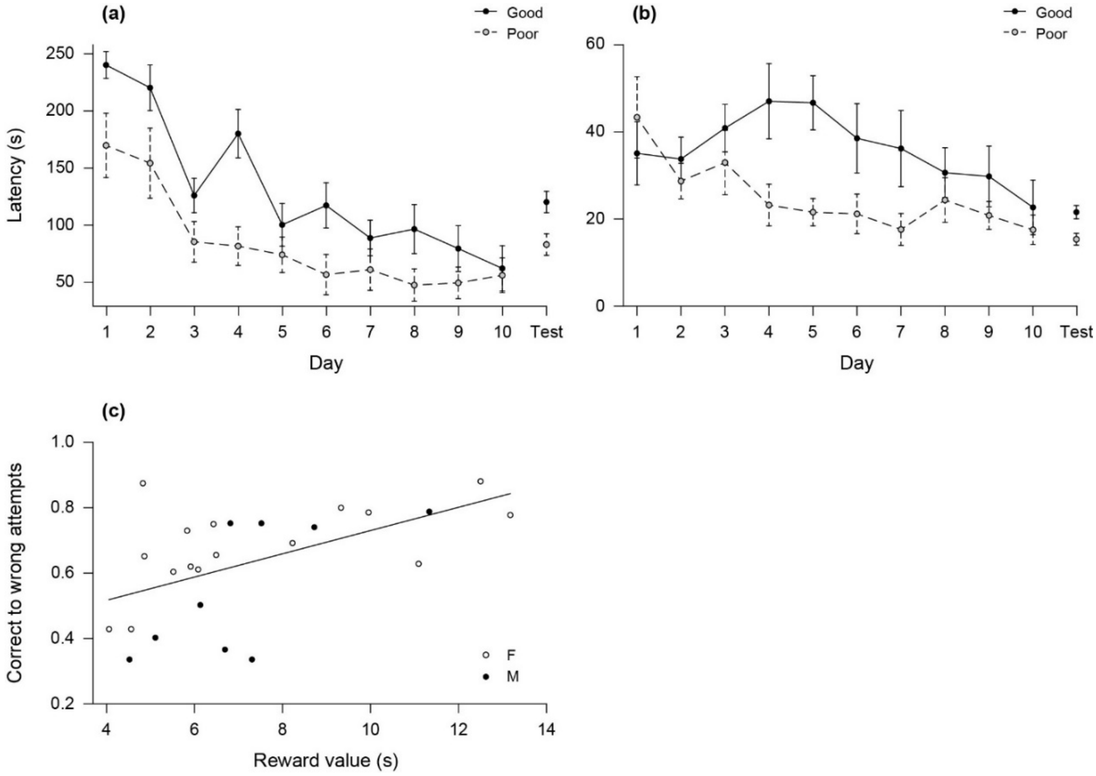

Figure 8. Comparison between observer sharks paired with ‘good’ (black dots, solid lines) or with ‘poor’ (grey dots, dashed lines) demonstrators, with regards to **(a)** latency to swim through the partition (mean ± SEM) and **(b)** latency to the first attempt to cross the partition (mean ± SEM). **(c)** Relationship between individual reward value (time fighting the aquarium tongs to retrieve the reward) and average ratio of correct to wrong attempts (F: females, white dots; M: males, black dots).

Discussion

During this study, naïve juvenile Port Jackson sharks were allowed to observe and interact with either ‘demonstrator’ sharks (trained to gain access to food through one of two arbitrary routes), or ‘sham demonstrators’ (no previous experience in the task), to investigate the use of social information in learning a new foraging task in this benthic species. We found that a similar proportion of observer sharks from the demonstrator group and the sham demonstrator group learnt the task (65% against 60%, respectively), and took approximately the same number of days to reach learning criterion, suggesting that social facilitation enhanced learning abilities in both group conditions.

Less than 40% of observer sharks in each group performed well when tested without a companion. These results suggest that the mere presence of a conspecific was sufficient to facilitate learning and shows some evidence of social information use. It is likely that sharks in pairs show higher individual search rates generated by social facilitation and/or competition (Grand and Dill, 1999; Ward, 2012), or through local enhancement processes, which in turn leads to an increased probability that at least one of the two individuals discovers the solution to the problem (here, crossing the partition). It is well established that many teleost fishes find food, or the route to a food source, and escape predators more efficiently in a group than alone (Ryer and Olla, 1992; Laland and Williams, 1997; Brown and Warburton, 1999; Brown and Laland, 2011; Webster and Laland, 2017). Even in non-group-forming species, individuals are likely to be exposed to, and act on, social cues, and might provisionally aggregate when exploiting resources. Juvenile Port Jackson sharks are usually seen in the wild solitary or in dyads, and occasionally in small, loose aggregations in sheltered locations (Powter and Gladstone, 2009). Therefore, these juveniles may exploit social information in addition to other cues to increase their chance of survival.

About half the sharks that failed the test session showed high response rates and low choice latency, indicating that these sharks learnt that they were required to cross the partition, but failed to identify the correct hole. An interpretation for this result is a release of social constraints in the absence of demonstrators. This ‘social release hypothesis’ postulates that animals will show conformity to social norms when in the presence of demonstrators, but relax their tendency to conform in the absence of clear demonstration (Brown and Laland, 2002b). Our casual observations during the trials suggest that, in most trials, demonstrators were drawing attention to the location of the hole, and thus providing a ‘tip-off’ to the observers; in fewer instances, observers would follow the demonstrator directly along the route.

Contrary to expectations, lower training frequency facilitated learning, with sharks from the ‘3T’ schedule taking fewer trials to reach learning criterion and showing an overall higher proportion of correct responses. Loss of motivation over trials might have affected individuals from the high intensity group, however the proportion of null trials was very similar between both treatments and daily food intake was proportionally the same for all individuals. An alternative explanation for this result is a ‘distributed-practice effect’ phenomenon. Learning techniques have been widely researched in educational psychology. Distributed practice, defined as a schedule of practice that spreads out study activities over time, is known to improve student success compared to massed practice (Delaney *et al.*, 2010; Dunlosky *et al.*, 2013). While this phenomenon is quite complex and relates mostly to consolidation of learning and memory retention (Dunlosky *et al.*, 2013), it suggests that ‘more isn’t always better’ regarding cognitive tasks. It is possible that most of the learning occurs between sessions (and to a lesser extent within sessions), thus having a smaller number of trials per day and a long interval of time spacing the session improves learning. It would be interesting to compare the accuracy in the first half vs. the last half of the same session, and the last half of the session with the first half of the next session and look for the higher increase in accuracy. Unfortunately, our small number of trials per day (3 or 6) does not allow for such comparison, but this seems to be the case in typical matching-to-sample tasks with pigeons (I. Fortes, T. Zentall, pers. comm.).

Even though the sharks we tested were juveniles, we found that males performed worse than females in multiple trial statistics; a result that was unanticipated. Sex differences in cognitive ability or cognitive style are usually related to ecological or physiological demands, and could result from sexual selection pressures (Jones *et al.*, 2003; Lucon-Xiccato and Bisazza, 2016). In this population, adult Port Jackson shark females have slightly larger home ranges compared to males (C. Brown, unpubl. data); however, both males and females show high levels of philopatry after a migration of hundreds of kilometres (Bass *et al.*, 2016) and gene flow in the population seems to be facilitated through male-biased dispersal, likely during juvenile stages (C. Brown, unpubl. data). Differences in metabolic rate, and thus motivational state, between juvenile males and females are also unlikely to explain the results found here (C. Gervais, unpubl. data). These differences between males and females requires further investigation.

Several studies from a wide range of taxa have shown that exposure to ‘low quality’ demonstrators improves learning in observers. For example, pigeons learnt discrimination tasks better, and zebra finches faster, when exposed to less proficient demonstrators (Biederman and Vanayan, 1988; Beauchamp and Kacelnik, 1991). Guppies paired with poorly trained demonstrators also showed a faster rate of increased performance compared to fish paired with well-trained demonstrators (Swaney *et al.*, 2001). Our results also showed that observers paired with ‘poor’ demonstrators were faster in engaging and responding to the task, but had similar learning abilities compared to observers paired with ‘good’ demonstrators. Our ‘good’ demonstrators were likely swimming through the hole too quickly to be followed, but still spent considerable time near the correct hole during the trial and made more correct crossings, and thus might have directed the observer’s attention to the correct hole through local enhancement processes, even if later in the trial.

In some trials, observer sharks would attack the aquarium tongs and take a long time to consume the reward; we hypothesised that a reward would be more salient if the shark spent some time ‘fighting’ the tongs. Observer sharks that took longer to retrieve the reward engaged more in the task, showing a higher ratio of correct to wrong attempts, but did not have better performance.

We also found no association between a proactive-reactive behavioural syndrome, or laterality, and social learning abilities in this species. Behavioural syndromes and cerebral laterality are tightly linked to ecological factors such as the presence of predators or food abundance (Rogers *et al.*, 2004; Réale *et al.*, 2010; Bisazza and Brown, 2011). It is possible that the traits we measured in our sharks, which were born in captivity, bear little ecological significance compared to wild juveniles (Archard and Braithwaite, 2010) and future experiments should assess if personality and laterality of captive-born sharks reflects the same traits observed in their wild counterparts.

In conclusion, our results suggest that juvenile Port Jackson sharks can use social information to learn a new foraging route to a food source. To our knowledge, only two other studies have investigated social learning in elasmobranchs – in lemon sharks and freshwater stingrays, two elasmobranchs with very different biological and ecological traits to Port Jackson sharks (Guttridge *et al.*, 2013; Thonhauser *et al.*, 2013). Vertical transmission of information can lead to cross-generational changes in behaviour that result in unique population culture (Helfman and Schultz, 1984; Laland and Williams, 1998; Fernö *et al.*, 2011; Rendell *et al.*, 2011), which means that populations may lose some flexibility to locally adapt to changes in the environment. This has significant implications for movement ecology, mating systems, and population vulnerability, especially in current days with increasing overexploitation of elasmobranch populations and dramatic climate change pressures. Further knowledge of what drives elasmobranch aggregations and social groups, and what role social learning and social information diffusion plays in their ecology and behaviour, will therefore prove an important avenue for future research.

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CHAPTER IV

Incubation under climate warming affects behavioural lateralisation in Port Jackson sharks

Incubation under climate warming affects behavioural lateralisation in Port Jackson sharks

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Abstract

Climate change is warming the world’s oceans at an unprecedented rate. Under predicted end-of-century temperatures, many teleosts show impaired development and altered critical behaviours, including behavioural lateralisation. Since laterality is an expression of brain functional asymmetries, changes in strength and direction of lateralisation suggest that rapid climate warming might impact brain development and function. However, despite its implications for cognitive functions, the potential effects of elevated temperature in lateralisation of elasmobranch fishes are unknown. We incubated and reared Port Jackson sharks at current and projected end-of-century temperatures (20.6 or 23.6 ºC) and measured preferential detour responses to left or right. Sharks incubated at elevated temperature showed stronger absolute laterality and were significantly biased towards the right relative to sharks reared at current temperature. We propose that animals reared under elevated temperatures might have more strongly lateralized brains to cope with deleterious effects of climate change on brain development and growth. However, far more research in elasmobranch lateralisation is needed before we can fully comprehend the significance of these results. This study provides further evidence that elasmobranchs are susceptible to the effects of future ocean warming, though behavioural mechanisms might allow animals to compensate some of the challenges imposed by climate change.

Keywords

Laterality; climate change; temperature; development; elasmobranchs

Introduction

Climate change has been identified as one of the major human-induced environmental changes to ecosystems worldwide (Collins *et al.*, 2013). The average temperature of the upper layers of the ocean has increased by 1.0°C over the past 120 years, and is predicted to increase by 1–3ºC in the next century if the current trajectory of greenhouse gas emissions is maintained (Collins *et al.*, 2013; Pörtner *et al.*, 2014). In addition, oceanic carbon dioxide (CO_2) levels have now reached historically high levels (Stocker *et al.*, 2013). Such rapid changes in important environmental parameters will have big impacts on marine ecosystems.

Elevated temperature and CO_2 levels in the ocean can significantly impair sensory functions and alter critical behaviours in teleost fish and elasmobranchs. For example, coral reef fish and benthic sharks exposed to elevated CO_2 levels showed impaired olfactory and auditory responses, important for predator/prey recognition and homing behaviour (Dixson *et al.*, 2010; Cripps *et al.*, 2011; Simpson *et al.*, 2011; Munday *et al.*, 2014; Rosa *et al.*, 2014; Pistevos *et al.*, 2015). Additionally, exposure to elevated temperatures resulted in higher developmental rate and metabolism as well as limited growth, aerobic scope, reproductive output, and foraging (Munday *et al.*, 2008; Nilsson *et al.*, 2009; Donelson *et al.*, 2010; Rosa *et al.*, 2014; Pistevos *et al.*, 2015). While highly mobile species will likely shift their distributions polewards (Perry *et al.*, 2005), less mobile species will have to cope through rapid evolution or phenotypic plasticity. Ectotherms are especially vulnerable to global warming because their body temperature and basic physiological functions are regulated by the external environment. In addition, many elasmobranch species are oviparous and have long gestation periods of several months, thus embryos will be exposed to prevailing environmental conditions and have little choice other than to adapt or die. One mechanism more sedentary species might use to compensate for the increase in developmental and metabolic rates is a reallocation of energy resources during development, which is expected to affect highly metabolically expensive systems such as neural development and processing (Soengas and Aldegunde, 2002; Sheridan and Bickford, 2011; Brown, 2012). Indeed, the detrimental effects in a range of sensory modalities and behaviours already observed in fish (e.g. Cripps *et al.*, 2011; Simpson *et al.*, 2011) suggest that predicted climate change conditions might disproportionately impact brain development and function.

Behavioural lateralisation, the tendency to favour the left or right side in a given context, results from a functional asymmetry between the two hemispheres of the brain (Rogers and Andrew, 2002; Vallortigara and Rogers, 2005; Rogers *et al.*, 2013; Vallortigara and Versace, 2017). Cerebral and behavioural lateralisation are widespread in the animal kingdom and have been well studied in teleost fish (Bisazza and Brown, 2011; Rogers *et al.*, 2013). Teleosts generally prefer to use the left eye/right hemisphere to process biologically relevant stimuli such as predators or potential mates and emotional responses such as fear and aggression, while the right eye/left hemisphere is generally linked to stimuli categorisation and object manipulation. Nonetheless, we often see species, population, or individual differences which arise through a mixture of genetic and experiential effects (Bisazza *et al.*, 2000; Bisazza and de Santi, 2003; Dadda and Bisazza, 2006b; Bibost and Brown, 2013).

Laterality in elasmobranchs is much understudied, with only two studies to our knowledge investigating behavioural lateralisation in benthic sharks (Green and Jutfelt, 2014; Byrnes *et al.*, 2016a). Byrnes *et al.* (2016a) observed individual levels of laterality bias in rotational swimming and T-maze turn preference in juvenile Port Jackson sharks, with females more strongly lateralised than males, and Green and Jutfelt (2014) reported a population-level laterality bias in double T-maze turn in small-spotted catsharks. Lateralisation of behaviour and cognitive functions has been suggested to offer selective advantages (Rogers *et al.*, 2004; Vallortigara and Rogers, 2005). For example, laterality enhances schooling behaviour, which can have important fitness-related implications in foraging and anti-predator behaviour (Krause *et al.*, 2000). Schools of lateralised fish were more cohesive and coordinated than schools of non-lateralised fish (Bisazza and Dadda, 2005), and individuals tended to take up positions in the school that correspond to their visual hemifield preferences for observing conspecifics (Bibost and Brown, 2013). A laterality bias might also provide them with advantages in multitasking situations such as foraging while being vigilant to predators and enhance cognitive efficiency (Rogers *et al.*, 2004; Sovrano *et al.*, 2005; Dadda and Bisazza, 2006a; Bibost and Brown, 2014).

Since behavioural lateralisation is an expression of brain function, it can be used as a barometer of normal brain development and function in some contexts, namely exposure or development under climate change conditions. Indeed, an increasing number of studies in recent years has reported an impact of increased CO_2

levels and elevated temperature in behavioural lateralisation in some teleost species, though with varying direction and magnitude (Domenici *et al.*, 2011; Jutfelt *et al.*, 2013; Domenici *et al.*, 2014; Lopes *et al.*, 2016; Sundin and Jutfelt, 2018). The behavioural effects of elevated CO_2 levels in teleosts seem to stem from a dysfunction of the GABA-A neurotransmitter receptor in the brain (Nilsson *et al.*, 2012). In elasmobranchs, only one study investigated the effects of future climate change conditions in behavioural lateralisation (Green and Jutfelt, 2014). Small-spotted catsharks aged 4-24 months old exposed to increased CO_2 for as little as four hours showed stronger absolute lateralisation at the population level when compared to control individuals (Green and Jutfelt, 2014). Such short-term responses are likely indicative of phenotypic plasticity and might mimic responses to brief environmental changes (e.g. day *vs.* night, intertidal zone conditions). The impacts of long-term exposure to elevated temperature on cerebral lateralisation, especially during critical developmental periods, have not been assessed in elasmobranchs yet. With so many reported consequences on development and physiology in elasmobranchs (Di Santo and Bennett, 2011; Rosa *et al.*, 2014; Pistevos *et al.*, 2015; Rosa *et al.*, 2016), it is likely that rapid climate warming might also impact brain function in this group. In the present study we test the hypothesis that predicted end-of-century temperature during embryogenic and hatchling development affects behavioural lateralisation in a benthic shark species.

Materials and methods

Ethics statement

Egg collection occurred under NSW Fisheries permit P08/0010-4.2. The experiments were approved by the Macquarie University Animal Ethics Committee (ARA 2016-027). All animals were euthanised at the end of the experiment with a lethal dose of MS-222 (tricaine methane-sulfonate; 1.5g/L seawater) for brain anatomy studies (to be reported in a separate paper).

We collected Port Jackson shark eggs via snorkelling from Jervis Bay, NSW. Females lay their eggs in shallow rocky reefs during late winter, mostly over August-September (McLaughlin and O'Gower, 1971). Freshly laid egg capsules are clean, soft, pliable and olive green in colour, but become brittle in two weeks and change to a dark brown colour in 3-5 weeks (Rodda and Seymour, 2008) enabling an estimation of laying date. Under ambient conditions, embryos have a long incubation period of 10 to 11 months (Rodda and Seymour, 2008). Eggs were collected on 11 October and 2 November 2016 and we estimate all had been laid recently (within 6 weeks of collection). Eggs were transported to Macquarie University, Sydney Australia, and held in a temperature-controlled laboratory until hatching. The eggs were placed in 40 L tanks containing natural filtered seawater and temperature was maintained using a custom-design Seawater Environmental Control Mixing Chamber. Following transport, eggs were left to rest for 7 days, then temperature was steadily increased by 0.5 °C/day to the elevated temperature treatment in half of the tanks. We randomly divided eggs among two treatments: a control temperature treatment (‘C’; n = 12) incubated at 20.6 ± 0.5°C, consistent with the annual average maximum temperature in Jervis Bay; and an elevated temperature treatment (‘ET’; n=12) incubated at 23.6 ± 0.5°C, representing an end of century projected sea-surface temperature increase under the representative concentration pathway (RCP) 8.5 climate model (Collins *et al.*, 2013). The CO_2 of the system reflected ambient conditions (ρCO_2 ~ 418 ppm).

When the egg capsules’ mucous plug opened, approximately four months into development, the embryos were removed from the egg and placed in individual containers within the housing tank for close monitoring.

Husbandry and rearing

Approximately one month after hatching (stage 15 (Rodda and Seymour, 2008); external yolk completely exhausted, internal yolk virtually depleted, and disappearance of slime coat), individuals were moved to the Sydney Institute of Marine Science (SIMS). Sharks were housed in groups of six animals in 1000L tanks maintained at incubation temperatures using submersible heaters (one 2000W titanium stick heater or four 300W AquaOne glass heaters). Tanks had continuous supply of fresh seawater pumped directly from Sydney harbour, aeration, and PVC structures and fake kelp to provide shelter and enrichment. Tanks were scrubbed clean at least once a week. The room had a natural light/dark cycle.

Immediately after hatching, Port Jackson sharks were weighed, measured (total length, TL), and individually tagged beneath the dorsal fin (Passive Integrated Nano-Transponder, Trovan® ID-100A/1.25). Sharks were fed *ad libitum* on a mixed diet of squid, cuttlefish, whitebait, and prawns three times per week.

Five sharks from the elevated temperature treatment did not survive the first month after hatching (three deaths and two were euthanased because they were not feeding). We therefore started the procedure with seven ‘ET’ and twelve ‘C’ sharks, 58.3 and 100 % of our initial sample size for each group respectively. The median age of the test subjects from the ‘ET’ group was 63.5 days post-hatching compared with 85.5 of the ‘C’ group.

Procedure

The experimental tank (180 × 100 × 40 cm, Fig. 1) was maintained at incubation temperatures using four to six 300W AquaOne glass heaters. For four days prior to the laterality assay, sharks were allowed to familiarise with the experimental tank to allow them to overcome any stress associated with moving between the housing and experimental tanks and being in a novel environment. During the familiarisation phase, the shark could swim freely in the tank for a 30-min period after which it was fed 2% of its body weight.

To assess behavioural laterality, sharks were tested individually in a detour test using a Y-maze (Bisazza *et al.*, 1997). The test consisted of 10 trials conducted on a single day. For each trial, the subject was ushered down a corridor and its turn direction at the end of the maze was recorded. Based on results from a pilot study, a small piece of food was placed behind the partition at the end of the corridor and individuals were fed at the end of the 5th and 10th trials to encourage directed swimming along the maze and ensure motivation in the task. After each run, the shark was temporarily constrained in the choice zone. The subject was then released and allowed to swim down the runway in the opposite direction. This approach reduces handling stress and reduces the possibility of extraneous cues inducing side biases. The shark was allowed 30 s to recover between runs.

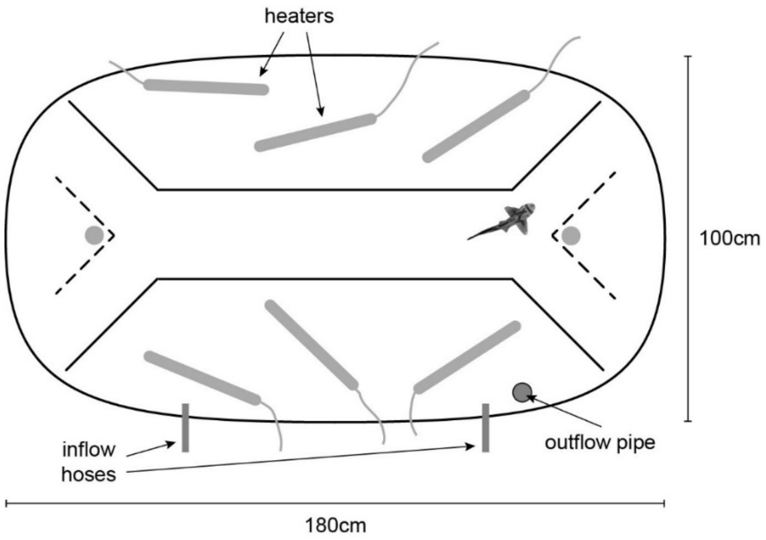

Figure 1. Diagram of the experimental tank.

Data analysis

We calculated 'laterality index' as follows: L_I = (number of right turns – number of left turns)/(total number of turns). L_I is a continuous value from -1 to 1, in which a positive score indicates a preference for rightward turns and a negative score indicates a preference for leftward turns. 'Laterality strength' (L_S) was calculated by taking the absolute value of L_I.

Statistical analyses were conducted in R v. 3.4.3 (R Core Team, 2017). We used non-parametric tests due to low sample sizes. Separate Mann–Whitney U tests were used to compare L_I and L_S scores between 'C' and 'ET' individuals, and to test if sharks within each group are significantly lateralised (distribution with $\mu \neq 0$).

Results

Sharks from the elevated temperature treatment ('ET') showed stronger absolute laterality (L_S) compared to control temperature ('C') sharks (Fig. 2a; $W = 19$, $P = 0.047$), along with higher laterality index (L_I) values (Fig. 2b; $W = 10.5$, $P = 0.0067$). 'ET' sharks displayed a significant rightward bias ($V = 28$, $P = 0.021$), while 'C' sharks showed no population-level preference of either side ($V = 12.5$, $P = 0.746$). Individual turn preferences are given in Table 1.

We examined the possible effect of age within the control group and found no relationship between age at testing and L_I (d.f. = 10, $t = - 0.06$, $P = 0.953$, $R^2 = 0.00036$).

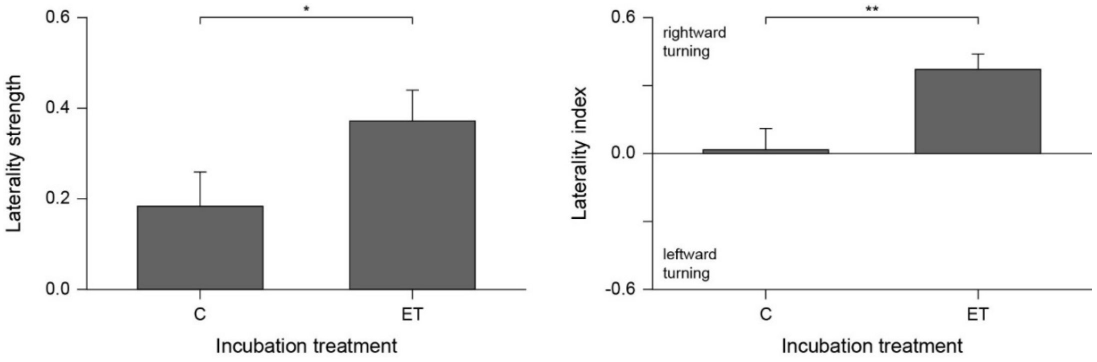

Figure 2. (a) Laterality strength (group mean ± SEM); and **(b)** laterality index (group mean ± SEM) in sharks incubated at control temperature (‘C’; n = 12) or elevated temperature (‘ET’; n = 7).

Table 1. Summary information on experimental subjects and individual left or right turn preference in the detour task.

Shark ID	Sex	Weight (g)	Treatment	# right turns	# left turns	L_I	L_S
C489	M	86	C	1	9	-0.8	0.8
C451	M	87	C	4	6	-0.2	0.2
C430	M	81	C	5	5	0	0
C437	M	70	C	5	5	0	0
C456	F	53	C	5	5	0	0
C469	M	101	C	5	5	0	0
C492	F	79	C	5	5	0	0
C500	F	89	C	5	5	0	0
C407	M	83	C	6	4	0.2	0.2
C452	F	76	C	6	4	0.2	0.2
C459	M	94	C	6	4	0.2	0.2
C460	F	95	C	8	2	0.6	0.6
ET455	M	64	ET	6	4	0.2	0.2
ET369	M	50.5	ET	6	4	0.2	0.2
ET373	F	59	ET	6	4	0.2	0.2
ET480	M	64.5	ET	7	3	0.4	0.4
ET400	F	78.5	ET	7	3	0.4	0.4
ET433	F	79	ET	8	2	0.6	0.6
ET468	F	62	ET	8	2	0.6	0.6

M, male; F, female; C, control temperature; ET, elevated temperature; L_I, Laterality index; L_S, Laterality strength.

Discussion

In this study we show that incubation temperatures predicted for the end of the century affect behavioural lateralisation in Port Jackson sharks. This is the first documented case of a change in lateralised behaviour induced by elevated temperature in any elasmobranch. Our hatchling sharks incubated and reared in elevated temperature showed stronger absolute laterality and a rightward bias compared to control individuals. Byrnes *et al.* (2016a) observed high individual variation in laterality in wild-caught juvenile Port Jackson sharks similar to our control group, suggesting the results from our sharks reared at current temperature in captivity reflect those in wild populations and are not influenced by captive rearing.

It is unclear if the shift in laterality to the right observed in the present study was due to plastic responses during development or the deaths of left biased or non-lateralised sharks during early ontogeny (42% of sharks reared in elevated temperatures died before testing). Behavioural lateralisation (in particular handedness) is linked to immune response in humans, rodents, and dogs (Neveu, 2002; Quaranta *et al.*, 2006; Siniscalchi *et al.*, 2010). It is possible immune responses might differ between our two groups. However, to our knowledge the link between immune competency and lateralisation has not been investigated in teleosts or elasmobranchs. Elevated temperature significantly increases developmental rates and metabolism (Rosa *et al.*, 2014; Pistevos *et al.*, 2015), with associated costs in terms of energy allocation to growth and physiological processes (e.g. Rosa *et al.*, 2016). It is therefore possible that stronger lateralisation arises as an energy saving mechanism. Functional asymmetries in the brain are thought to enable separate and parallel information processing in each hemisphere, which might increase the brain's capacity to carry out simultaneous processing resulting in enhanced cognitive efficiency (Rogers *et al.*, 2004; Bisazza and Brown, 2011). Neural processing is metabolically expensive, thus higher parallel processing abilities could allow animals to save energy during brain development and information processing without compromising function. We predict, therefore, that animals reared under elevated temperatures might have smaller but more strongly lateralized brains. Interestingly, juvenile small-spotted catsharks exposed short-term to elevated CO_2 levels also showed stronger absolute laterality in a detour task (Green and Jutfelt, 2014). Laterality can vary with age (Dharmaretnam and Andrew, 1994; Jozet-Alves *et al.*, 2012), but we examined the possible effect of age within the control group and found no correlation. It is worth noting that the variation in age within the control group was 35 days, which covers the average age difference between the control and elevated temperature treatments. Future research is needed to determine if laterality varies with age in sharks, perhaps over larger time frames. Regardless of the mechanism, it is apparent that climate change will impact elasmobranchs in many ways and that early developmental stages are particularly vulnerable, thus further work is required specifically in the context of brain development and cognition under future climate scenarios.

With so few studies investigating laterality in elasmobranchs, it is difficult to comment on the variability one might expect to see at the population or individual level, let alone context specific individual variation. Teleost fish show high variability in laterality strength and direction at the individual, population, and species level (Bisazza *et al.*, 1997; Bisazza *et al.*, 2000; Brown *et al.*, 2004). Additionally, laterality in teleosts is linked to life history traits and environmental variables (Bisazza *et al.*, 1998; Bibost *et al.*, 2013). Fish from high predation areas, for example, show stronger laterality than those from low predation areas and this has been linked to schooling behaviour in several species (Bisazza *et al.*, 2000; Brown *et al.*, 2004; Bisazza and Dadda, 2005; Bibost and Brown, 2013). To further muddy the waters, exposure to elevated temperature or CO_2 levels resulted in varying directions and magnitude of change in laterality in different teleosts (Domenici *et al.*, 2011; Jutfelt *et al.*, 2013; Domenici *et al.*, 2014; Lopes *et al.*, 2016; Sundin and Jutfelt, 2018). Some of these different effects might be due to the context of the task or a consequence of altered emotional states in the animal. For example, Domenici *et al.* (2014) observed a reversal from right-turning bias in damselfish detouring around an opaque barrier to a left-turning bias when exposed to elevated CO_2 levels. The authors suggest that elevated stress could induce this shift since stressed animals predominantly use the right hemisphere to control motor functions (Rogers, 2010; Domenici *et al.*, 2014). It is possible that this was also true in the present study but would assume that Port Jackson sharks predominantly use the left hemisphere to control motor function when under stress. Further studies are required to determine if this is the case.

To conclude, this study provides strong evidence that predicted end-of-century temperature affects behavioural lateralisation in sharks. It is likely that at combination of elevated CO_2 and temperature might have synergistic effects on laterality. We propose that enhanced lateralisation could help animals cope with deleterious effects of climate change on development and growth. Evidently, far more research is needed in multiple elasmobranch species to provide us with an adequate picture of brain lateralisation in elasmobranchs to test this hypothesis. Future studies should combine laterality and cognitive tasks to assess if cognitive functions in elasmobranchs are lateralised, and evaluate the effect of climate change conditions on cognitive performance.

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Author contributions

Conceptualisation: CVP, CG and CB; Methodology: CG, CVP and CB; Investigation: CG, CVP and JR; Analysis: CVP; Writing: CVP and CB.

Conflict of interest

The authors declare no conflict of interest.

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CHAPTER V

Quantity discrimination in sharks incubated under climate warming

Quantity discrimination in sharks incubated under climate warming

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Abstract

Climate change is driving natural systems toward conditions not seen for millions of years. Global warming can induce physiological and behavioural effects in terrestrial and marine organisms, especially during embryonic and juvenile development, which affect ecosystems on multiple levels. Changes in physiological traits and critical behaviours of apex and mesopredators can alter predation and risk effects on prey populations and lead to cascading effects through the ecosystem. However, research on the impacts of global warming in marine predatory species, especially regarding behavioural traits, is extremely scarce. To date, there is no information on the possible effects of elevated temperature during embryonic development on cognitive skills in elasmobranchs. In this study, Port Jackson shark (*Heterodontus portusjacksoni*) eggs were incubated and reared at current and projected end-of-century temperatures (20.6 and 23.6 ºC). We tested hatchlings’ learning ability with a quantity discrimination task and assessed laterality levels using a detour task. Here we show, for the first time, that sharks can discriminate between two quantities. We recorded 41.7 % mortality in the elevated temperature group against 0 % mortality in the control group. Against expectations, we found that hatchlings that survived incubation at elevated temperature participated in the task at a higher rate and performed better compared to sharks incubated at current-day temperatures. In addition, we observed that individuals with stronger rightward bias overall reached learning criterion faster. Our results suggest that learning and behaviour might may play a role in allowing elasmobranchs to overcome some of the deleterious effects of global warming.

Keywords

Climate change; elasmobranchs; numerical abilities; animal cognition; cerebral lateralisation

Introduction

Climate change is driving natural systems towards conditions not seen for millions of years. Global average air temperatures are predicted to increase by 2–5ºC and global sea surface temperatures by 1–3ºC in the next century under ‘business-as-usual’ scenarios (Collins *et al.*, 2013; Pörtner *et al.*, 2014). In addition, climate change is not being felt equally around the world. Some ‘hotspot’ regions where warming is most rapid, such as south-east Australia, will be among those to experience impacts first (Hobday and Pecl, 2014). Global warming has already impacted multiple terrestrial and marine organisms around the globe, causing shifts in abundance, distribution, and phenotypic changes, among others (Parmesan, 2006).

Ectothermic animals are especially vulnerable to global warming because their body temperature and basic physiological functions are regulated by the external environment. Exposure to elevated temperatures impacts many morphological and physiological traits, with the most obvious influences on variables such as metabolic rate, growth, and locomotor performance (Cano and Nicieza, 2006; Calosi *et al.*, 2008; Munday *et al.*, 2008). Thermal stress during embryogenic development is particularly harmful, and likely causes significant and long-lasting effects in brain development and cognitive performance (Jonson *et al.*, 1976; Wang *et al.*, 2007; Dayananda and Webb, 2017). For example, geckos incubated in future (warmer) nest temperatures took longer to locate a shelter and made more mistakes compared to hatchlings from current-day temperatures (Dayananda and Webb, 2017). In addition, individuals with lower learning scores had lower survival rates after being released into the wild, suggesting that hampered cognitive performance influenced fitness and survival (Dayananda and Webb, 2017). Teleost fish reared in future warming conditions also showed restricted growth and reproductive output, decreased antipredator and foraging behaviour, and modified activity and group social structure (Munday *et al.*, 2008; Nilsson *et al.*, 2009; Donelson *et al.*, 2010; Nowicki *et al.*, 2012; Colchen *et al.*, 2017).

Changes in growth, metabolic demands, density, and foraging behaviour of predatory species are likely to have cascading effects through entire ecosystems (Estes *et al.*, 2011). Apex and mesopredators shape ecosystem structure and function by inflicting mortality or inducing costly antipredator behaviour in their prey (Heithaus *et al.*, 2008). Because of their important ecological influence, it is vital to understand how predatory species will be affected by global warming from a physiological and behavioural perspective. However, research in marine apex and mesopredators in the context of climate change is still very scarce.

A few recent studies have reported changes in physiology and behaviour of elasmobranchs reared under predicted end-of-century temperature. Bamboo sharks incubated under elevated temperature showed lower survival rates, decreased body condition, higher metabolic and growth rate, and decreased digestive capacity (Rosa *et al.*, 2014; Rosa *et al.*, 2016). Epaulette sharks had lower survival and abnormal coloration and patterns (Gervais *et al.*, 2016). Elevated temperature also increased the rate of embryonic development, food consumption and growth rate in Port Jackson sharks (Pistevos *et al.*, 2015). In addition, little skates reared under simulated ocean warming showed lower aerobic performance and scope and decreased escape responses (Di Santo and Bennett, 2011; Di Santo, 2016). With such consequences on development, physiology and behaviour, it is likely that rapid climate change might also impact cognitive skills in elasmobranchs. While learning and behaviour may play a leading role in allowing individuals to adapt to the rapidly changing environmental conditions (Brown, 2012; Wong and Candolin, 2015), there is a tremendous gap in empirical studies testing the impacts of climate warming in the learning abilities of marine predators.

The capacity to make relative quantity judgements is one among the many learning abilities animals evolved to deal with the ecological and social challenges they face (Geary *et al.*, 2014). Choosing to forage in a patch with the larger number of items or less competitors can improve foraging efficiency, and joining a larger social group can reduce sexual harassment or predation risk (Hager and Helfman, 1991; Boysen *et al.*, 2001; Agrillo *et al.*, 2007; Panteleeva *et al.*, 2013). The capacity to discriminate numerosities is already present at birth in vertebrates (Cantrell and Smith, 2013) and seems to be linked in part to cerebral lateralisation (Kilian *et al.*, 2005; Dadda *et al.*, 2015). Numerical abilities have been observed in a wide range of species, from mammals (Boysen *et al.*, 2001; Ward and Smuts, 2007), birds (Hunt *et al.*, 2008; Rugani *et al.*, 2013), reptiles (Petrazzini *et al.*, 2017), amphibians (Krusche *et al.*, 2010), teleost fish (Agrillo *et al.*, 2014), and some invertebrates (Chittka and Geiger, 1995; Carazo *et al.*, 2009), but remain to be tested in elasmobranchs. From an evolutionary perspective, it is likely that the selective pressures driving this cognitive ability in all these animal groups was also present for elasmobranchs, especially considering they seem to share a basic cognitive toolbox with other vertebrates (Schluessel, 2015).

The partitioning of information processing in either hemisphere of the brain has been argued to enhance processing capability, and thus increase cognitive efficiency (Rogers *et al.*, 2004; Magat and Brown, 2009; Bisazza and Brown, 2011). For example, guppies with more strongly lateralized brains are better at numerical discrimination (Dadda *et al.*, 2015). On par with other vertebrates, sharks also show individual lateralisation levels (Green and Jutfelt, 2014; Byrnes *et al.*, 2016a; Byrnes *et al.*, 2016b). In addition, one study has found that just four hours of exposure to elevated carbon dioxide levels increased lateralisation strength in juvenile small-spotted catsharks (Green and Jutfelt, 2014). It seems likely, therefore, that there may be a link between rearing temperature, laterality and cognitive performance.

Species that are unable to shift their distribution range will be particularly at risk with rapidly rising temperatures (Root *et al.*, 2003; Calosi *et al.*, 2008), and might have to rely on phenotypic plasticity and behavioural mechanisms to adjust to these conditions. The Port Jackson shark (*Heterodontus portusjacksoni*) is an epibenthic oviparous species widely abundant in temperate Australian waters (Last and Stevens, 2009). Port Jackson sharks are an important mesopredator (Powter *et al.*, 2010) and might play a role in regulating coastal reef environments. Females lay their eggs on shallow rocky crevices and under ambient conditions embryos have an incubation period of 10 to 11 months (Rodda and Seymour, 2008). On the east coast of Australia, Port Jackson sharks undertake a long-distance migration every year from potential foraging areas to their breeding reef, and show extremely high site fidelity to their breeding grounds within and between years (Bass *et al.*, 2016). High site fidelity might result in reduced range dispersion ability in face of rapidly changing conditions. Moreover, with such a long incubation period, Port Jackson shark embryos will be exposed to prevailing environmental conditions and have little choice to adapt or die. These two factors combined mean that Port Jackson shark populations will be susceptible to global warming and their decimation or removal is likely to cause trophic cascades in the ecosystem. Since Port Jackson sharks are reasonably small and do well in captivity, they are a suitable species to test the effect of climate warming on behaviour and cognition. In this study we tested the hypothesis that juvenile *H. portusjacksoni* can discriminate between two quantities, and predicted that elevated temperature during embryonic development impairs learning ability. In addition, we examined the proposed cognitive benefits of laterality by investigating the link between cerebral lateralisation and numerical abilities.

Methods

Egg collection and incubation

We collected Port Jackson shark eggs via snorkelling from Jervis Bay, NSW. Females lay their eggs in shallow rocky reefs during late winter, mostly over August-September (McLaughlin and O'Gower, 1971). Freshly laid egg capsules are clean, soft, pliable and olive green in colour, but become brittle in two weeks and change to a dark brown colour in 3-5 weeks (Rodda and Seymour, 2008) enabling an estimation of laying date. Under ambient conditions, embryos have a long incubation period of 10 to 11 months (Rodda and Seymour, 2008). Eggs were collected on 11 October and 2 November 2016 and we estimate all had been laid within 6 weeks of collection. Eggs were transported to Macquarie University, Sydney Australia, and held in a temperature-controlled laboratory until hatching. The eggs were placed in 40 L tanks containing natural filtered seawater, and temperature was maintained using a custom-design seawater environmental control mixing chamber. Following transport, eggs were left to rest for 7 days, then temperature was steadily increased by 0.5 °C per day to the elevated temperature treatment in half of the tanks. We randomly divided eggs among two treatments: a control temperature treatment (‘C’; n = 12) incubated at 20.6 ± 0.5°C, consistent with the annual average maximum temperature in Jervis Bay; and an elevated temperature treatment (‘ET’; n=12) incubated at 23.6 ± 0.5°C, representing an end of century projected sea-surface temperature increase under the representative concentration pathway (RCP) 8.5 climate model (Collins *et al.*, 2013).

When the egg capsules’ mucous plug opened, approximately four months into development, the embryos were removed from the egg and placed in individual containers within the housing tank for close monitoring.

Husbandry and rearing

Individuals were moved to the Sydney Institute of Marine Science (SIMS) one month after hatching (stage 15, external yolk completely exhausted, internal yolk virtually depleted, and disappearance of slime coat; Rodda and Seymour, 2008). Sharks were maintained at incubation temperatures throughout the whole experiment. Individuals were housed in groups of six animals in 1000L tanks and temperature was maintained using submersible heaters (one 2000W titanium stick heater or four 300W AquaOne glass heaters). Tanks had continuous supply of fresh seawater pumped directly from Sydney harbour, aeration, and PVC structures and fake kelp to provide shelter and enrichment. Tanks were scrubbed clean at least once a week. The room had a natural light/dark cycle.

Once sharks reached developmental stage 15, they were weighed, measured (total length, TL), and individually tagged beneath the dorsal fin (Passive Integrated Nano-Transponder, Trovan® ID-100A/1.25). Sharks were fed *ad libitum* on a mixed diet of squid, cuttlefish, whitebait, and prawns three times per week.

Five sharks from the ‘elevated temperature’ treatment did not survive the first month after reaching developmental stage 15 (three deaths and two euthanized because they were not feeding). We therefore started the procedure with seven ‘ET’ and twelve ‘C’ sharks, 58.3 and 100 % of our initial sample size for each group respectively.

Experimental apparatus

The experimental tank (180 × 100 × 40 cm, Fig. 1a) was maintained at incubation temperatures using four to six 300W AquaOne glass heaters. The tank contained an opaque, enclosed start box (40 × 20 × 40 cm) at one end, with a sliding door facing the middle of the tank, and a black wall at the opposite end. Water inflow was provided from both sides of the tank and water outflow was located on the left side of the start box area. Stimuli were printed within a 9 × 9 cm white square and were displayed on either side of the black wall, adjacent to the bottom of the tank (since PJ sharks usually swim close to the ground; Fig. 1b). The daily food intake per individual was equivalent to 2% of its wet body weight in squid (*Loligo opalescens*) pieces.

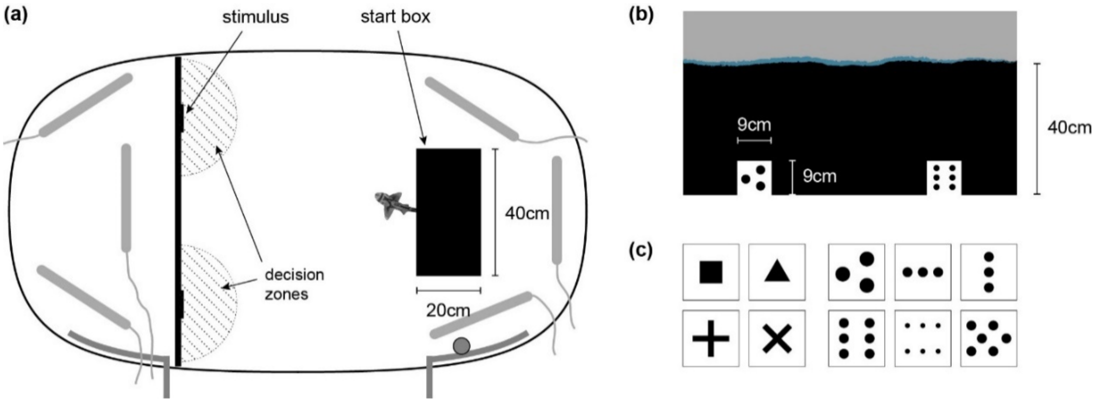

Figure 1. (a) Diagram of the experimental tank during the quantity discrimination phase. **(b)** Stimuli were displayed on either side of a black wall opposite the start box, adjacent to the bottom of the tank. **(c)** Set of stimuli used during the familiarisation and quantity discrimination phases.

Procedure

Familiarisation

Familiarisation took place four days preceding the laterality assay, and was set up to allow the shark to overcome any stress associated with moving tanks, and become familiar with how stimulus and reward would be presented during the quantity discrimination task. During the familiarisation phase, the shark could swim freely in the tank for a 30-min period. We presented them with two ‘sham stimuli’ – a black geometric shape in white background, randomly selected from a set of four (square, triangle, cross, or x mark; Fig. 1c). After 30 min elapsed, the shark was fed with long aquarium tongs within a 20-cm radius of either option (‘decision zone’, Fig. 1a).

Laterality assay

Sharks were tested individually in a detour test using a Y-maze (see Chapter IV for details). Briefly, each shark was moved from the housing tank to the experimental tank and the test consisted of 10 trials conducted in a single day. For each trial, the subject was ushered down a corridor and its turn direction at the end of the maze was recorded. A small piece of food was placed behind a partition at the end of the corridor and individuals were fed at the end of the 5th trial to encourage directed swimming along the maze and ensure motivation in the task.

Quantity discrimination (3 vs. 6)

Training sessions started the day following the laterality test and were conducted once a day, always at the same time. All sharks were trained with the same numerical contrast: 3 versus 6. For half of the individuals in each treatment group, the smaller numerosity (3) was chosen as the positive stimulus, and for the other half the larger numerosity (6) was chosen as the positive stimulus. To avoid or minimise correct identification of the positive stimulus based on pattern recognition, different stimulus pairs (black circles on a white background with variable arrangement and/or size) were shown on each trial, pseudo-randomly chosen from a set of three options *per* numerosity (Fig. 1c). To decrease the difficulty of the task, we did not control for ‘continuous quantities’ (non-numerical cues such as cumulative surface area, sum of perimeter of the figures, overall space occupied by the array, or luminance); therefore, the sharks could solve the task by using numerical and/or quantity information. The position of the stimuli (left–right) was counterbalanced over trials, with each numerosity never shown more than twice consecutively on the same side. Each session consisted of 5 min of acclimation in the experimental tank, followed by six training trials. Before each trial, the shark was gently moved into the start box. After 30 s elapsed, the door was opened and the trial began once the shark emerged. On days 1-3, sharks were rewarded upon entering the positive decision zone (head and pectoral fins over demarcation line), and approaches to the negative stimulus were disregarded. On days 4-6, sharks were only rewarded when they pressed their nose against the positive stimulus, and approaches to the negative stimulus were disregarded. From day 7, sharks were only rewarded when they pressed their nose against the positive stimulus, and nose contact with the negative stimulus was recorded as an incorrect choice. The trial would end when the shark pushed against one of the two stimuli, or 90 s elapsed. For each trial, we recorded the sharks’ choice and latency to push against the stimulus, latency to eat the food reward, and cumulative time spent in both correct and incorrect decision zones. The shark could receive a maximum of six rewards per session totalling 1% of its body weight. After the last trial, the shark was given a random interval of 3–6 min to settle, and then fed the remainder of the food allocated for the day in random locations within the experimental tank.

Data analysis

Trials were video recorded and trial statistics were collected by two observers using BORIS v. 2.62 (Friard and Gamba, 2016). Statistical analyses were conducted in R v. 3.4.3 (R Core Team, 2017). Prior to conducting analyses, we explored the data following Zuur *et al.* (2009), and assumptions of normality of residuals and homogeneity of variance were always verified.

Laterality assay

We calculated 'laterality index' as follows: L_I = (number of right turns – number of left turns)/(total number of turns). L_I is a continuous value from -1 to 1, in which a positive score indicates a preference for rightward turns and a negative score indicates a preference for leftward turns. 'Laterality strength' (L_S) was calculated by taking the absolute value of L_I. Separate Mann–Whitney U tests were used to compare L_I scores between 'C' and 'ET' individuals, and to test if sharks within each group are significantly lateralised (distribution with $\mu \neq 0$). Individual L_I and L_S scores were used in the analysis of learning performance.

Quantity discrimination (3 vs. 6)

Lack of motivation was apparent in some trials, in which the sharks did not press their nose against one of the stimuli (hereafter referred to as null trials). Separate Mann–Whitney U tests were used to compare the overall proportion of null trials of 'C' and 'ET' sharks and of sharks that learnt or did not learn the task.

We considered that a shark was successful during training if it made a correct choice in 9 out of 12 consecutive trials. If a shark did not reach learning criterion after 35 days it was excluded from the experiment. We assigned those individuals a ceiling value of 70 days (double the maximum allowed number of days) to the number of trials taken to learn the task.

To assess performance during training, we separately performed Gaussian linear mixed-effects models (LMM) with restricted maximum likelihood (REML) estimation for each of the following response variables: (1) number of days to learn the task; (2) proportion of correct choice; (3) proportion of correct choice excluding null trials; (4) latency of choice; (5) latency of correct choice; (6) latency to retrieve food reward; and (7) ratio of trial time spent in correct vs. incorrect decision zone. To improve model fit, response variables 2 and 3 were logit transformed, and variables

1, 4, 5, and 6 were log transformed. Potential predictor variables included incubation treatment (categorical with two levels, ‘C’ and ‘ET’), positive stimulus (‘3’ and ‘6’), training day (continuous), weight (continuous), sex (‘M’ and ‘F’), L_I (continuous), and L_S (continuous). Individual ID was included as a random factor. Due to a small sample size within groups, we used an information-theoretic approach to select potential predictor variables that might have influenced each response variable. The coefficient of determination (R^2) was used to assess goodness-of-fit and estimate the amount of variance explained by the models following Nakagawa and Schielzeth (2013), using the package *piecewiseSEM* (Lefcheck, 2016).

To investigate if individuals that failed to reach learning criterion developed a side bias, and if side choice and outcome in one trial would influence side choice on the following trial, we estimated Discrete Time Markov Chain (DTMC) transition probability matrices between trials (t - 1) and t for each individual shark (package *markovchain*, Spedicato *et al.*, 2016). Transition matrices were computed excluding days 1-6 (when incorrect choices were not scored). Confidence intervals of individual transition matrices should be considered cautiously due to low raw counts of transition steps.

Ethics statement

Egg collection occurred under NSW Fisheries permit P08/0010-4.2. The experiments were approved by the Macquarie University Animal Ethics Committee (ARA 2016-027). All animals were euthanised at the end of the experiment with a lethal dose of MS-222 (tricaine methane-sulfonate; 1.5g/L seawater) for brain anatomy studies.

Results

Five sharks were excluded from the quantity discrimination task: two ‘ET’ individuals did not acclimatise and were not eating in the experimental setup and three ‘C’ individuals a did not participate in the experiment. Nine ‘C sharks and five ‘ET’ sharks remained in the experiment. Of these, ‘ET’ sharks participated in the task more often than ‘C’ sharks (Fig. 2; W = 80, $P < 0.001$). We found no differences in the proportion of null trials between sharks that learnt or did not learn the task (W = 23, $P = 0.179$).

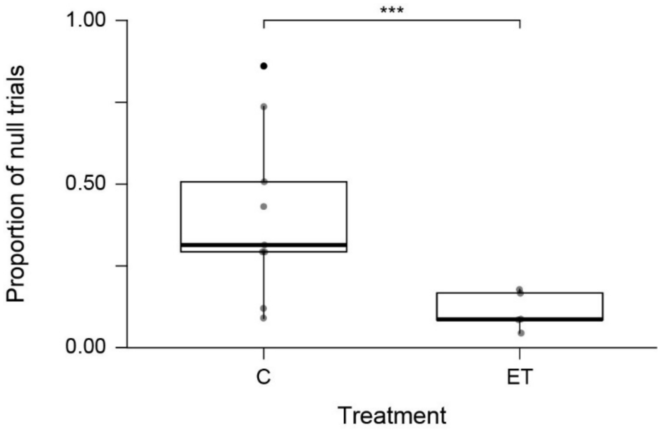

Figure 2. Proportion of null trials of ‘C’ (n = 9) and ‘ET’ (n = 5) individuals over all training sessions.

Learning outcome

Three out of nine (33.3%) ‘C’ sharks and three out of five (60%) ‘ET’ sharks reached learning criterion. All three ‘C’ sharks were trained to select the larger numerosity (6) and all three ‘ET’ sharks were trained to select the smaller numerosity (3). For this reason, incubation treatment and positive stimulus were confounded; however, positive stimulus was not linked to the proportion of successful sharks or the number of days to reach learning criterion and thus seems to have been less influential than incubation treatment. We therefore used incubation treatment alone when analysing performance of successful sharks, but are aware that we cannot fully disentangle the effects of each variable individually. Of the sharks that failed learning criterion, a similar proportion had been trained to ‘3’ and ‘6’.

The number of days to learn the task was best explained by incubation treatment, L_I scores, and individual ID. In contrast to our prediction, ‘ET’ sharks took significantly fewer days to reach learning criterion (12.7 ± 1.3 days) compared to ‘C’ sharks (31.0 ± 2.7 days; $\chi^2 = 7.871$, d.f. = 1, $P = 0.005$; Fig. 3), and sharks with higher rightward bias took slightly fewer days to learn, but the difference was not statistically significant even though it was an important parameter in the best-fit model ($t_{10} = 1.432$, $P = 0.183$). The predictor variables accounted for 41.2% of the variance in number of days to reach criterion, and individual ID for 51.5% of the variance.

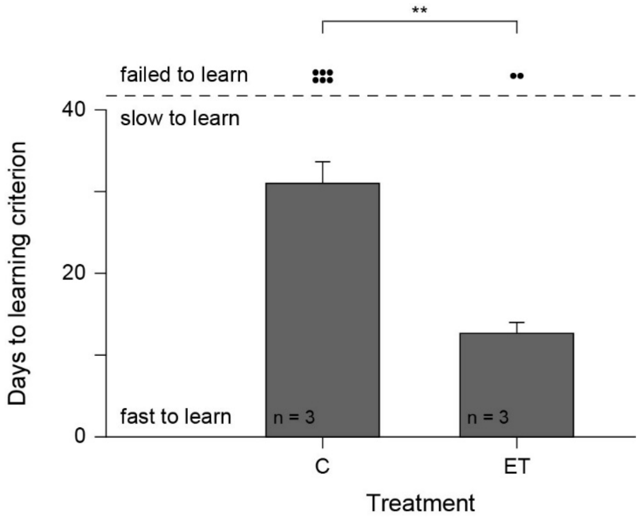

Figure 3. Number of days (mean ± SEM) taken to reach learning criterion by incubation treatment. Bar plot shows average over the three individuals that learnt the task per group, out of nine in 'C' and out of five in 'ET'.

Correct choice performance

'ET' sharks had a higher proportion of correct choice over all trials compared to 'C' sharks (Fig. 4a; $\chi^2 = 29.723$, d.f. = 1, $P < 0.001$), and sharks improved their choice performance over training days ($t_{88} = 7.682$, $P < 0.001$). Most of the variance in proportion of correct choice explained by the model was accounted for by the predictor variables (43.5%), with individual ID explaining only 5.6% of the variance. However, when we excluded null trials, incubation treatment was not linked to the proportion of correct choices, nor any other variable ($P > 0.05$ in all cases). This indicates that the effect in proportion of correct choices over all trials was driven by the low participation of 'C' in the task (Fig. 2), mostly in the initial days. This result suggests the sharks did not show a significant positive trend in the proportion of correct choices (excl. null trials) over training days (Fig. 4b). Visual inspection of individual data indicates that most animals had a stable, random performance most of the training days, followed by a steep increase in performance over two/three days prior to reach learning criterion.

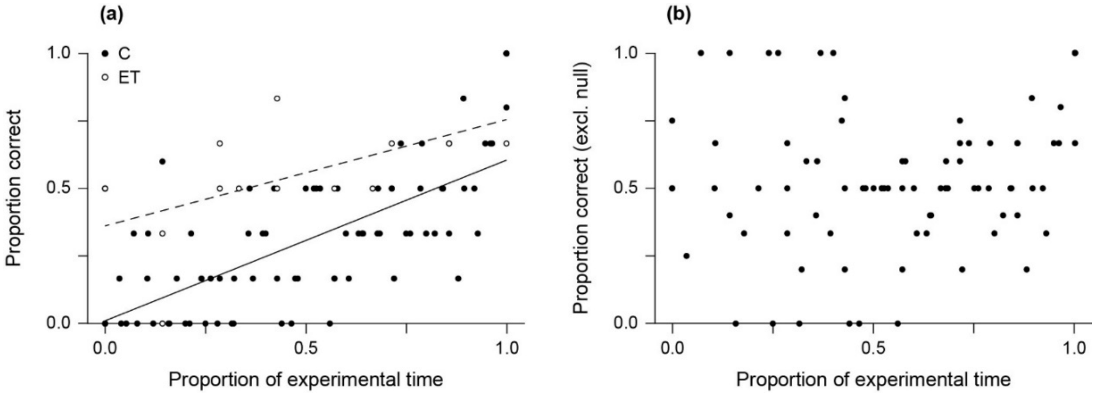

Figure 4. Proportion of correct choices **(a)** over all trials, for ‘C’ (full dots) and ‘ET’ (open dots) individuals; and **(b)** excluding null trials, for individuals of both treatments.

Choice latency

We found no differences between treatments in latency to push against one of the two stimuli (choice latency) or in latency to push against the correct stimulus (correct choice latency). Choice latency was best explained by day and sex (Fig. 5a), while correct choice latency was best explained by day alone with a marginally non-significant effect (Fig. 5b). The sharks became faster in responding over training days (all trials: $t_{75} = -4.563$, $P < 0.001$; correct trials: $t_{69} = -1.984$, $P = 0.051$), showing that individuals learnt that they had to push against a stimulus displayed on the wall, and females were faster in responding compared to males (all trials: $\chi^2 = 6.161$, d.f. = 1, $P = 0.013$). This difference between males and females was driven by choice latency in incorrect trials: females had low choice latency overall regardless of choice, while males showed high latencies associated with incorrect, but not correct, choices.

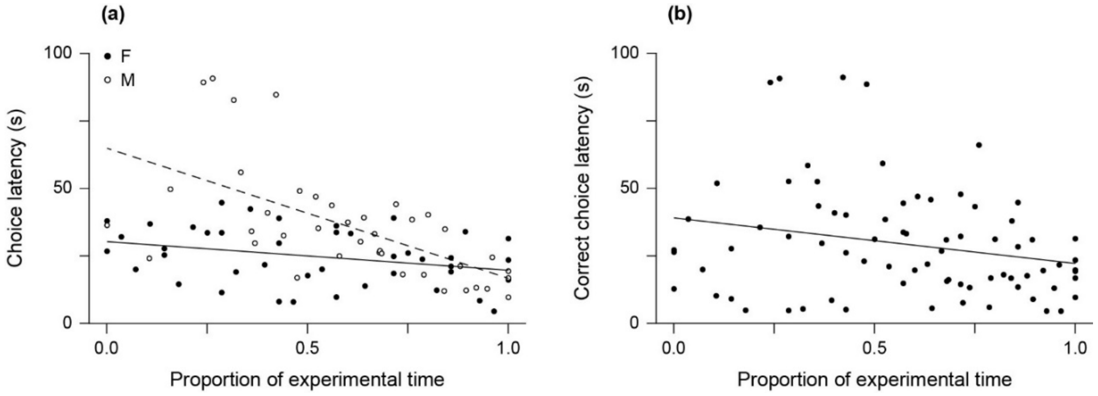

Figure 5. Latency of **(a)** all choices by sex (females, black dots; males, open dots); and **(b)** of correct choices only for all individuals.

Reward latency

The latency to retrieve the food reward also did not differ between treatments and was best explained by training day alone, with sharks becoming faster in retrieving the reward over time ($t_{75} = -2.108$, $P = 0.039$). This result further supports that the sharks learnt the contingencies of the task.

Trial time in decision zones

Considering all experimental individuals (including sharks that did not reach learning criterion), sharks from the 'ET' group had a higher ratio of trial time near the correct vs. incorrect decision zone compared to 'C' sharks (Fig. 6a; W = 352.5, $P = 0.024$). In both treatment groups, the ratio of trial time near the correct vs. incorrect decision zone was correlated with the proportion of correct choices (Fig. 6b; linear model: $t_1 = 11.231$, $P < 0.001$, $R^2 = 0.502$), suggesting that time spent near the rewarded target is a good proxy for successfully completing the trial, but does not require the sharks to touch the card.

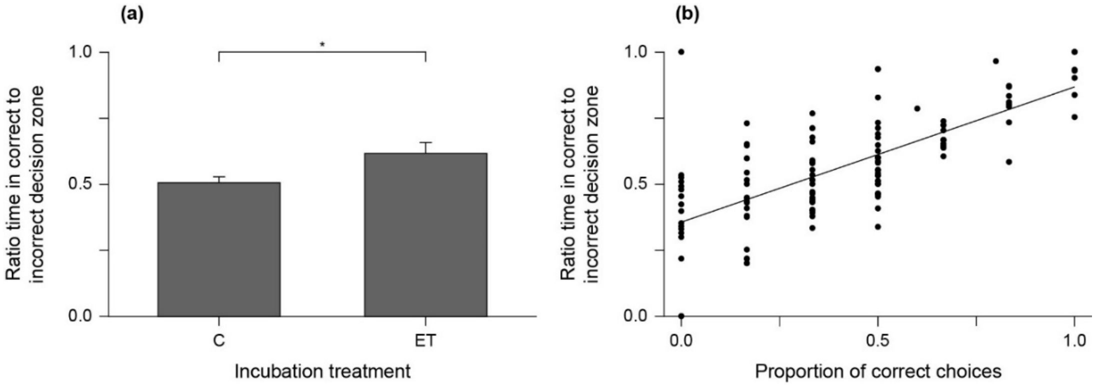

Figure 6. Ratio of trial time in the correct vs. the incorrect decision zones **(a)** for control temperature (‘C’; n = 12) and elevated temperature (‘ET’; n = 7) individuals (group mean ± SEM); and **(b)** as a function of proportion of correct choices.

Side bias

Choice/outcome DTMC transition probabilities for seven sharks that failed to learn the task show an overall bias to choose the stimulus on the right (Fig. 7; shark C407 was not included since it made very few consecutive choices). Individual variation in choice strategy was also apparent: ET433 and C430 both had a higher probability of choosing right after a right-side choice and of choosing left after a left-side choice; ET455 and C460 had an overall bias to choose right, but C460 chose left more often after gaining a reward on the left and ET455 after missing a reward on the left; and C456 tended to alternate to the right after choosing left and choose both sides randomly after a right-side choice.

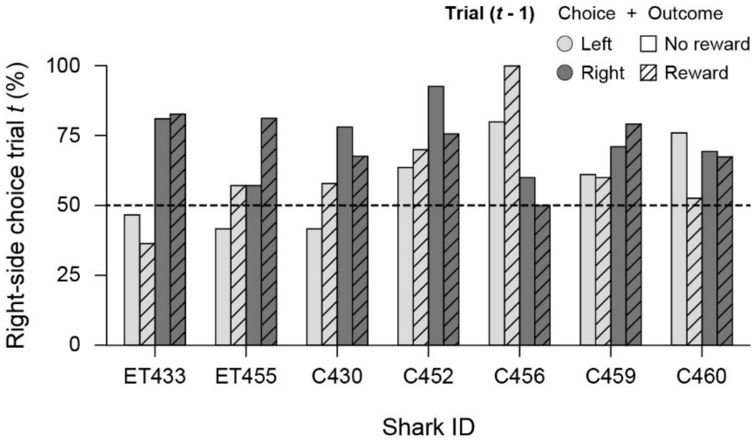

Figure 7. Choice/outcome DTMC transition probabilities for seven sharks that failed to learn the task (shark C407 was not included since it made very few consecutive choices).

Discussion

In this study we show that sharks can discriminate between two quantities, and found that individuals that survived incubation at predicted end-of-century temperature performed better compared to sharks incubated at current-day conditions. In addition, there was an indication that individuals with stronger rightward bias reached learning criterion faster.

All fourteen sharks, regardless of treatment temperature, showed a decrease in latency to choose one of the stimuli and latency to retrieve the reward, and six individuals reached learning criterion within 30 training days. This study provides the first evidence of quantitative abilities in elasmobranchs, the only vertebrate group not investigated to date. While the result shows that the species is likely equipped with the neuro-cognitive systems required to learn this rule, about half of the individuals did not acquire the discrimination. Two main hypotheses could be advanced. First, it is possible that those individuals required further training to reach learning criterion. This hypothesis is supported by the very high individual-level variability we observed in the number of days to reach criterion. As an alternative, learning and memorising attributes such as quantities (that in the wild translate to patch quality, for example) is costly (Fagan *et al.*, 2013), and could contribute to explain why some sharks failed to learn. It is possible that for some individuals attribute memory incurs extensive costs based on interindividual differences (e.g. physiological or internal states) that strongly influence the net fitness benefits of memory (Fagan *et al.*, 2013). Additionally, the forced-choice training procedure with 2D stimuli presented in a card could also present an impediment, as learning an association between the numerosity of arbitrary stimuli and a reward is unlikely to occur in nature. Spontaneous choice tests with groups of conspecifics or food are the typical alternative to operant training procedures in numerical competency tasks (Agrillo and Bisazza, 2014), but we are not convinced they would be a better alternative for this species. Juvenile Port Jackson sharks do not actively associate with conspecifics (Chapter II of this thesis), and the aquatic environment presents difficulties in controlling for olfactory cues, added to satiation effects, if pieces of food were used as stimuli.

Interestingly, we found that the proportion of correct choices correlated positively with the ratio of time spent near the correct vs. the incorrect stimulus.

While the proportion of correct choices is the most often used variable to assess learning in training procedures, it seems that association time with the stimuli can be used as a reliable predictor of choice in Port Jackson sharks, on par with results found in guppies (Petrazzini *et al.*, 2015).

Incubation at elevated temperature is known to hamper survival, brain development, and learning ability in some invertebrate and vertebrate species (Jonson *et al.*, 1976; Jones *et al.*, 2005; Rosa *et al.*, 2014; Dayananda and Webb, 2017). However, despite higher mortality in our 'ET' group (41.7 % did not survive the first month against 0 % mortality in the control group), the surviving 'ET' hatchlings showed faster learning and higher proportion of correct choices over the course of the experiment, largely driven by their increased participation in the task. Our study therefore adds learning ability to a growing list of physiological and phenotypical traits that incubation temperature can modify during early development in elasmobranchs. Three-lined skinks incubated at higher temperatures also outperformed 'cold'-incubated individuals in multiple learning tasks (Amiel and Shine, 2012; Clark *et al.*, 2014), though both thermal regimes tested were typical natural nest conditions from low (hot) or high (cold) elevations. Elevated temperature during incubation induces significant metabolic and ventilatory costs, as well as a three-fold increase in food consumption rates, in a range of ectotherms, including reptiles, teleosts and elasmobranchs (Cano and Nicieza, 2006; Nilsson *et al.*, 2009; Di Santo and Bennett, 2011; Rosa *et al.*, 2014; Pistevos *et al.*, 2015). It is possible that in this study 'ET' hatchlings also had increased metabolic requirements, and might have valued rewards at a higher level compared to sharks from ambient conditions. In addition, thermal regimes during incubation might cause a change in endocrine pathways linked with brain development. For example, changes in temperature can affect endocrine homeostasis responsible for gonadal differentiation in reptiles and fish (Pankhurst, 1997; Amiel and Shine, 2012). Thus, thermal effects on hormone levels or receptors may also induce structural variation of brain regions. Indeed, incubation treatment has been shown to cause differences in size and volume of specific brain regions, and in neuron size, number, and density, yet the mechanisms underlying these changes are still largely unknown (Jonson *et al.*, 1976; Jones *et al.*, 2005; Wang *et al.*, 2007; Amiel *et al.*, 2017).

In each treatment group, the three sharks that acquired the discrimination were trained towards the same numerosity ('ET' sharks trained to '3' as positive stimulus, and ‘C’ sharks trained to ‘6’ as positive stimulus). We are, however, unable to assess if this was a chance event or a result of the experimental treatments. Many authors now agree that vertebrates have two numerical information processing systems (Agrillo et al., 2014; Geary et al., 2014). The first is an accurate ‘object tracking system’ that operates on a small number of items to quickly track individual objects; it is precise but limited by short-term memory to 3–4 objects. The other is a generalist ‘analogue magnitude system’ that allows estimation of large quantities, and follows Weber’s Law – as numerical magnitude increases, a larger disparity is needed to obtain the same level of discrimination. In our task, the smaller numerosity falls under the object tracking system while the larger numerosity is likely outside working memory capabilities; we therefore suspect the sharks had to use the analogue magnitude system to solve the discrimination problem. In relative quantity judgments, animals can use both numerical and non-numerical information that covaries with number (‘continuous quantities’), and most studies suggest that individuals will spontaneously use continuous quantities if they are available (Agrillo *et al.*, 2011). Animals also tend to use relative numerosity rules over absolute contrasts, even though they can learn with either criteria (Petrazzini *et al.*, 2015). In this study, we did not control for non-numerical information, therefore the sharks could solve the task by using numerical and/or quantity information. Since a similar number of animals succeeded in the task with ‘3’ or ‘6’ as positive stimulus, and considering our low sample size, we are unable to determine whether one quantity would be easier to learn compared to the other.

It is interesting to note that, in this study, sharks that failed to learn the quantity discrimination developed a side bias towards the stimuli shown on the right side of the wall, including individuals from the control group. We observed a similar bias to choose the option on right in individuals that failed to discriminate between two auditory stimuli (Chapter II of this thesis). These side bias might arise in a decision-making context, since having a default option in a two-choice situation yields a higher payoff compared to random choice.

Research with a range of vertebrates supports the hypothesis that cerebral lateralisation can enhance cognitive efficiency (Vallortigara and Rogers, 2005; Bisazza and Brown, 2011). Strongly lateralized birds outperform non-lateralized individuals in pebble-seed discrimination tasks, problem-solving tasks, and in multitasking situations such as foraging while being vigilant to predators (Rogers *et* *al.*, 2004; Magat and Brown, 2009). In a numerical discrimination context, strongly lateralised guppies showed enhanced numerical abilities compared to non-lateralised individuals (Dadda *et al.*, 2015). Our results indicate that stronger right-lateralised sharks might have some cognitive benefits and the observed shift to the right in sharks raised in elevated temperatures is likely responsible for their improved performance relative to control sharks. Laterality in elasmobranchs is severely understudied, with only two studies to our knowledge investigating individual and population-level behavioural lateralisation in benthic sharks (Green and Jutfelt, 2014; Byrnes *et al.*, 2016a). It is therefore difficult to comment on the variability one might expect to see at the individual or population level, let alone its link to cognitive performance and fitness. Significantly more work is required on cerebral lateralisation and its link to learning ability in elasmobranchs.

In conclusion, our results show that juvenile sharks are capable of quantity discrimination, and provide evidence that elevated temperature during embryonic development alters behavioural and cognitive abilities. This study provides further evidence that elasmobranchs are susceptible to the effects of future ocean warming, though it seems that behavioural and cognitive mechanisms might allow those individuals that survive exposure to elevated temperatures during embryogenesis to compensate some of the challenges imposed by climate change. Increased learning performance might allow apex and mesopredators to increase foraging efficiency and match increased energetic demands. However, this might imply that with rapidly rising temperatures predators may exert a stronger control on their prey populations, with cascading effects through entire ecosystems (Estes *et al.*, 2011).

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Conflict of interest:

The authors declare no conflict of interests.

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GENERAL DISCUSSION

Cognition helps animals face a wide range of challenges and requirements in the environment. Elasmobranchs have historically been less represented in cognition research than most vertebrate groups and many behaviours and skills remain understudied in this group, despite a recent expansion in the field (Guttridge *et al.*, 2009b; Schluessel, 2015). The present thesis explored three research areas that have been little investigated in elasmobranchs – social cognition, sound discrimination learning, and numerical competency; examined potential mechanistic links between individual personality and/or laterality traits and learning performance; and investigated the effect of projected end-of-century incubation temperature in hatchlings' behavioural lateralisation and quantity discrimination skills.

Learning and behaviour in the Port Jackson shark

Many aquatic animals use biotic and abiotic sounds for vital activities such as foraging, predator avoidance, communication, and orientation. Underwater sound cues might be ecologically relevant to benthic elasmobranchs, especially nocturnal ones, to aid in locating prey and in navigating between reefs. Here I've shown that juvenile Port Jackson sharks are capable to associate a sound stimulus with a food reward, on par with the few large coastal shark species that have been examined in a similar task (Kritzler and Wood, 1961; Nelson, 1967). However, not all sharks were able to learn the association. None were successful when later required to discriminate between two sound cues and all developed strong side biases.

Sociality is a widespread phenomenon across multiple taxa, but species and populations vary in their degree of sociality depending on the trade-off between fitness benefits and costs of living in a group (Krause and Ruxton, 2002). Here I found that juvenile Port Jackson sharks did not actively choose to associate with one or three conspecifics and that sex, size, swimming activity, or foraging motivation had no effect on association preference. These findings suggest that predation pressure might not play an important role in shaping the social behaviour of juvenile Port Jackson sharks, unlike other shark species. In addition, our results indicate sociality in is not stable during ontogeny in this species, considering that adults form aggregations and have consistent social networks in the wild (Powter and Gladstone, 2009; Mourier *et al.*, 2017).

All animals are regularly exposed to other individuals, conspecifics or heterospecifics, and their products; thus, social cues should be one among many cues that can lead to changes in behaviour, even in species that show low sociality levels (Heyes, 2012; Webster and Laland, 2017). I found that observer sharks interacting with a trained demonstrator or with a sham demonstrator learnt the task in a similar proportion and in approximately the same number of days, suggesting that social facilitation enhanced learning abilities in both group conditions. When tested without demonstrators some individuals in both groups showed marked reduction in performance, which could indicate a release of social constraints as described in guppies (Brown and Laland, 2002b). Training intensity and quality of demonstration also influenced learning ability; sharks with lower training intensity and interaction with ‘poor’ demonstrators showed better learning performance, similar to what has been observed in other species (Biederman and Vanayan, 1988; Beauchamp and Kacelnik, 1991; Swaney *et al.*, 2001).

The ability to estimate and compare quantities is likely to confer important fitness benefits in a number of contexts (Geary *et al.*, 2014). Numerical abilities have been observed in a wide range of species, among mammals (Boysen *et al.*, 2001; Ward and Smuts, 2007), birds (Hunt *et al.*, 2008; Rugani *et al.*, 2013), reptiles (Petrazzini *et al.*, 2017), amphibians (Krusche *et al.*, 2010), teleost fish (Agrillo *et al.*, 2014), and some invertebrates (Chittka and Geiger, 1995; Carazo *et al.*, 2009), but had not been tested in elasmobranchs. I found that juvenile Port Jackson sharks can discriminate between two quantities, showing that the species is likely equipped with the neuro-cognitive systems required to estimate and discriminate different quantities.

Sharks were capable of reaching learning criterion in three learning experiments included in this thesis – social learning, food approach conditioning with sound, and quantity discrimination. These results provide new insights on the cognitive abilities of a benthic elasmobranch species, the Port Jackson shark, and contribute to support the idea that elasmobranchs share a cognitive toolbox with the examined representatives from other vertebrate groups. It is important to mention, however, that some of the experimental subjects in the three chapters failed to learn the tasks. It is possible that those individuals required further training to reach learning criterion. Bisazza *et al.* (2014) showed that guppies trained with an extended procedure could discriminate numerosities up to a 0.8 ratio (4 vs. 5 objects), a much higher limit than those reported with short training procedures. The observed high individual-level variability in the number of trials to reach criterion in each task provides some evidence in support of this hypothesis. In addition, the sharks used in each learning task were experimentally naïve. It is often the case in animal cognition experiments that the animals serve as experimental subjects in multiple tasks and for some years (e.g. Chase, 2001), thus are likely less stressed and more attentive to task details than subjects that have never been involved in a learning experiment. For example, bamboo sharks needed significantly fewer sessions in a second task that required them to discriminate between a square and a circle compared to the first task discriminating between a square and a 'blank' stimulus (Schluessel and Duengen 2015). On the other hand, experimentally naïve bamboo sharks were capable of learning a spatial task within a small number of training days (Schluessel and Bleckmann, 2012). The type of ability being assessed and sensory modality of the stimulus thus seem to be important variables as well.

Cerebral lateralisation and personality traits

While side preference did not seem to be a major factor in the food approach conditioning task with sound or the social learning task, most sharks that failed the sound discrimination and the quantity discrimination tasks developed a rightward bias. Interestingly, Graeber and Ebbesson (1972) report a similar rightward bias in a visual discrimination task (black vs. white; horizontal vs. vertical stripes) with nurse sharks. Strong side-bias are often seen in animal learning experiments with two-choice responses (e.g. Vallortigara *et al.*, 1996; Laland and Williams, 1997; Szabo *et* *al.*, 2017), and perhaps arise from an animal's default-option when facing indecision, which yields a higher payoff compared to random choice.

Side preferences might also be linked to cerebral lateralization, a functional asymmetry between the two brain hemispheres (Rogers and Andrew, 2002). We did not observe a population level bias in direction of laterality bias among our studies when sharks were incubated and reared in ambient, current conditions. Similar results have been found in wild-caught juvenile Port Jackson sharks tested in rotational swimming and turn preference tasks (Byrnes *et al.*, 2016a). We found some indication that sharks with stronger rightward bias reached learning criterion faster in the quantity discrimination task. Nonetheless, in the remaining experiments our measures of laterality direction and strength did not contribute to explain any of the learning performance measurements we assessed. Several cognitive skills seem to be lateralised in teleost fish and other vertebrates (Vallortigara and Rogers, 2005; Bisazza and Brown, 2011), including numerical abilities (Dadda *et al.*, 2015). Further studies investigating behavioural lateralization and potential links with cognitive abilities are required in multiple elasmobranch species to provide conclusive evidence in this subject.

We also found no association between single personality traits or behavioural syndrome and learning performance measurements. While we attempted to investigate traits that seemed reasonably relevant to each experiment (e.g. activity levels in social preference; boldness and stress reactivity in learning tasks that required leaving a start box and approach a target zone), we cannot exclude the possibility that such traits had little or no significance for the sharks and/or experimental tasks. In addition, our sample size in most experiments was generally low and our subjects were born in captivity, thus these measures might not directly compare to wild juveniles (Brown *et al.* 2007; Archard and Braithwaite 2010). Future research should assess if personality and laterality of captive-born sharks reflects the same traits observed in their wild counterparts. Moreover, additional studies are needed examining personality in the context of learning in elasmobranchs, considering the substantial body of research showing that personality traits are linked to fitness-related behaviours and cognitive traits in multiple vertebrate groups (Budaev and Brown, 2011; Sih and Del Giudice, 2012).

Cognition in a changing world

Climate change is one of the major human-induced environmental changes to ecosystems worldwide and is occurring at unprecedented rates (Collins *et al.*, 2013). For example, the average sea surface temperature is predicted to increase by 1–3ºC in the next century (Collins *et al.*, 2013; Pörtner *et al.*, 2014). Behaviour and learning may therefore play a leading role in allowing individuals to adapt and keep track of environmental variation (Brown, 2012; Wong and Candolin, 2015).

We observed lower survival rates in Port Jackson sharks incubated and reared at forecasted end-of-century temperature compared to current conditions (58.3 % survival against 100 % in the control group). Interestingly, the surviving hatchlings from the elevated temperature group showed stronger absolute laterality and a rightward bias compared to control sharks. In addition, elevated temperature sharks performed better in the quantity discrimination task. While negative effects have been reported in sharks reared at elevated temperature, including lower survival rates, impaired condition, and significant metabolic and ventilatory costs (Rosa *et al.*, 2014; Di Santo, 2016), our results suggest that they were able to adjust their behaviour to compensate those costs. We found some indication that rightward lateralisation was linked to faster learning, though more studies are needed to clarify this. Interestingly, sharks exposed to elevated CO_2 levels also showed stronger laterality (Green and Jutfelt, 2014). In light of the proposed benefits of cerebral lateralisation (Rogers *et al.*, 2004; Bisazza and Brown, 2011), it is possible that increased laterality arises a mechanism to cope with the added challenges these animals face.

Concluding remarks

In recent years, an increasing number of studies has investigated cognitive functions in sharks and rays, but we are still far from a detailed picture. This thesis extends the known cognitive abilities of elasmobranchs and provides additional experimental support to the view that elasmobranchs share most of the cognitive toolbox of teleosts and other vertebrates.

Some species of sharks and rays are not very suitable for behavioural and cognitive studies in the laboratory and/or in the wild, but there is still a wide range of families and species not investigated to date that could serve as useful subjects in these studies. For example, medium-sized reef species such as black-tip reef and bonnethead sharks do well in captivity and could provide good comparisons to the models commonly used so far. In addition, many more research areas lack proper investigation in elasmobranchs. Social intelligence is one of them; species that often form social groups and show territorial behaviour would be good candidates to examine a number of cognitive abilities. Interesting questions in the topic of social intelligence include: Can elasmobranchs recognise and remember specific individuals? Can they recognise themselves? When assessing a social hierarchy, are they able to use transitive inference skills to infer social rank? Do they behave differently according to specific social context and audience? Future studies should aim at a combination of controlled laboratory studies to examine the basic principles and mechanisms of learning, together with semi-captive or wild experiments that can place cognitive skills in the ecological and evolutionary context of the species.

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APPENDIX I – communication of research during candidature

I. **Vila Pouca C.**, Heinrich H., Huveneers C. & Brown C. (accepted) *Social facilitation of foraging behaviour in juvenile Port Jackson sharks.* Sharks International 2018, João Pessoa, Brazil

II. **Vila Pouca C.** & Brown C. (2017) *Spatial and social behaviours in the Port Jackson shark*. Laland Lab, School of Biology, University of St. Andrews & Schluessel Lab, Institute of Zoology, University of Bonn

III. **Vila Pouca C.** & Brown C. (2017) *Can sharks learn and discriminate sound cues?* Behaviour 2017: 35th International Ethological Conference & Summer Meeting of the ASAB, Estoril, Portugal. Poster contribution

IV. **Vila Pouca C.**, Chambers S., Mourier J. & Brown C. (2016) *How do Port Jackson sharks find their home?* 4th ASFB conference, Hobart, Australia

V. **Vila Pouca C.** & Brown C. (2016) *Lack of social preferences in juvenile Port Jackson sharks.* ASSAB 2016, Katoomba, NSW Australia.

APPENDIX II – additional research published during candidature

I. **Vila Pouca C.** & Brown C. (in press) *Fish: How to ask them the right questions. In:* Field and laboratory methods in animal cognition (Bueno-Guerra N. & Amici F., eds.), Cambridge University Press, UK.

II. **Vila Pouca C.** & Brown C. 2017. *Contemporary topics in fish cognition and behaviour*. Current Opinion in Behavioral Sciences 16, 46-52. 10.1016/j.cobeha.2017.03.002

III. Queiroz N., **Vila Pouca C.**, Couto A., Southall E., Mucientes G., Humphries N., Sims D. 2017. *Convergent Foraging Tactics of Marine Predators with Different Feeding Strategies across Heterogeneous Ocean Environments.* Frontiers in Marine Science 4, 239. 10.3389/fmars.2017.00239

IV. Byrnes E., **Vila Pouca C.** & Brown C. 2016. *Laterality strength is linked to stress reactivity in Port Jackson sharks* (Heterodontus portusjacksoni). Behavioural Brain Research 305, 239-246. 10.1016/j.bbr.2016.02.033

V. Byrnes E., **Vila Pouca C.**, Chambers S. & Brown C. 2016. Into the wild: developing field tests to examine the link between elasmobranch personality and laterality. Behaviour 153, 1777-1793. 10.1163/1568539X-00003373

APPENDIX III – funding obtained during candidature

During the course of thesis, I secured a number of competitive grants totalling over AUD $20,000 to conduct research and attend relevant conferences. Selected grants include:

I. 2017. MQ Post-Graduate Research Fund, AUD $4925

II. 2017. MQ Biological Sciences grant matching scheme, AUD $3000

III. 2017. PADI Foundation Grant, USD $6325

IV. 2015. Vemco Student Special Offer, AUD $3420

APPENDIX IV – animal ethics & fisheries approval

The research done in this thesis was conducted in accordance with Macquarie University Animal Ethics Committee and NSW Fisheries. Obtained approvals are given in the following pages.