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Shark personality within the predator-prey context

Valentina Scarponi

Submitted for the degree of Doctor of Philosophy

University of Sussex

School of Life Sciences

Department of Evolution, Behaviour and Environment

September 2019

Declaration

I confirm that the work submitted is my own, except where work which has formed part of jointly-authored publications has been included. The contributions of other authors are indicated for the yet to be published chapters below. This thesis has not been and will not be submitted to another University for the award of any other degree.

List of chapters and contributions

Chapter 2. Scarponi, V., Gennari, E., Hughes, W.O.H., 2019. Physiological response to capture stress in endemic Southern African benthic catsharks.

VS designed the experiments and carried out the experimental work, analysed the data, and wrote the manuscript. WOHH supervised the work, and the design of the experiments, assisted with the data analysis, and proofing the manuscript. EG supervised the experimental work and assisted with proofing the manuscript.

Chapter 3. Scarponi, V., Cartolano-Leal, J.G., McCord, M.E., Drobniewska, N., Hughes, W.O.H. Personality in a mesopredator and the effects of the social environment on behavioural syndromes.

VS designed the experiments and carried out the experimental work with JGCL, analysed the data, and wrote the manuscript. WOHH supervised the work and the design of the experiments, assisted with the data analysis, and proofing the chapter. MEM and ND supervised the experimental work and will assist with proofing the manuscript before submission for publication.

Chapter 4. Scarponi, V., Gennari, E., Hughes, W.O.H. Personality and its effects on hunting behaviour and spatial use of the foraging landscape in a marine apex predator.

VS designed the experiments and carried out the experimental work, analysed the data, and wrote the manuscript. WOHH supervised the work and the design of the experiments, assisted with the data analysis, and proofing the chapter. EG supervised the experimental work, assisted with the design of the experiments and will assist with proofing the manuscript before submission for publication.

Signed _____   Date_____

Acknowledgments

Abstract

Consistent behavioural variability between individual animals ('personalities') and behavioural correlations ('syndromes') are widespread across the animal kingdom, and are likely of major conservation and ecological importance. Although there has been growing interest in understanding how animal personalities influence fitness and are maintained over time, the effects of this phenomenon on complex ecological processes like predator-prey interactions are still poorly understood, especially for large marine vertebrates like sharks. The gap in our understanding is particularly concerning due to the worldwide decline in shark population numbers, threatened by fishing and environmental changes. In this thesis, I first describe the physiological response to capture in endemic Southern African catsharks, and observe how capture stress significantly lowered pH and K^+, while it severely increased lactate and pCO_2 concentrations in their peripheral circulation. I then present novel evidence that a marine mesopredator, the dark shyshark, and a marine apex predator, the white shark, show consistent inter-individual variability in hunting and movement behaviour within the foraging landscape, that are not simply due to individual differences in hunger. Dark shysharks also showed individuality in coping styles, and repeatability in anti-predator responses. Their behaviour was further constrained by syndromes, although individuals appeared to maintain a certain level of behavioural plasticity in response to their social environment. Our results have important implications for public perception of sharks, and suggest that personality will directly affect shark fitness by mediating foraging behaviour and trophic interactions with other species. If personality is likely to have an effect on marine ecosystems as a whole, considering how sharks, through predation, have important roles in shaping prey populations, then its consequences on shark fitness should be included in conservation strategies for these species.

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1. General Introduction

In recent years, behavioural ecologists have recognized that animal behaviour is not infinitely plastic, and that many animal species, from ants to fish and primates, show consistent individual differences in their behaviour (Gosling 2001, Sih et al., 2004a,b; Réale et al., 2007). Differences in behaviour between individuals that are consistent across time and different contexts are termed personalities, and are of great evolutionary, ecological and conservation interest (Gosling, 2001; Bolnick et al., 2003; Sih et al., 2004a,b; Réale et al., 2007; Biro and Stamps, 2008; Byrnes and Brown, 2016; Finger et al., 2017). They are believed to influence many aspects of an animal's fitness and survival, including movement patterns and habitat use, foraging strategies and how individuals interact with their environment within the predator-prey context (Gosling, 2001, Sih et al., 2004a,b; Réale et al., 2007; Smith and Blumstein, 2008; Carere and Locurto, 2011; Vaudo et al., 2014; Matich and Heithaus, 2015; Byrnes and Brown, 2016; Finger et al., 2016; Finger et al., 2017; Chapter 3; Chapter 4). The phenomena of personality and individual specialization are important concepts that depart from the optimal behaviour theory often used to describe animal behaviour, which assumes the ability of animals to adapt their use of food resources according to their current ecological conditions (Pyke et al., 1977; Toscano et al., 2016).

1.1 Personality and behavioural syndromes

Personality is defined as one-dimensional, consistent individual variability in behaviour between individuals (Sih et al., 2004a,b, Øverli et al., 2007). Consistent personality traits have been observed for aggression towards conspecifics or threats, boldness in exploring novel habitats, activity, sociability, reactivity to stimuli, hunting mode, and a variety of other behavioural variables (Sih et al., 2004a,b, Øverli et al., 2007; Carere

and Locurto, 2011; Vaudo et al., 2014; Matich and Heithaus, 2015; Byrnes and Brown, 2016; Finger et al., 2016; Finger et al., 2017; Chapter 3; Chapter 4). Personalities often generate trade-offs, providing significant benefits under certain circumstances, while being suboptimal under other selective pressures (Sih et al., 2004a,b; Bergmüller, 2010). For example, individuals that are consistently more aggressive than others may thrive in competitive situations (e.g. quest for resources), but will be more vulnerable in contexts where caution and care are favoured (e.g. anti-predator behaviour; Sih et al., 2003; Sih et al., 2004a,b). A further constraint on behavioural plasticity is the tendency of different personality traits to be correlated among individuals within a population or a species (Sih et al., 2004a,b, Dingemanse and Réale, 2005; Mittelbach et al., 2014). When different personality traits are correlated, then individuals are characterized by a multidimensional, consistent variability in behaviour or 'behavioural syndrome' (Sih et al., 2004a,b; Dingemanse and Réale, 2005; Conrad et al., 2011; Sih, 2013; Mittelbach et al., 2014). Behavioural syndromes can extend across different contexts such as feeding, mating, parental care and movement behaviour under similar environmental conditions (Figure 1.1), or can be domain specific when behavioural traits are correlated within a functional context but across different situations (e.g. feeding in the presence and in the absence of a predator; Sih et al., 2004b; Smith and Blumstein, 2008). For example, animals that are relatively more aggressive towards conspecifics may also be bolder when exploring new environments or hunting under variable predation-pressure (Dingemanse and Réale, 2005; Smith and Blumstein, 2008). A certain behavioural syndrome can provide fitness benefits within some situations, while being suboptimal under other selective pressures (Réale and Festa-Bianchet, 2003; Sih et al., 2004a,b; Bergmüller, 2010; Mittelbach et al., 2014). As a result, behavioural correlations and limited plasticity can generate trade-offs across different functional behavioural

categories that can limit the adaptability of a species to rapidly changing environments such as human-dominated habitats (Schlaepfer et al., 2002; Sih et al., 2004a; Mittelbach et al., 2014).

Nonmating $\longrightarrow$ **Mating** $\longrightarrow$ **Parental Care**
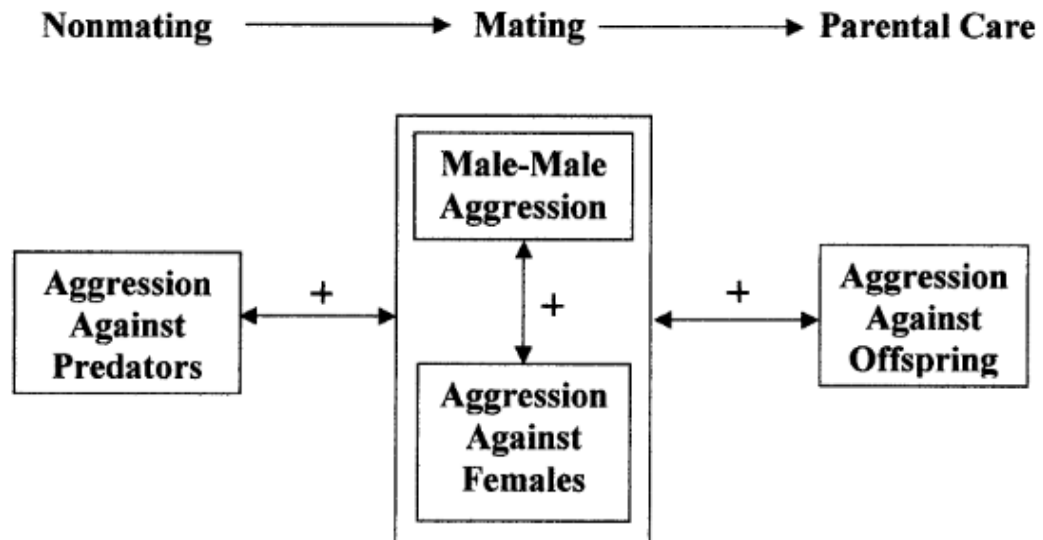
Figure 1.1: Example of behavioural syndrome across different situations. Behavioural correlations created by the tendency of a certain personality type (aggressiveness) to carry over across situations (non-mating, mating, parental care). The positive symbol indicates a positive behavioural correlation. In this case, aggressiveness might be optimal and increase fitness in a non-mating situation (i.e. aggression against predators), but is likely suboptimal when mating and in the context of parental care. Aggressiveness against females or against offspring would therefore have a negative impact on individual fitness (Sih et al., 2004b).

Recent research efforts have strengthened our understanding of the possible origins of inter-individual differences in animal behaviour, and helped explain how such variability is maintained (Sih et al., 2004a,b; Dingemanse and Réale, 2005; Øverli et al., 2007; Pruitt et al., 2012; Baugh et al., 2013). A mechanistic approach has been employed to explain animal personality and to describe what mechanisms are likely to generate behavioural correlations (Dingemanse and Réale, 2005). Researchers seek to explain how different personalities emerge from the combined action of the neuroendocrine system (coping styles, section 1.2), genetics, and environmental

experiences (Sih et al., 2004b; Dingemanse and Réale, 2005; Øverli et al., 2007; Pruitt et al., 2012; Baugh et al., 2013).

1.2 The physiological basis of personality: coping styles

Although the underlying role that the neuroendocrine system has in controlling the development and expression of personality is highly complex, individual variability in behaviour is believed to be strongly associated with and individual 'coping style', defined as the differences in the physiological response to stress (Koolhaas et al., 1999; Øverli et al., 2007; Baugh et al., 2013). Coping styles in most animals vary between species, and is likely different between individuals of the same species (Øverli et al., 2007; Skomal and Mandelman, 2012). Most evidence for the hormonal basis of behavioural correlations has found that proactive individuals generally show low hypothalamic-pituitary-adrenal axis reactivity to stress but high sympathetic reactivity, and hence experience a less acute hormonal response to stress when compared to shyer, more reactive animals (Sih et al., 2004b; Øverli et al., 2007; Archard et al., 2012; Atwell et al., 2012). Consequently, when presented with stimuli that can elicit a stress response, individuals with a bolder personality are expected to show smaller increases in glucose, lactate, and potassium, and a smaller decrease in blood pH (Øverli et al., 2007; Atwell et al., 2012). They would also be expected to show less significant changes in swimming behaviour when compared to individual with a shyer personality, who are expected to be affected by higher levels of glucose, lactate and potassium, and a greater decrease in blood pH. The study of stress-coping styles has primarily focused on mammals, and little is known about other animal taxa (Øverli et al., 2007). Among aquatic organisms, much of the research investigating the physiological response to stress is concentrated on freshwater salmonids and marine teleost fish, while limited

information is available on elasmobranchs, despite population declines threatening most species worldwide (Heithaus and Dill, 2006; Frick et al., 2010b; Martin and Hammerschlag, 2012; Skomal and Mandelman, 2012; Worm et al., 2013; Danylchuk et al., 2014).

1.3 Genetic basis of personality

Recent findings support the hypothesis that animal personalities have a strong genetic basis, as they are often heritable, and suggest that behavioural syndromes are under strong polygenetic control with frequent pleiotropy and epistasis (Price and Langen, 1992; Sih et al., 2004b; Dingemanse and Réale, 2005; van Oers and Sinn, 2013). Behavioural correlations and inter-individual variability in behaviour are likely maintained by evolutionary mechanisms such as frequency- and density-dependent selection, mutation-selection or migration-selection balance and overdominance (Sih et al., 2004a; Réale et al., 2007). If behavioural correlations are genetically linked, then behavioural syndromes are likely to evolve as a package, explaining the persistence of strategies that appear to be suboptimal under certain circumstances (Price and Langen, 1992; Brodin and Johansson, 2004; Sih et al., 2004b). In most cases, genetic factors play a key role in maintaining personality traits especially at the individual level, but environmental selection pressure becomes an important driver of the evolution of personality traits and behavioural syndromes at the population and species level (Réale and Festa-Bianchet, 2003; Brodin and Johansson, 2004; Pruitt et al., 2012).

1.4 The role of the environment

Personality and behavioural correlations are frequently shaped by an animal's environment. The development of personality is influenced by an individual's

experience within its lifetime, such as early experience and parental choices even in species without extensive parental care. Moreover, the contextual experience of an individual's environment in terms of predation pressure or social interactions with conspecifics can influence personality. For example, experiencing predation risk can alter, decouple or generate correlations between anti-predator behavioural responses and other personality traits, and contribute to the maintenance of high inter-individual variability in behaviour (Bell and Sih, 2007; Réale et al., 2007; Stamps and Groothuis, 2010; Conrad et al., 2011; Mittelbach et al., 2014). Also, observing conspecifics can mitigate individual personality traits: animals observing shy individuals of their own species are found to increase caution when reacting to novelty (Frost et al., 2007). The environment can shape personality also via natural selection, and personality traits will in turn influence fitness in environments that might have different selective pressures (Réale and Festa-Bianchet, 2003; Brodin and Johansson, 2004; Pruitt et al., 2012). For example, an individual who experiences high predation pressure might develop strong anti-predator behaviours that are maintained even after predation risk is relaxed, limiting the time the animal would otherwise spend foraging and searching for resources (Sih et al., 2003). Such time budget conflicts are also a major source of behavioural correlations, as the various activities within a conflict are generally negatively correlated because the more time an individual spends engaging in one behaviour directly decreases the time spent on other mutually exclusive activities (Werner and Anholt, 1993; Sih et al., 2003; Brodin and Johansson; 2004). Since animals cannot simultaneously maximize all activities to increase fitness, certain behavioural correlations may not be optimal in all circumstances (Sih et al., 2004b).
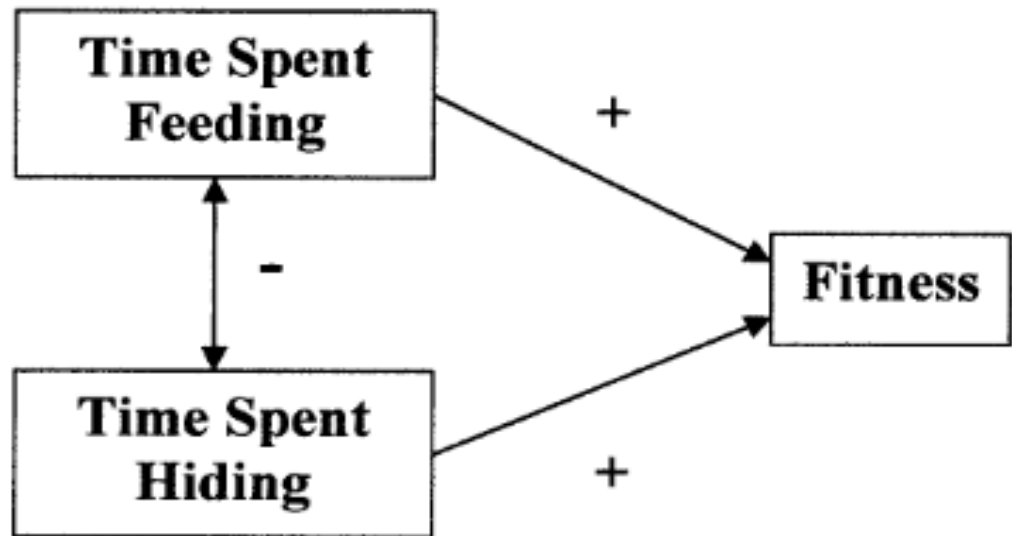
Figure 1.2: Example of a time budget conflict.
Time spent feeding and spent hiding would increase fitness (indicated by the positive symbols i.e. positive correlations), but are in conflict since the animal cannot engage in both simultaneously (indicated by the negative symbol i.e. negative relationship), (Sih et al., 2004b).

Behavioural correlations and different personality types tend to coexist and be maintained in natural environments when the costs of flexibility are high (Dall et al., 2004; Sih et al., 2004a). Because organisms make unavoidable errors, a species or population may benefit from maintaining low behavioural plasticity, especially if individuals have weak information about their environment (Sih et al., 2004a). Limited behavioural plasticity may also be favoured by natural selection when the benefits of consistent behaviours and hence predictability are large (e.g. Hawk-Dove game, cooperation), and can be maintained either by the direct connection between a fixed phenotypic trait (e.g. morphology or physiology) and behaviour, or could be the result of a behavioural positive feedback loop (Dall et al., 2004; Sih et al., 2004a,b). The key role that the environment has in shaping behaviour can be easily observed in studies that compare wild populations living under different environmental conditions (Réale and Festa-Bianchet, 2003; Sih et al., 2003; Dingemanse et al., 2007; Réale et al., 2007;

Dingemanse et al., 2009). For instance, fish populations living under higher predation pressure tend to develop stronger anti-predator behaviours, bolder personalities and tighter behavioural syndromes compared to populations found in safer areas (Dingemanse et al., 2007; Réale et al., 2007; Dingemanse et al., 2009). In addition to individual experiences, neuroendocrine profiles have been found to influence the development and maintenance of behavioural correlations.

1.5 Sharks and their physiological response to stress

Most elasmobranchs species are long lived and display strong K-selected life history strategies, with slow growth, late age at maturity, low fecundity, long gestation periods and a strong relationship between the number of offspring and the size of the breeding biomass (Stevens et al., 2000; Cailliet et al., 2005; Myers et al., 2007; Afonso and Hazin, 2014; Danylchuk et al., 2014; Finger et al., 2016; Musyl and Gilman, 2018). Although sharks can be found in a wide range of habitats, including inshore estuaries and lagoons, coastal waters and the open sea, most species tend to have a restricted geographical distribution and to aggregate by age, sex and reproductive stage (Cailliet et al., 2005). Within marine ecosystems, elasmobranchs play keystone roles through top-down control of population number, habitat use and foraging behaviour of prey species, while enhancing biodiversity (Cailliet et al., 2005; Byrnes et al., 2016). These biological and ecological characteristics have developed in this taxon over 400 million years of evolution, and are key concerning implications for the ability of shark population to survive and recover from declines caused by fisheries and other anthropogenic activities (Stevens et al., 2000; Cailliet et al., 2005; Afonso and Hazin, 2014; Danylchuk et al., 2014; Musyl and Gilman, 2018). Recent estimates of shark and ray total landings reach over 750 thousand tons, or ~100 million individuals, caught annually to supply the

increasing demand for lucrative shark products (fins, livers, gill rakers) or as by-catch in fisheries targeting other teleost species (Myers et al. 2007; Worm et al., 2013; Dulvy et al., 2014; Davidson et al., 2015).
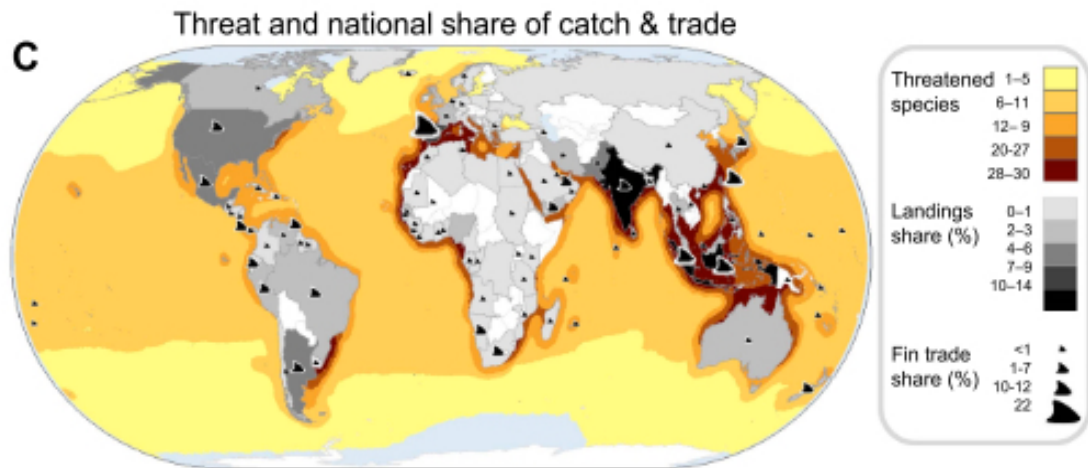
Figure 1.3: Countries involved in shark fisheries, exporting of shark fins to Asia. The nations involved in shark and ray fisheries are grey shaded to reflect their share (in %) of their average annual landings reported to FAO between 1999 and 2009. The size of the fin symbols represents their relative share of shark and ray fin exported to Hong Kong in 2010 (Dulvy et al., 2014).

Often the real number of landed individuals goes unrecorded, as animals caught-and-released by either commercial or recreational fisheries are omitted from official reports to national and international management agencies (Stevens et al., 2000; Cooke and Schramm, 2007; Cooke et al., 2013; Worm et al., 2013). Another key problem in shark and ray fisheries management remains the assumption that capture will have no impact on the survival of discarded animals, even when they are released alive. Because of the stress experienced by most sharks during the capture event, many may be are unlikely to survive, further threatening the survival of shark populations worldwide, particularly in the larger species (Stevens et al., 2000; Afonso and Hazin, 2014; Butcher et al., 2015).

From the current understanding of the integrated physiological response to stress in elasmobranchs, the exposure to acute stressors in this taxon is known to activate a series of sequential responses, which have been divided in three categories: primary, secondary and tertiary (Skomal and Mandelman, 2012). The initial encounter of a stressor elicits a primary neuroendocrine response mediated by the hypothalamic-pituitary-interrenal axis, upon which a rapid increase in the concentration of stress hormones occurs in the circulating blood (Gelsleichter, 2004; Skomal and Mandelman, 2012). Catecholamines (epinephrine and norepinephrine) increase in concentration as they are secreted by specialized cell clusters present on the dorsal surface of the kidneys (Randall and Perry, 1992), along with corticosteroids (primarily 1α-hydroxycorticosterone) produced by an adrenocorticoid gland found between two posterior lobes of the kidney (Hight et al., 2007). The release of stress hormones elicits the secondary response, which consists of a significant change in the physiological and biochemical composition of the circulating blood (Skomal and Mandelman, 2012). Features of the secondary response include the rapid increase of glucose in the blood that is mobilized from hepatic glycogen to meet the energetic requirements of 'fight or flight', a decrease in blood pH upon accumulation of metabolic H^+ and respiratory pCO_2 (Mandelman and Farrington, 2007; Frick et al., 2012), and an increase in lactate concentration (Hight et al., 2007; Mandelman and Skomal, 2009). Acidosis induced by acute stress is expected to cause an increase in blood K^+ following the efflux of potassium ions from the intra- to extracellular compartment (Cliff and Thurman, 1984; Skomal and Mandelman, 2012). The secondary stress response will elicit the tertiary response, which can impact fitness variables such as growth, reproduction, immune response and behaviour. However, many effects of the tertiary response to stress on the fitness and survival of elasmobranch species are still poorly understood or completely

unknown (Skomal and Mandelman, 2012). As the secondary response to stress has received some attention in the assessment of survival post capture-and-release, it provides the possibility of investigating the individuality of shark coping styles using similar methods (Mandelman and Skomal, 2009; Brooks et al., 2012; Cooke et al., 2013; Chapter 2).

1.6 Personality within the predator-prey context

As experience and the neuroendocrine system affect the expression of behavioural correlations within individuals and the maintenance of consistent behavioural differences between individuals, their role in shaping personality should be taken into consideration when investigating the potential ecological consequences of behavioural correlations to widen our understanding of the ability of wild populations to respond to habitat degradation and global climate change (Gates and Gysel, 1978; Sih et al., 2004b; Archard and Braithwaite, 2010; Conrad et al., 2011; Jacoby et al., 2014; Mittelbach et al., 2014). Activity levels, aggressiveness, boldness and exploration among other personality traits have been observed to influence important indicators of individual fitness such as hunting strategy, diet preference and habitat use, with significant implications for several ecological mechanisms including population dynamics and predator-prey interactions (Sih et al., 2004b; Dingemanse and Réale, 2005; Réale et al., 2007; van Oers and Sinn, 2013; Byrnes et al., 2016; Toscano et al., 2016). For example, differences in individual boldness were found to affect foraging behaviours in scrounging barnacle geese and in pumpkinseed sunfish (Coleman and Wilson, 1998; Kurvers et al., 2010). Populations of many species that are seemingly composed by generalists are rather constituted by specialised individuals with little overlap in diet preferences (Toscano et al., 2016). This has been shown with isotope analysis in some

sharks (Bolnick et al., 2003; Toscano et al., 2016; Hussey et al., 2017; Matich et al., 2019). As these personality traits also covary with individual dispersal and migration patterns, it is likely that individual specialization and diet preference directly influence an animal's use of the foraging landscape, especially when resources are distributed heterogeneously (Toscano et al., 2016). A bold individual would benefit from moving between habitat patches if exploring led to greater foraging success, then manifested in improved fitness through faster growth and higher fecundity (Fraser et al., 2001). However, the net advantages of such behavioural type would we nullified if predation pressure was higher in certain habitats or if the risk of mortality while exploring was large (Fraser et al., 2001). Consistent individual differences in foraging mode and diet specialization hence help explain the persistence of behaviours in natural populations that appear maladaptive under certain ecological circumstances (Bolnick et al., 2003; Toscano et al., 2016).

Species interactions within the food web arena create the underlying structure of ecological networks and maintain stable communities, therefore playing a central role in the preservation of healthy predator and prey populations and their ecosystems (Toscano and Griffen, 2014). Predator-prey interactions in aquatic ecosystems influence the survival, size structure, growth, distribution, foraging behaviour of prey species and the overall biodiversity of their communities (Beauchamp et al., 2007; Byrnes and Brown, 2016). Predator-prey interactions have traditionally been modelled at the species or population level from the averages of important behavioural measures, treating individuals as ecologically equivalent units (Bolnick et al., 2003; Bolnick et al., 2011; Toscano and Griffen 2014). When looking at intraspecific variability in behaviour within animal populations, most researchers focus on sex, age class, morphology and genetics as the main sources of this variability, while personality and individual

specialization are often overlooked, despite among-individual variation in many behavioural traits being independent of an animal's biology (Kimber et al., 2009; Bolnick et al., 2011; Kock et al., 2013; Toscano et al., 2016; Towner et al., 2016; Hussey et al., 2017; Matich et al., 2019). For example, studies on great tits and on a piscivorous cichlid fish found how individual variation in exploration behaviour and hunting technique were not linked to sex, age, and size, suggesting that personality plays a key role in mediating animals' fitness, distribution and ecological interactions between species (Toscano et al., 2016). Disregarding intraspecific variability has resulted in critical gaps in our understanding of how species interactions within the predator prey-context shape the structure and stability of important ecosystems (Bolnick et al., 2011; Kalinkat, 2014). This limitation, and the fact that personality studies often focus on one species irrespective of its ecological interactions with others, reinforces the need to investigate the links between inter-individual behavioural differences and trophic interactions, especially for highly vulnerable taxa like elasmobranchs (Conrad et al., 2011; Kalinkat, 2014; Mittelbach et al., 2014).

The difficulty of reliably identifying individual animals and obtaining controlled replicate behavioural measures from them in the wild has greatly constrained our ability to answer complex ecological questions, such as the role that personality has in interspecific interactions within contexts such as predator-prey cycles (DiRienzo et al., 2013). Moreover, as two or more observations of a behavioural response for a set of multiple individuals are needed to characterize and quantify within-individual and between-individuals consistency in behaviour (Sih et al., 2004b), developing an accurate framework for the identification of single individual animals in the wild over time is necessary for empirical studies. Individuals of some species may be more difficult to identify and score than others, simply because their behaviour may be hard

to track, or because it is not feasible to collect replicate data for the same individual over time (Gosling, 2001). To overcome some of the challenges posed by obtaining repeated behavioural measures for each individual in the natural environment, measuring personality in the laboratory has provided valuable observations of behavioural consistency in many species (e.g. Byrnes et al., 2016; Finger et al., 2017). Even if laboratory studies introduce animals into situations that may be different from their natural conditions, researchers have been able to obtain evidence of the differences in personality between individuals in a broader context, focusing less on the specific stimuli tested per se (Réale et al., 2007). Studies in captivity also offer the possibility to observe the same individuals across different experimental treatments and to quantify behavioural correlations across situations (Sih et al., 2003; Byrnes and Brown, 2016; Finger et al., 2016; Finger et al., 2017; Finger et al., 2018), and have been found to closely reflect results of animals observed in the field (Herborn et al., 2010), paving the way for a broader investigation of the presence of animal personality in wild populations and in relation to complex ecological questions within the food web arena.

1.7 Chapters overview

As the persistent gap in our understanding of personality in marine predators and its effects over the long-term survival of these species has been raising concerns for the health of marine ecosystems (Finger et al., 2016; Finger et al., 2017), in this thesis I aim to investigate the occurrence of personality traits in two shark species within the predator-prey context, using lab experiments benthic Southern African dark shysharks under controlled conditions, and a field study of white sharks. In Chapter 2, I describe the physiological response to capture stress in Southern African benthic catsharks as a basis onto which I build the assessment of the individuality of coping styles in these

sharks in Chapter 3. In this chapter, I expose dark shysharks to a series of different prey

and predator stimuli to determine the repeatability, inter-individual variability and

physiological component of their behavioural responses. I also test whether social

environment has an effect on shyshark responses to predator-prey stimuli and on

behavioural syndromes. In Chapter 4, I present two controlled food stimuli to white

sharks in the field to determine whether white sharks in their natural environment show

consistent inter-individual variability in their behavioural responses. I also collect

movement data from a subset of the white sharks in an attempt to investigate individual

differences in habitat use within the foraging landscape in this species. Finally, in

Chapter 5 I discuss the overall findings of this thesis and their implications for the study

of shark behaviour and the conservation of shark populations worldwide.

2. Physiological response to capture stress in endemic Southern African benthic catsharks

2.1 Abstract

Fishing is a major cause of global decline of shark populations, and in many cases fishing practices involve the release of sharks caught accidentally as by-catch or deliberately for recreational purpose. While important data has been collected for some sharks, capture stress is still poorly understood for most species, and reliable estimates of capture-and-release mortality are yet to be incorporated in fisheries and conservation strategies. Here, we quantified the physiological effect of capture on benthic catsharks endemic to Southern Africa, which are regularly discarded as by-catch and targeted by recreational fisheries. 15 pyjama sharks, 9 leopard sharks, and 9 shysharks were captured, left on a circle hook for 3 min, and blood samples used to measure their response to capture. Stressed blood chemistry values were compared to unstressed levels obtained after the sharks had recovered for 24 h in an underwater pen. Capture stress significantly lowered pH and K^+, severely increased lactate of 55-63% and pCO_2 concentrations in the peripheral circulation of the sharks. Species-specific differences in capture stress were detected, along with effects of size and sex on some of the bioindicators. The severe response elicited by even the relatively quick capture event experienced in our study suggests that common fishing practices will have an even stronger impact on catshark physiological homeostasis due to longer hooking times and the use of more disruptive fishing gear. While the relationship between survival and the physiological changes elicited by capture needs further investigation, minimising stress during capture-and-release fishing practices would be prudent to maximise shark survival.

2.2 Introduction

Overfishing has been the leading cause of the steep declines in shark population numbers globally (Frick et al., 2010a; Worm et al., 2013; Danylchuk et al., 2014). Recent estimates of total landings are in excess of 750 thousand tons of sharks and rays, or ~100 million individuals, caught annually due to the increasing demand for shark products and fisheries by-catch (Myers et al., 2007; Worm et al., 2013; Davidson et al., 2015). A key problem in shark and ray fisheries management remains the incomplete reporting of catches, with individuals caught-and-released by either commercial or recreational fisheries not usually being reported to national and international management agencies (Cooke and Schramm, 2007; Cooke et al., 2013 Worm et al., 2013). Sharks and rays are particularly vulnerable to over-exploitation because of their K-selected life-history strategy (Afonso and Hazin, 2014; Danylchuk et al., 2014; Musyl and Gilman, 2018). Concerns have been raised over the impact that fishing has on the populations of sharks and rays worldwide and on the overall health of marine ecosystems affected by the dramatic decrease of apex and mesopredators (Stevens et al., 2000; Myers et al., 2007; Afonso and Hazin, 2014; Danylchuk et al., 2014). In general, commercial fisheries are managed either by input controls in terms of gear restrictions and spatio-temporal limitations, or by output controls that limit the legal sizes and quotas of animals and species that can be retained, with others generally being discarded as by-catch (Afonso and Hazin, 2014; Butcher et al., 2015). Recreational fishing is generally managed by either forbidding capture or requiring release after capture of designated species (Cooke and Schramm, 2007; Cooke et al., 2013; Gallagher et al., 2017b). Such management practices are based fundamentally on the assumption that the effects of the capture event on discarded individuals have no impact on their survival (Cooke and Schramm, 2007; Cooke et al., 2013; Butcher et al., 2015;

Gallagher et al., 2017b; Musyl and Gilman, 2018). It is therefore important to assess the physiological effects that capture-and-release has on sharks and their subsequent survival (Cooke and Schramm, 2007; Frick et al., 2010a,b; Cooke et al., 2013; Bouyoucos et al., 2018a; Musyl and Gilman, 2018). More reliable estimates of mortality rates could then be incorporated into yearly landing quotas to provide a more realistic picture on shark global declines.

The necessity for a comprehensive and global assessment of the long-term effects of fishing on shark survival has motivated many recent studies (e.g. Skomal, 2007; Brooks et al., 2012; Cooke et al., 2013; Butcher et al., 2015; Bouyoucos et al., 2017; Adams et al., 2018; Bouyoucos et al., 2018a; Bouyoucos et al., 2018b). The evidence strongly suggests that, depending on the capture method and duration (the 'fight' time the animal spends resisting capture), the physiological consequences of fishing capture can negatively affect growth, feeding and swimming behaviours, resistance to disease, reproductive output, can cause reflex impairment, capture-induced parturition and abortion in viviparous species, and can eventually lead to death (Skomal, 2007; Frick et al., 2010a; Brooks et al., 2012; Gallagher et al., 2014; Adams et al., 2018; Bouyoucos et al., 2018a; Bouyoucos et al., 2018b). Moreover, the physiological response to capture stress and consequent survival rate differs in sharks depending on biotic factors such as sex and size (Cooke et al., 2013; Dapp et al., 2016; Ellis et al., 2017). Sex-related differences in shark mortality and physiological response to capture have been reported in scalloped and bonnethead hammerhead sharks (Manire et al., 2007; Lotti et al., 2011), blue sharks (Coelho et al., 2013) and *Carcharhinus* spp. *(*Butcher et al., 2015). In these species, males are more likely to die than females, possibly because females have a thicker skin that offers more protection against injuries (Dapp et al., 2016; Musyl and Gilman, 2018). The relationship between size, capture stress and consequent survival

remains ambiguous with size having been found to influence the physiological response to capture in some species (e.g. Lotti et al., 2011; Kneebone et al., 2013; Gallagher et al., 2014) but not in others (e.g. Hoffmayer and Parsons, 2001; Mandelman and Farrington; 2007), and remains unknown for many species (e.g. Morgan and Carlson, 2014; Gallagher et al., 2017b; Musyl and Gilman, 2018). A better understanding of the physiological effects of capture stress as a function of sex and size is therefore important for the conservation of shark populations and their cascading trophic effects as predators in many marine ecosystems, especially considering that most fishing regulations are based on size and location restrictions (Myers et al., 2007; Mandelman and Farrington, 2007; Bornatowski et al., 2014; Morgan and Carlson, 2014; Dapp et al., 2016; Butcher et al., 2015; Musyl and Gilman, 2018).

The strong evidence of species-specific relationships between the physiological effects of capture and subsequent survival (Skomal, 2007; Mandelman and Skomal, 2009; Brooks et al., 2012; Worm et al. 2013; Ellis et al., 2017; Bouyoucos et al., 2018b), reinforces the need for the assessment of a broad range of shark species affected by commercial and recreational fisheries to determine the relationship between the physiological changes experienced by the animals and their condition and mortality rate post capture-and-release (Skomal, 2007; Mandelman and Skomal, 2009; Brooks et al., 2012; Bouyoucos et al., 2018b). Despite these growing concerns about the consequences of capture-and-release stress on shark survival, and the recent increase in research efforts aimed to characterize the physiological response to capture in some shark and rays species (reviwed in Skomal and Mandelman, 2012; Dapp et al., 2016; Ellis et al., 2017), there remains an enormous gap in knowledge for the vast majority of species, including the effects of gear types, hooking times and biotic factors like sex and size. This is due to the difficulties in obtaining species-specific, baseline physiological

profiles of unstressed animals (controls) that are based on empirical data rather than on mathematical or theoretical predictions and that can be used for comparison with the physiological profiles of stressed animals (Skomal, 2007; Cooke et al., 2013; Barragán-Méndez et al., 2019). Given the diversity of shark life-history, metabolic rate, respiratory mode, and swimming behaviours, it would be inaccurate to apply a stress response model from one species to another based on the limited knowledge available, strongly reinforcing the need for species-specific studies (Skomal and Mandelman, 2012; Dapp et al., 2016; Jerome et al., 2018).

Pyjama sharks (*Poroderma africanum*), leopard sharks (*Poroderma pantherinum*), puffadder shysharks (*Haploblepharus edwardsii*) and dark shysharks (*Haploblepharus pictus)* are benthic catsharks (family Scyliorhinidae) endemic to Southern Africa. Catsharks such as these are caught both by recreational fisheries and commercial fisheries, generally being discarded due their low commercial value (Fowler et al., 2005; DAFF, 2013; da Silva et al., 2018; Barragán-Méndez et al., 2019; Silva and Ellis, 2019). Despite the recent concern for the long-term survival of these endemic species due to increasing fishing pressure (DAFF, 2013; da Silva et al., 2015; da Silva et al., 2018), no studies have investigated the physiological consequences of capture stress on these species, hence there is no clear assessment of the possible consequences that capture stress may have on their populations. The primary objective of this study was therefore to quantify the physiological effects that capture stress has on these benthic catsharks and to determine whether there are interspecific differences in the effects. Congeneric species can have different stress responses and survival rates as a result of capture (Butcher et al., 2015; Ellis et al., 2017). The second objective was to examine whether the stress response to capture varies with size or sex, as these biotic factors have been found to influence the change in physiological homeostasis in other sharks.

2.3 Material and methods

This study was conducted between 11th May and 30th October 2017, in Mossel Bay, South Africa (34.1747^o S 22.0834^o E). All experimental procedures were approved by the University of Sussex Ethical Review Committee (ARG/2/3), and all research was carried out under research permit RES2017-47 issued by the South African Department of Agriculture, Forestry and Fisheries.

2.3.1 Study area and shark capture

The fishing rod-line technique was used to catch all individuals for the study. The research vessel was anchored at one of the two local reefs in Mossel Bay (The Point Reef 34.189° S 22.1616° E or Hartenbos Reef 34.13° S 22.13° E) depending on weather conditions. A Tinytag Aquatic 2 temperature logger (Gemini Data Loggers manufacturers) was suspended from the research vessel to collect water temperature readings at the time that the sharks were hooked (mean ± s.e. 15.1 ± 0.18 °C). After anchoring, four to six, 180 cm graphite fishing rods equipped with 14 kg breaking strength line and 5/o circle hooks were baited and dropped to reach the ocean substrate (depth: min = 5.6 m, max = 19.6 m, mean ± s.e. = 9.4 ± 0.8 m). Once hooked, a shark was reeled aboard the vessel in less than 1 min, and then placed individually in a plastic container (80 x 40 x 40 cm) filled with fresh salt water if belonging to the species of interest and free of hook scars indicating previous capture. Each shark was kept for 3 min on the hook in order to standardize the duration of the exposure to capture stress. After 3 min, the hook was removed. Two individuals of any of the four species were caught on any given day. Pyjama and leopard catsharks are easily recognized by their skin markings. Puffadder and dark shysharks can be hard to distinguish phenotypically, so were pooled as *Haploblepharus* shysharks (Figure 2.1). A total of 15 pyjama sharks (4 females TL = 67-94.5 cm, 9 males; TL = 57.5-95 cm), 9 leopard sharks (2 females

TL = 67.5 cm, 7 males; TL = 52.5-69 cm), and 9 *Haploblepharus* shysharks (4 females

TL = 58.5-62, 5 males TL = 55-71 cm) were captured during the study period.

Figure 2.1 Species of sharks included in this study.
P. africanum (A), *P. pantherinum* (B), *Haploblepharus* spp. (C). Panel C shows the
example of a spaghetti tag introduced in the muscle of the sharks to avoid later
recapture.

2.3.2 Blood analysis

As the secondary stress response was found to peak 30 min after the stress stimulus in

another species of the family Scyliorhinidae (DeRoos and DeRoos, 1978), the captured

sharks was left in the plastic container (covered by a dark towel to minimize other

external stressors) for 30 min before blood collection. After 30 min, the shark was

gently removed from the container, its eyes covered with a wet towel and placed with

its ventral side upwards on a flat surface to induce tonic immobility (Brooks et al.,

2011). Once in tonic immobility (<1 min), 0.5 ml of blood was harvested through

caudal venipuncture using 22-gauge hypodermic needles. This quick procedure (< 2

min), is considered to be the least invasive and physiologically taxing method of

collecting blood from sharks, and has been used in many studies assessing the

physiological profile of other shark species (Cooper and Morris, 1998; Mandelman and Skomal, 2009; Skomal and Mandelman, 2012). The blood samples were then analysed immediately with EPOC reader (Woodley Equipment Company ltd.) to obtain measures of glucose, pH, K^+, lactate and pCO_2 as biomarkers of the stress response (Mandelman and Skomal, 2009; Skomal and Mandelman, 2012; Cooke et al., 2013). Similar blood readers thermostatted at 37°C have been used in other recent studies on teleost and elasmobranch fishes, and all pH and pCO_2 values were corrected for temperature (Ashwood et al., 1983, Mandelman and Skomal, 2009; Kneebone et al., 2013; Lennox et al., 2016; Toledo-Guedes et al., 2016).

2.3.3 Post-recovery physiological profiles

The sharks were transported to, and placed as a pair in, an underwater holding pen (transportation time: 79 ± 4.8 min; placement then took < 2 min), where they were given a 24 h recovery period at 14.9 ± 0.17 °C (Tinytag Aquatic 2 temperature logger, Gemini Data Loggers manufacturers). Blood biochemistry parameters have been observed to return to normal after 24 h after a stressor is perceived (DeRoos and DeRoos, 1978; Kneebone et al., 2013; Barragán-Méndez et al., 2019). The holding pen was built of PVC pipes and plastic chicken mesh and was hexagonal, 1 m in height and 3 m in diameter (1.5 m length of each side; Figure 2.2). It was moored at the bottom of a sheltered reef in Mossel Bay (Dollose Reef 34.18° S 22.14° E), at 4 m depth using six small tires filled with cement. One side of the pen had a lockable door to enable sharks to be added or removed by a SCUBA diver.

Figure 2.2: Holding pen for shark 24 h recovery.
Moored at the bottom of a sheltered reef in Mossel Bay, the holding pen (1 m in height, 3 m in diameter) was used to house the sharks for 24 h and allow them to recover from the stress elicited by capture and transport.

The advantage of our approach of using blood samples collected from sharks kept in the underwater pen is that all sharks had been kept under standardised, controlled conditions, in addition to these being relatively stress-free. The validity of our approach was confirmed by the differences we observed in the physiological indicators of stress (see Results). Because all sharks were kept under the same conditions in captivity, this doesn't confound our results indicating that the stress levels in these species are much higher soon after capture than after shark had been left to recover for 24 h in the underwater pen. Moreover, baseline or unstressed physiological profiles of elasmobranch fish are often collected when sharks are kept in captivity (Kneebone et al., 2013; Barragán-Méndez et al., 2019). The evidence from previous studies showing that sharks kept in captivity do provide baseline and unstressed physiological profiles to

use when assessing the response to capture stress suggests that being placed within enclosures after capture is not stress inducing (Frick et al., 2009; Frick et al., 2010a; Kneebone et al., 2013).

Benthic catshark species show strong social behaviour and tendency to aggregate in the wild and under laboratory conditions (Jacoby et al., 2012); therefore we housed the sharks as a pair to create a more natural environment and maximise their recovery after capture. As it was not always possible to catch two individuals of the same species, 12 of the 18 pairs were conspecifics and 6 heterospecifics. After 24 h, one shark at a time was retrieved from the holding pen, and a second sample of 0.5 ml of blood collected. As the blood sample was collected not more than 5 min from the first contact with the shark, it was considered to be a valid approximation of the control profile of blood chemistry profiles that had returned to normal (Brooks et al., 2012). After blood collection, the shark was sexed, its total length measured from the tip of the snout to the tip of the caudal fin, a spaghetti tag inserted on the side of the first dorsal fin to avoid recapture, and the shark released at the site where it was captured.

2.3.4 Statistical analysis

We used linear mixed effects models (lme4 package for mixed model analysis; Bates et al., 2015) to investigate whether there was a significant difference in the concentration of blood biomarkers between stressed and unstressed blood samples for each of the three species. Treatment (stressed vs. unstressed), sex and size were coded as fixed factors to investigate their effects on the change in the concentration of blood biomarkers. Pairing type (conspecific vs. heterospecific) was included as a fixed covariate to prevent our conclusions from being confounded by any unaccounted variability in the data. Shark ID was included as a random factor. Minimum adequate

models were obtained by stepwise deletion of non-significant terms from the full factorial models. The significance of the fixed factors was derived using the package lmerTest based on Satterthwaite's approximations (Kuznetsova et al., 2015). Likelihood ratios tests and AIC values were used to select the best model. Tukey's HSD post-hoc tests were used to compare the three shark species for each of the four biomarkers (glht function of multcomp package; Hothorn et al., 2008). All analyses were performed in R v3.5.1 (R Core Team, 2018).

2.4 Results

Pyjama catsharks showed the highest concentrations in pH and lowest levels of K^+ and lactate in both post-capture and post-recovery blood samples, while leopard catsharks showed highest values of lactate and pCO_2; shysharks showed the highest concentrations of K^+ and similar levels of pCO_2 to pyjama catsharks (Table 2.1). Higher levels of lactate and pCO_2, and lower pH and K^+ were found in stressed compared to sharks post-recovery (Table 2.1). The level of glucose was also somewhat lower in stressed compared to shysharks post-recovery (Table 2.1). Glucose concentrations for pyjama and leopard catsharks were too low for reliable quantification (the EPOC machine was not able to give exact concentrations lower than 1.1 mmol/L).

Table 2.1: Blood values (mean ± s.e.) obtained from the samples taken 30 min after capture (stressed) and after the 24 h recovery period (unstressed), and percentage change between these (negative values indicate a decrease while positive values represent an increase).

Species	n	Treatment	pH	K^+ (mmol l^{-1})	Lactate (mmol l^{-1})	pCO_2 (mm Hg)	Glucose (mmol l^{-1})
P. africanum	15	Stressed	7.09 ± 0.01	4.31 ± 0.08	1.233 ± 0.13	5.19 ± 0.27	<1.1
		Unstressed	7.231 ± 0.02	4.53 ± 0.07	0.476 ± 0.041	3.25 ± 0.14	<1.1
		% change	- 1.99 ± 0.29	- 5.65 ± 2.2	55.1 ± 5.4	36.2 ± 2.39	NA
P. pantherinum	9	Stressed	6.99 ± 0.02	4.62 ± 0.17	2.36 ± 0.33	5.56 ± 0.26	<1.1
		Unstressed	7.15 ± 0.03	4.99 ± 0.14	0.980 ± 0.13	3.49 ± 0.16	<1.1
		% change	- 2.27 ± 0.46	- 8.74 ±3.4	59.9 ± 3.4	36.3 ± 8.8	NA
Haploblepharus spp.	9	Stressed	7.05 ± 0.03	4.64 ± 0.12	1.83± 0.18	5.14 ± 0.13	2.99 ± 0.19
		Unstressed	7.25 ± 0.03	5.07 ± 0.22	0.603 ± 0.10	3.29 ± 0.23	3.57 ± 0.39
		% change	- 2.74 ± 0.28	- 9.08 ± 3.8	63.0 ± 8.6	35.7 ± 4.8	- 19.6 ± 11.8

For all three species, treatment had a significant effect on all biomarkers, except for glucose in shysharks, while pair type had no effect on any of the biomarkers in any of the three species (Table 2.2).

Table 2.2: LMM run on the concentration of bioindicators as a function of treatment (stressed vs. unstressed), size, sex and pair type (conspecific vs. heterospecific) for pyjama sharks (dF = 14), leopard sharks (dF = 8), and shysharks (dF = 8).

Species	Bioindicator	Statistics	Treatment	Size	Sex	Pair type
Pyjama	pH	F	49.4	3.83	0.36	0.34
		p	**< 0.001****	0.072	0.56	0.57
	K+	F	6.24	0.062	0.21	0.22
		p	**0.03****	0.81	0.66	0.065
	Lactate	F	47.1	15.7	0.008	0.039
		p	**< 0.001****	**< 0.001****	0.93	0.84
	pCO2	F	97.6	0.72	0.48	0.43
		p	**< 0.001****	0.41	0.5	0.52
Leopard	pH	F	24.2	0.05	0.34	0.013
		p	**< 0.001****	0.85	0.58	0.92
	K+	F	9.001	18.9	0.32	2.15
		p	**0.01***	**< 0.001****	0.59	0.17
	Lactate	F	26.8	0.28	22.6	1.31
		p	**< 0.001****	0.62	**< 0.001****	0.304
	pCO2	F	4.97	0.002	0.14	0.58
		p	**0.05***	0.97	0.72	0.48
Shysharks	pH	F	83.9	0.85	1.76	0.043
		p	**< 0.001****	0.39	0.24	0.85
	K+	F	6.62	0.21	6.12	0.08
		p	**0.03****	0.66	**0.04***	0.79
	Lactate	F	34.4	0.66	1.81	0.82
		p	**< 0.001****	0.43	0.21	0.38
	Glucose	F	2.48	0.68	0.84	0.22
		p	0.15	0.45	0.4	0.66
	pCO2	F	52.5	5.67	0.037	2.1
		p	**< 0.001****	**0.03****	0.95	0.17

Pyjama and leopard catsharks differed in levels of pH, K^+ and lactate but not in pCO_2 when controlling for treatment (respectively, $z = 2.68$, $p = 0.003$; $z = -2.45$, $p = 0.038$; $z = -4.86$, $p < 0.001$; $z = -1.85$, $P = 0.15$). Leopard catsharks and shysharks differed in levels of pH, lactate and pCO_2 but not in K^+ (respectively, $z = 2.45$, $p = 0.04$; $z = -2.38$, $p = 0.05$; $z = -2.63$, $p = 0.02$; $z = 0.29$, $P = 0.95$). Pyjama catsharks and shysharks differed in levels of K^+ and lactate, but not in pH or pCO_2 (respectively, $Z = 2.78$, $P =$

0.015; Z = 2.41, P = 0.05; Z = - 0.64, P = 0.79; Z = - 1.09, P = 0.52). Pyjama catsharks showed the lowest effect of stress for all the blood biomarkers except pCO_2, which was the lowest in shysharks (Table 2.1). Shysharks showed the greatest effect of stress for all other blood biomarkers (Table 2.1).

In pyjama sharks, the change in the concentrations of lactate was affected significantly by size (Table 2.2), with larger individuals accumulating more lactate in response to capture (Figure 2.3). There was no effect of size on pH, K^+, pCO_2, or of sex on any of the blood biomarkers (Table 2.2; Figure 2.3).
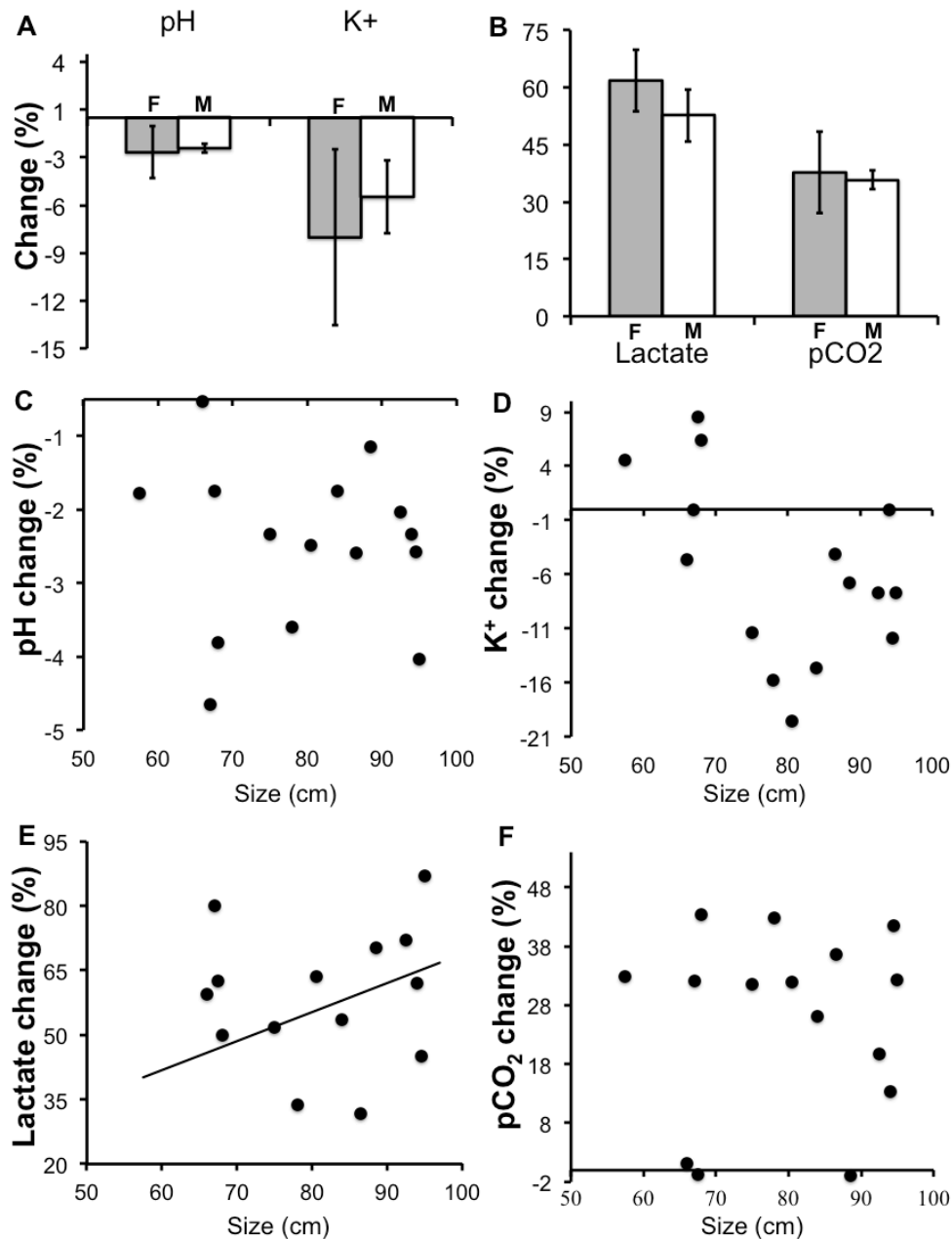
Figure 2.3: Response to stress in pyjama sharks.
Mean ± s.e. percentage change in blood biomarkers (stressed – unstressed) for pyjama sharks. A) and B) show the response for females (F; grey) and males (M; white) for pH and K^+, and for lactate and PCO_2 respectively. C), D), E), and F) show the change in the four blood biomarkers in relation to the size (cm) of the pyjama sharks. Line of best fit indicates a significant relationship.

In leopard sharks, there was a significant effect of size on K^+, with larger sharks

showing the strongest responses to capture for this blood biomarker. There was also an

effect of sex on lactate, as males showed a stronger response to capture compared to

females (Table 2.2; Fig. 2.4). No other effect of size and sex was recorded for the

remaining blood biomarkers.
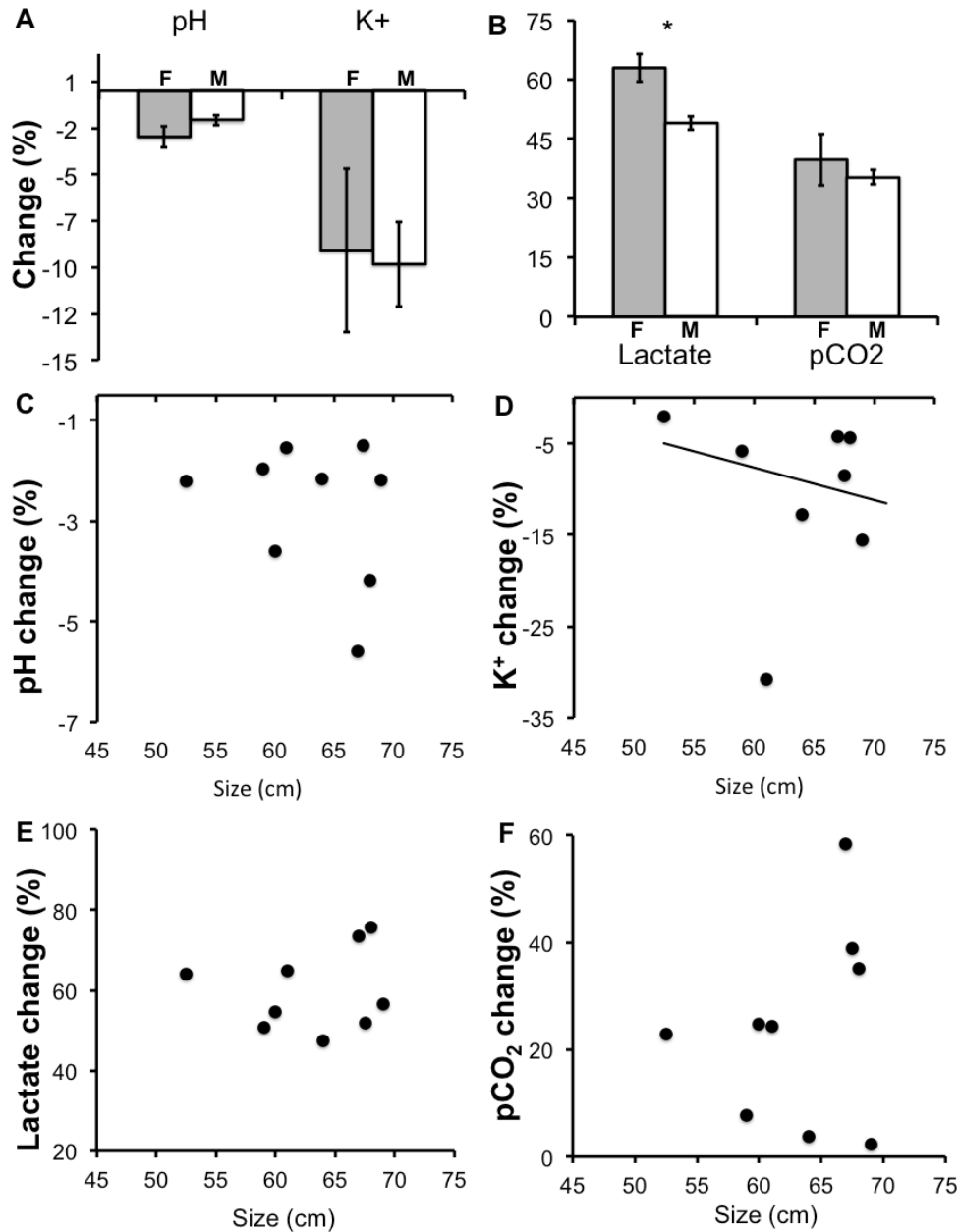
Figure 2.4: Response to stress in leopard sharks.
Mean ± s.e. percentage change in blood biomarkers (stressed – unstressed) for leopard sharks. A) and B) show the response for females (F; grey) and males (M; white) for pH and K^+, and for lactate and PCO_2 respectively. C), D), E), and F) show the change in the four blood biomarkers in relation to the size (cm) of the catsharks. Asterisk (*) and line of best fit indicate a significant relationship.

In shysharks, the change in K^+ was affected significantly by sex with males undergoing the highest change in concentration, but not by size (Table 2.2; Figure 2.5). Larger animals experienced a larger effect of stress on blood pCO_2 (Table 2.2). Sex and size did not affect any of the other biomarkers (Table 2.2).
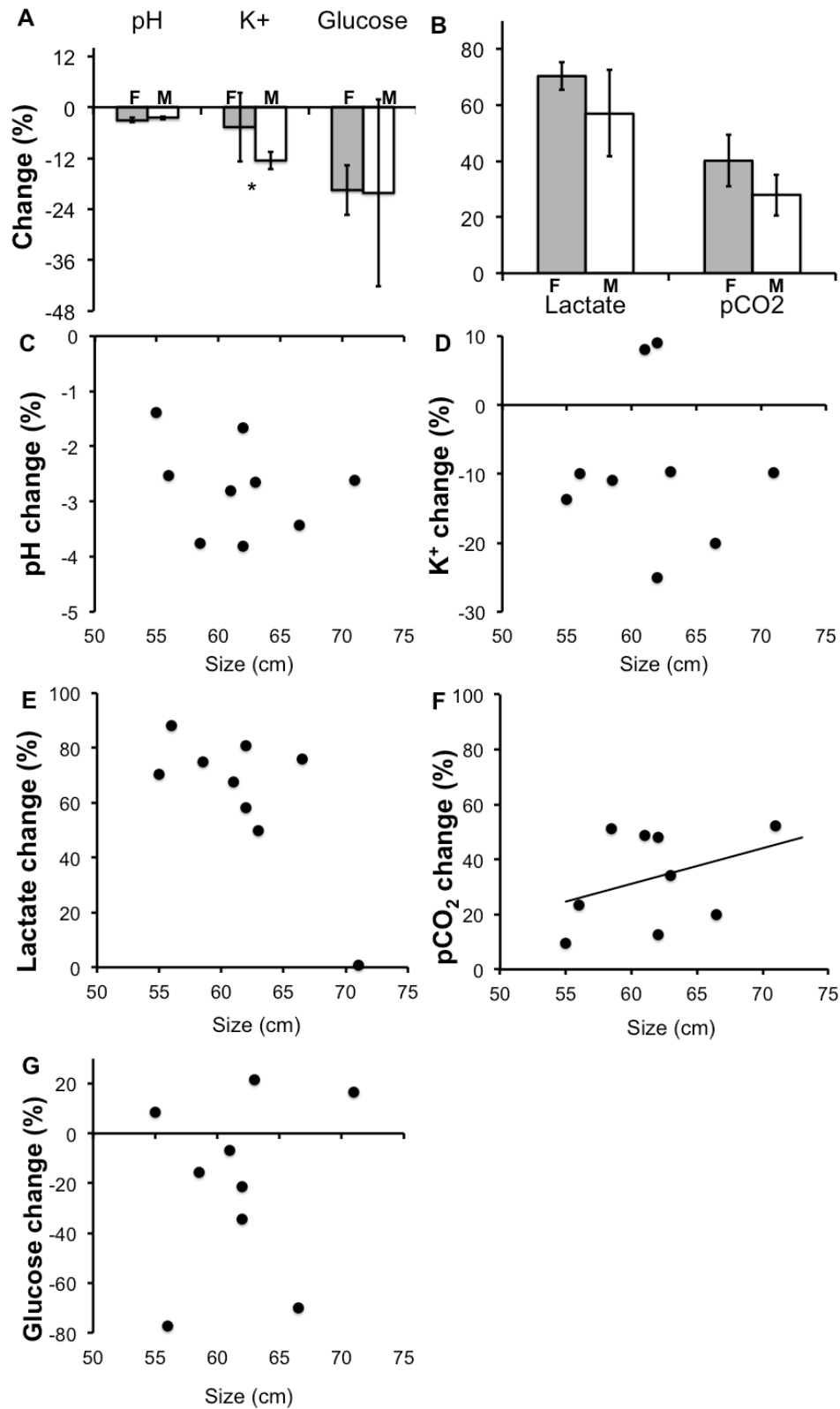
Figure 2.5: Response to stress in shysharks.

Mean ± s.e. percentage change in blood biomarkers (stressed − unstressed) for shysharks. A) and B) show the response for females (F; grey) and males (M; white) for pH, K^+ and glucose, and for lactate and PCO_2 respectively. C), D), E), F) and G) show the change in the four blood biomarkers in relation to the size (cm) of the shysharks. Asterisk (*) and line of best fit indicate a significant relationship.

2.5 Discussion

Capture stress elicited significant changes in blood pH, K^+, lactate and pCO_2 in all sharks studied. The strength of the stress response differed interspecifically and in some cases was affected by the size and sex of the sharks, depending on the biomarker and shark species.

Acute exercise and anaerobic glycolysis likely drove the movement of La^- ions from the muscle into blood circulation, therefore causing an increase in mean lactate concentration after capture (Brooks et al., 2011; Skomal and Mandelman, 2012; Jerome et al., 2018; Barragán-Méndez et al., 2019). Lactate is a highly informative predictor of shark stress because it often correlates with impairment and modifications of behaviour after release (Jerome et al., 2018). As a result, finding a 55-63% increase in lactate in catsharks after a short (3 min) capture event strengthens the concerns about their survival when captured with common fishing practices, which will likely have a stronger impact on shark physiological homeostasis due to longer hooking times and the use of more disruptive fishing gear. Individuals of different size and sex were not equally affected by these metabolic and respiratory perturbations, as their effect depended upon shark species. This is in agreement with previous research finding differences in shark response to capture stress both within and between species (rev. Skomal and Mandelman, 2012). The highest change in concentration of lactate was experienced by larger pyjama catsharks. Larger individuals might exhibit greater struggle and acute exercise while on the hook compared to smaller sharks, or might be affected from a higher rate of accumulation of this metabolite during and after capture. The same trend was observed in male leopard sharks. Males are likely more affected by capture as they have a thinner skin compared to females, which offers less protection against injuries (Dapp et al., 2016; Musyl and Gilman, 2018).

Acute exercise and struggle during capture were the likely cause of the overall increase in blood pCO_2 upon O_2 depletion, lowered gas exchange and ventilation inefficiency (Skomal and Mandelman, 2012). Similarly to pyjama sharks, larger shysharks experienced a greater accumulation of pCO_2, probably because the rate of struggle on the hook or of pCO_2 accumulation in the peripheral circulation increases as sharks increase in size or because larger individuals suffered more acute ventilation inefficiency while on the hook. Consequently, larger shysharks may be more affected to capture stress than smaller individuals because their physiological homeostasis is more disrupted by the acute metabolic and respiratory perturbations indicated by pH levels.

In all four species, the overall increase in lactate and pCO_2 resulting from capture stress likely elicited the decrease observed in blood pH, irrespectively of sex and size (blood acidosis; Skomal and Mandelman, 2012; Bouyoucos et al., 2018b). Acidosis induced by acute stress is expected to cause hyperkalaemia, an increase in blood K^+ following the efflux of potassium ions from the intra- to extracellular compartment (Cliff and Thurman, 1984; Skomal and Mandelman, 2012). However, we found K^+ levels in stressed sharks to be lower than in post-recovery sharks. Since the efflux of potassium ions into the blood only occurs as a result of blood acidosis, it is likely that changes in K^+ take longer to occur compared to the other blood bioindicators. The samples taken 30 min after capturing the sharks were likely too early to capture the change in K^+. In spiny dogfish for example, K^+ concentrations were found to peak 2 h after capture, while other bioindicators like pCO_2 and most electrolytes reached high concentrations far more quickly after capture (Mandelman and Farrington, 2007). Interestingly, the three smallest pyjama sharks and two of the smaller shysharks did show the expected hyperkalemia at the 30 min sampling point, suggesting that it may develop more rapidly

in smaller individuals. On the other hand, larger leopard sharks were affected by the highest change in K^+. This suggests that the rate of K^+ production and accumulation might decrease with size in pyjama catsharks and shyshark. On the other hand, larger leopard sharks might need more time to reabsorb K^+ ions from their peripheral circulations into the intra-extracellular compartment, taking longer to recover after capture stress. In agreement with previous studies, male shysharks showed the highest change in K^+ concentrations, suggesting that males may be suffer more as a result of capture stress as their skin offers less protection against injuries compared to females. Hyperkalemia can disrupt myocardial function and is the most likely cause of mortality in acutely stressed elasmobranchs (Skomal and Mandelman, 2012; Butcher et al., 2015). This aspect of the secondary response to capture stress in catsharks therefore warrants further investigation.

The change in glucose concentrations in captured pyjama and leopard catsharks also deserves further investigation. The ability to mobilize glycogen following a rise in cathecholamine levels to obtain the energy needed for the fight or flight response is integral to survival (Marshall et al., 2012). The average decrease in glucose concentration experienced by shysharks after capture suggests that they may be particularly slow at mobilizing glycogen after a stressor is perceived. Since the concentration of glucose was found to be highest 30 min after capture in nursehound catsharks, also in the family Scyliorhinidae (DeRoos and DeRoos, 1978), it is reasonable to expect the change in concentration of glucose to peak 30 min after capture even in the shysharks. Overall, the effect of stress on glycogen mobilisation needs further testing with more sensitive methods.

The results show that the stress of even a short, 3 min capture event elicits a substantial disruption of physiological homeostasis in all three of the catshark species investigated. Pyjama sharks experienced the lowest change in all the biomarkers of the species examined here, and may therefore be more resilient to capture stress compared to shysharks and leopard sharks. Conversely, shysharks experienced the greatest effect of capture on all the physiological biomarkers that are among the most important determinants of shark survival post capture-and-release (Mandelman and Skomal, 2012; Cooke et al., 2013; Jerome et al., 2018). When stressed, shysharks show an extreme and unique behaviour, as they fully tense their muscles to curl their body into a loop and use their tail to cover their snout. This behaviour might indicate that shysharks are more disturbed by stress compared to leopard and pyjama catsharks, causing shysharks to be the most affected and potentially the least resilient to capture stress.

Overall, all three species showed significant responses to capture stress even after a considerably shorter hooking event compared to demersal and benthic longline fisheries, by which benthic catsharks are mostly affected. Stronger effects will likely be caused by the more prolonged stress resulting from typical demersal fishing practices. While the relationship between physiological stress and survival in these species and sharks in general needs further investigation, it would be prudent for fishing management plans to reflect that a significant proportion of sharks, and indeed other fish, that are caught and released will be negatively affected by the stress of the event, also depending on their sex and size. Fishing quotas, areas, gear and handling practices may all potentially benefit from being adjusted to allow for or reduce capture stress and thereby achieve more realistic fishing management strategies.

3.   Personality in a mesopredator and the effects of the social environment on behavioural syndromes

3.1 Abstract

Consistent differences in behaviour between individuals ('personalities') and behavioural correlations ('syndromes') are ubiquitous across the animal kingdom, and are likely of major adaptive significance. Although there has been growing interest in understanding how animal personalities influence individual fitness and are maintained over time, the effects of this phenomenon on trophic interactions are still poorly understood, especially for vertebrates such as marine predators. Here, we show that a marine mesopredator endemic to Southern Africa, the dark shyshark, exhibits consistent individual behavioural variability in anti-predator responses, coping styles in their reaction to predation stress, and behavioural syndromes within the predator-prey context. We also show that social environment and the contextual experience of predation risk influence the expression of behavioural syndromes. Specifically, paired sharks that showed a weaker anti-predator response also spent longer foraging. Also, predation risk decoupled the correlation between ToE and Activity, but only in paired sharks, showing the effects of the interaction between predation risk and social environment on behavioural syndromes. Our results suggest that personality may directly affect shark fitness and survival by mediating foraging and anti-predator behaviour, which will have consequent multidirectional impacts on the many marine ecosystems where shark mesopredators are important components.

3.2 Introduction

One of the most important recent advances in behavioural ecology in recent years has been the recognition that many animals show individual differences in behaviour that are consistent across time and contexts (i.e. animal personality; Gosling, 2001; Sih et al., 2004a; Biro and Stamps, 2008). Such individual differences in behaviour are no longer simply seen random noise around a population mean, but as being of major adaptive significance in channelling behavioural responses and their interactions with selection pressures (Dall et al., 2004; Sih et al., 2004a; Wolf and Weissing, 2012). Animal personality has been shown in more than 200 species, from primates to fish, and are likely ubiquitous across the animal kingdom (Gosling, 2001, Sih et al., 2004a,b; Réale et al., 2007; Carere and Locurto, 2011; Byrnes and Brown, 2016). Despite the rapid increase in research interest, there remains a paucity of data for large animals, especially marine vertebrates that occupy high trophic positions (Byrnes and Brown, 2016, Finger et al., 2016; Finger et al., 2017).

Marine predators such as sharks are of particular importance because of their top-down trophic effects that enhance biodiversity and influence many aspects of marine ecosystem dynamics including prey population size, foraging behaviour and habitat use (Heithaus and Dill, 2006; Martin and Hammerschlag, 2012; Byrnes and Brown, 2016). Because of their K-selected life-history strategy, sharks are particularly vulnerable to anthropogenic pressures such as fishing (Stevens et al., 2000; Myers et al., 2007; Afonso and Hazin, 2014; Danylchuk et al., 2014; Finger et al., 2016; Musyl and Gilman, 2018). As personality influences species interactions within the predator-prey context, information on the ecological consequences of personality for shark fitness, foraging efficiency and anti-predator behaviour is key to broaden our understanding of their ecological role in marine ecosystems, especially considering the steep decline in

many shark populations exploited in global fisheries (Stevens et al., 2000; Réale et al., 2007; Conrad et al., 2011; Mittelbach et al., 2014; Vaudo et al., 2014; Byrnes and Brown, 2016; Finger et al., 2016; Towner et al., 2016; Finger et al., 2017; Finger et al., 2018).

Individual variability in sharks has been investigated for a few species: boldness and laterality in Port Jackson sharks (Byrnes and Brown 2016; Byrnes et al., 2016); social networks in spotted catsharks (Jacoby et al., 2014); diel movement and social axis in lemon sharks (Wilson et al., 2015; Finger et al., 2016; Finger et al., 2018); vertical movement in tiger sharks (Vaudo et al., 2014). Inter-individual differences in foraging strategies and ontogenetic trophic shifts have been inferred using isotope analysis in juvenile lemon sharks (Hussey et al., 2017), spurdogs, bullsharks and Caribbean reef sharks (Matich et al., 2019). Yet, other important sources of inter-specific variability including personality remain overlooked for most species (Kalinkat, 2014; Toscano and Griffen, 2014). Although some evidence has been found for the short-term individuality in hunting mode in white sharks (Towner et al., 2016), and in foraging strategies in juvenile lemon sharks (Hussey et al., 2017), to date, no study has systematically investigated the occurrence of shark personality within the predator-prey context. This is due to the difficulty of obtaining replicated behavioural measures for individuals in the field that are necessary to quantify personality (Jacoby et al., 2014; Wilson et al., 2015; Byrnes and Brown, 2016; Byrnes et al., 2016; Finger et al., 2016; Pouca and Brown, 2019).

One of the proximate causes of consistent personality types is the fundamental difference between individuals in their underlying physiological traits and response to stress (i.e. coping styles; Koolhaas et al., 1999; Øverli et al., 2007; Baugh et al., 2013; Mittelbach et al., 2014). The physiological basis of behaviour is likely one of the

underlying origins of the correlations between several ecologically important personality types (behavioural syndromes) that have been observed for many animal species in different situations (e.g. feeding in the presence or absence of predation risk; Sih et al., 2004a,b; Conrad et al., 2011; Mittelbach et al., 2014). In teleost fish, a connection has been evidenced between stress hormones and multiple behavioural axes including activity, fear response and exploration under predation pressure (Schulkin et al., 2005; Lowry and Moore, 2006; Carpenter et al., 2009; Archard et al., 2012). In elasmobranchs, there is evidence for individuality in coping styles in Port Jackson sharks, with boldness correlating with stress reactivity, although the physiological component of the stress response was not measured (Byrnes and Brown, 2016). The physiological response to stress in sharks has received significant attention in the assessment of survival post capture-and-release, offering the possibility of using similar techniques to investigate the individuality of coping styles in this taxon (Mandelman and Skomal, 2009; Brooks et al., 2012; Skomal and Mandelman, 2012; Cooke et al., 2013; Chapter 2).

Behavioural syndromes in teleost fish are often influenced by an individual's social environment (Conrad et al., 2011; Mittelbach et al., 2014). In sharks, the social environment is known to directly affect foraging behaviour (Kimber et al., 2009; Jacoby et al., 2012; Pouca and Brown, 2019), but behavioural syndromes still remain an unexplored area of their behaviour. Contextual experience has been observed to reshape and modify behavioural correlations in some teleost species, as individuals moderately adjust their behavioural response according to the perceived stimuli (Sih et al. 2004b, Frost et al., 2007). For example, experiencing predation risk can alter, decouple or generate correlations between behavioural axes and anti-predator behaviour (Sih et al., 2004b; Bell and Sih, 2007). Predation risk can affect long- and short-term phenotypic

variance through behavioural modifications, imposing opportunity costs for animals within the food web arena, as some might choose to avoid risky areas or seek refuge hence having to reduce the time spent foraging (Dingemanse et al., 2009; Sommer-Trembo et al., 2016). Information on the effect of predation risk as a relevant contextual experience on behavioural correlations can provide valuable insight on individuals' ability to adapt their behaviour in response to changes in predation pressure or abundance of resources (Stamps and Groothuis, 2010). Investigating the relationship between social environment, predation risk and behavioural syndromes will bring valuable insight on the ecology of sharks, especially for species like benthic catsharks that tend to aggregate and share their habitats with conspecifics and are often predated on by other sharks or mammals (Jacoby et al., 2012; Fallows et al., 2015; Pouca and Brown, 2019).

Here we used the dark shyshark as a model system to investigate shark personality and behavioural syndromes within the predator-prey context. Dark shysharks belong to the Scyliorhinidae family of catsharks endemic to Southern Africa, and are important mesopredators within coastal ecosystems formed by kelpbeds (Dainty, 2002; Human, 2007). Catsharks thrive in captivity and are often used as model systems in behavioural studies (Kimber et al., 2009; Kimber et al., 2014; Jacoby et al., 2014; Byrnes and Brown, 2016; Byrnes et al., 2016; Finger et al., 2017; Pouca and Brown, 2019). Moreover, benthic catshark species show strong social behaviour and tendency to aggregate in the wild and under laboratory conditions (Jacoby et al., 2012). We aimed to (1) establish whether dark shysharks show consistent inter-individual variability in anti-predator behaviour; (2) assess if the secondary response to stress relates to inter-individual variability in anti-predator behaviour (i.e. coping style); (3) establish the presence of behavioural syndromes across different contexts (feeding in the presence

and absence of predation risk), and (4) determine if differences in the social environment (solitary vs. paired) influence these correlations.

3.3. Material and methods

This study was conducted between the 8th of January and the 13th of June 2018, in Hermanus, South Africa (34.4207^{o} S 19.243^{o} E). All research was carried out in collaboration with The South African Shark Conservancy (SASC), under research permit RES2018/59 issued by the South African Department of Agriculture, Forestry and Fisheries.

The fishing hand-line technique was used to catch all individuals for the study. Between 2 and 6 baited lines with 6/o circle hooks were deployed from the shore near SASC's laboratory facility in Hermanus (South Africa; 34.4208^{o} S 19.244^{o} E). Once hooked, a shark was retrieved to the shore in about 1 min, and placed in a plastic container (80 x 40 x 40 cm) filled with fresh sea water. The sharks were quickly transported to the oval experimental tanks located in the laboratory facility (180 cm x 256 cm x 72 cm). The experimental tanks (Figure 3.1) contained approximately 1700 L water supplied by a continuous inflow and outflow of sea water, maintaining stable and natural oxygen, temperature and pH parameters (95.6 ± 0.92 %; 14.3 ± 0.22 °C; 7.84 ± 0.014 respectively). The tanks had a centre divide forming two connected sections. Their bottom surface was covered with sand, and the environment enriched with 6 large rocks and an opaque, plastic shelter (65 cm x 30 cm) to provide refuge. Dark plastic sheets were used to cover the ceiling over the tanks and the sides that were not adjacent to the wall in order to minimise external disturbance. A grid of 66 squares (17.5 x 17.5 cm) built with chicken mesh was placed over each of the two tanks to obtain a measure of

sharks' rate of movement (Finger et al., 2016). After capture, sharks were allowed to

acclimate for 24 h.
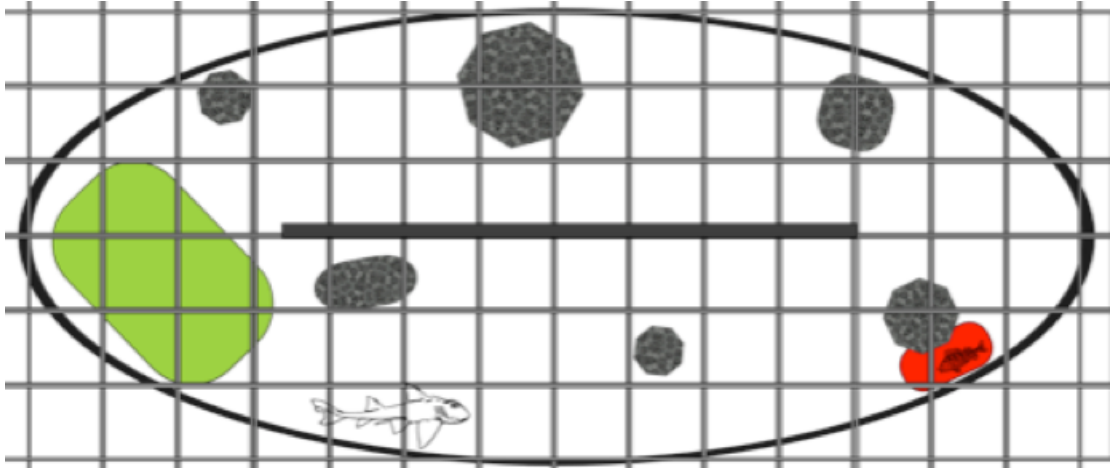
Figure 3.1: Schematic illustration of camera view of experimental tanks.
The red rectangle represents prey item, the green rectangle represents the opaque
shelter, and grey shapes represent rocks. Central line across diameter represents central
divide. Grey lines represent perforated plastic mesh grid.

A total of 48 individuals were caught (19 pairs, 10 solitary; 19 females and 29 males).

Shysharks were kept in the experimental tanks for a maximum of 5 days for the

experiments, and fed a portion of sardine (2% of body weight) after the experiments

shortly before being released back in the natural environment. At the end of the

experiments, sharks were sexed, their total length measured from the tip of the snout to

the tip of the caudal fin, a spaghetti tag inserted on the side of their first dorsal fin to

avoid subsequent recapture, and released where they were captured. The tanks were

emptied and rinsed to remove any chemical cues, the sand replaced by fresh sand, and

filled again with clean sea water.

3.3.1 Experiment 1

A total of 15 sharks were used in Experiment 1 (10 males TL = 59.5 ± 1.6 cm; 5

females TL = 48.4 ± 0.3), all housed as pairs (8 mixed sex, 3 same sex). We could

differentiate between individuals, as they were always slightly different in size. We first measured blood glucose levels to assess initial hunger state. To assess personality, on day 1we tested the behavioural response of sharks to a simulated predator threat (see below) on 10 occasions (Figure3.2)..We then determined the relationship between shark physiology or response to stress, and their behavioural responses to prey stimuli on day 2 and to predator stimuli on day 3 to help us investigate whether physiology predicts variation in personality across individuals and find evidence of coping styles in this shark species (Figure 3.2).

To measure blood glucose, a 0.5 ml blood sample was collected via caudal venepuncture 24 h after capture (Skomal and Mandelman, 2012; Chapter 2). This sample was taken within 2 min of disturbance and was therefore a realistic approximation of the stress-free physiological status of the sharks, because the secondary stress response takes longer than 2 min to become detectable in the peripheral circulation and is expected to cease within 24 h after capture (DeRoos and DeRoos, 1978; Cooper and Morris, 1998; Mandelman and Skomal, 2009; Brooks et al., 2012; Skomal and Mandelman, 2012; Chapter 2). Blood samples were analysed immediately with a EPOC reader (Woodley Equipment Company ltd.) to obtain levels of glucose (Lennox et al., 2016; Toledo-Guedes et al., 2016). Glucose levels in blood vary with appetite and satiation in sharks and other teleost fish (Fletcher, 1984; Sims, 1994; Boujard et al., 1993; Le Bail and Bœuf, 1997). Since gastric emptying in the shysharks and other catshark species occurs between 24 h and 100 h (Sims et al., 1996; Dainty, 2002), glucose concentrations obtained within a 24 h window are expected to accurately reflect shark hunger state. After blood collection, sharks were left overnight.
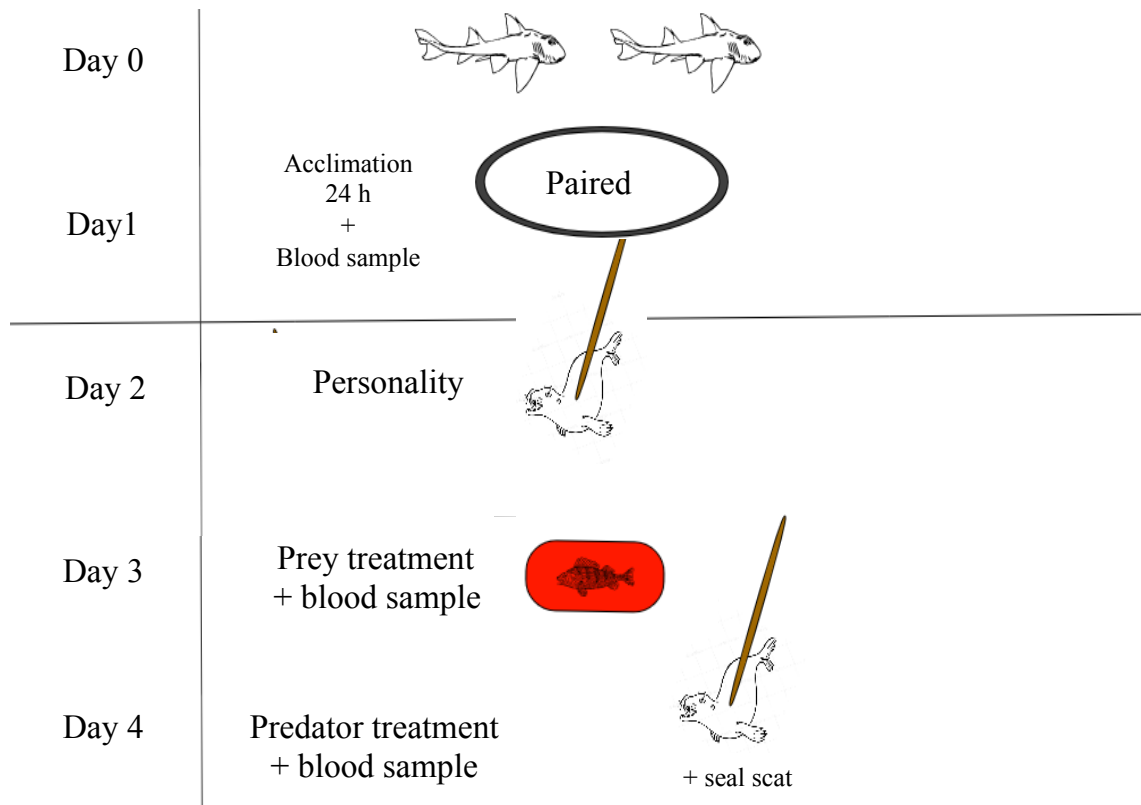
Figure 3.2: Schematic illustration of Experiment 1 layout.
On Day 0, sharks were caught, placed in the experimental tank and left to acclimate for 24 h. On day 1, the first blood sample was taken to quantify glucose as a measure of shark satiation. On day 2, shark behaviour was observed when exposed to predation risk (model predator i.e. inflatable seal) on 10 consecutive occasions to assess personality. On day 3, shark behavioural responses were recorded when given a prey stimulus (sardine head and live klipfish in perforated container) and then a blood sample was taken. On day 4, shark anti-predator responses were recorded when exposed to two cues of predation risk (visual cue i.e. model predator; olfactory cue i.e. 2 ml of seal scat diluted in sea water) and then a blood sample was taken. On days 3 and 4, physiology and behavioural responses were used to find evidence of the presence of coping styles in this species.

Table 3.1: Description of recorded behavioural responses for all 48 dark shysharks observed in the laboratory when presented to either a predator stimulus (inflatable seal). and/or a prey stimulus (sardine head and live klipfish).

Behaviour	Description	Trial
Rest	Total time (s) spent without swimming, either outside or inside the shelter (when present)	All
Locomotion	Total time (s) spent swimming either on the bottom or along the edges of the tank	
Rate of Movement	Total number of different grids visited during locomotion as a proxy of the shark's rate of movement (Finger et. al., 2016)	
Speed	Average speed during observation period calculated as grids/s	
Bottom swim	Total time (s) spent swimming right above the sandy substrate of the tank	
Nosing	Time spent by the shark using snouth to make contact with prey container	Prey, Exp 1 - Day 2 Prey, Exp 2 - Day 1 Prey + Predator, Exp 2 - Day 2
Force	Time spent by the shark using snouth to attempt pushing prey container	
Total duration of interaction	Sum of nosing and force	
Total # of interactions	Total number of times the shark makes contact with prey container	
Average Duration	Average duration of each nosing interaction	
Ignore	Shark behaviour remains unchanged upon presentation of model predator	Predator x 10, Exp 1 - Day 1 Predator, Exp 1 - Day 3 Prey + Predator, Exp 2 - Day 2
Swim Away	Shark swims away from model predator without changing swimming speed	
Escape	Shark swims away from model predator increasing swimming speed	

3.3.1.1 Day 1: do shysharks show personality?

On day 1 (Figure 3.2), we tested the behavioural response of the sharks to a simulated predator model on 10 consecutive occasions. The shelter was removed from the tank 30 min before the onset of the first assay to have full access to the sharks. As simulated predators have been used successfully to investigate fish responses to predation risk (Bell, 2005; Bell and Sih, 2007), a plastic inflatable seal was used as the model predator to provide a visual stimulus of predation risk. Cape fur seals are natural predators of shysharks (personal observations; Martin, 2004; Fallows et al., 2015). By maneuvering the model predator with a rigid bamboo cane from behind the dark plastic sheets, shark behavioural responses (i.e. escape swim, Table 3.1) to the model seal predator were

observed and could therefore be easily quantified to infer anti-predator behaviour. The model seal was presented to each pair of sharks 5 times, after which the behaviour of the sharks was recorded for 10 min with a GoPro (Hero 6 black, 1080 p; GoPro Inc.) mounted above the experimental tank. The sharks were then left for 30 min before being retested. We carried out this assay 10 times with each pair of sharks. Measures of their anti-predator behaviour were subsequently quantified from video recordings (Table 3.1). After the tenth assay, the shelter was reintroduced into the tank and the sharks were left overnight.

3.3.1.2 Days 2 & 3: does individual variation in physiology predict variation in personality?

On day 2 (Figure 3.2), we quantified the behavioural response of the sharks to prey. A sardine head (*Sardinops sagax*) and a live klipfish (*Clinus superciliosus*) were introduced in the tank within a perforated, clear plastic container at the opposite end to the shelter. A small rock was placed inside the clear plastic container to provide extra weight and stability, while one of the large rocks already in the tank was used to firmly hold the prey container externally against the wall and prevent the sharks from moving the container or gaining access to the prey stimuli. The blood from the sardine provided an olfactory cue, while the klipfish added visual and electromagnetic cues to induce foraging behaviour in the sharks (Kimber et al*.,* 2009; Kimber et al., 2014). Once the plastic container was introduced into the tank, shark foraging behaviour was recorded for 30 min with the GoPro mounted over the tank. The behavioural responses were subsequently quantified from the video recordings (Table 3.1). At the end of this period, a 0.5 ml blood sample was collected from each shark as before to measure glucose levels as an indicator of shark satiation when foraging behaviour was observed. After blood collection, the sharks were left overnight.

On day 3 (Figure 3.2), the model predator was presented to each shark. As teleost fish and other sharks show anti-predator responses when exposed to the faeces of their predators (Rasmussen and Schmidt, 1992; Brown et al., 1995a; Brown et al., 1995b), we added 2 ml diluted seal scat (5 g / 20 ml) to the tank with a syringe directly above the sharks as an olfactory cue to reinforce the risk of predation. We did not add seal scat on day 3 during the 'personality' part of Experiment 1 because we did not want to introduce bacteria and waste into the experimental tanks where sharks were kept for another 2 days after the experiment. Also, we did not want the results collected in the following experiments to be confounded by any residue of seal scat. The behaviour of the sharks was recorded for 30 min, and subsequently quantified from the video recordings (Table 3.1). At the end of the 30 min trial, a blood sample was collected from each shark as before to measure glucose, pH, K^+, and pCO_2 as bioindicators of the secondary stress response (Skomal and Mandelman, 2012; Cooke et al., 2013; Chapter 2). The response to a stressor becomes detectable in the peripheral circulation of sharks 30 min after a stressor is perceived (DeRoos and DeRoos, 1978; Chapter 2). All pH and pCO_2 values were corrected for temperature following previous studies on sharks and teleost fish (Ashwood et al., 1983, Mandelman and Skomal, 2009; Brooks et al., 2012; Kneebone et al., 2013; Lennox et al., 2016; Toledo-Guedes et al., 2016; Chapter 2).

3.3.2 Experiment 2: behavioural syndromes and social environment

A partially crossed experiment was carried out to investigate the effects of social environment on behavioural correlations (i.e. syndromes) of dark shysharks within the predator-prey context. Each shark was placed in one of the two social conditions: paired (9 females TL = 51.9 ± 4.0 cm; 13 males TL = 51.8 ± 1.4 cm) or solitary (4 females, TL = 53.5 ± 3.6 cm; 7 males = 55.7 ± 1.6 cm), and then exposed to two situations, 1) a prey

stimulus only on day 1, and 2) both prey and predator stimuli on day 2 (Figure 3.3). Following this design illustrated in Figure 3.3, we determined whether behavioural correlations between different aspects of the shark responses to prey and predator stimuli differed depending on the social environment i.e. on whether sharks were tested alone or when paired with a conspecific.
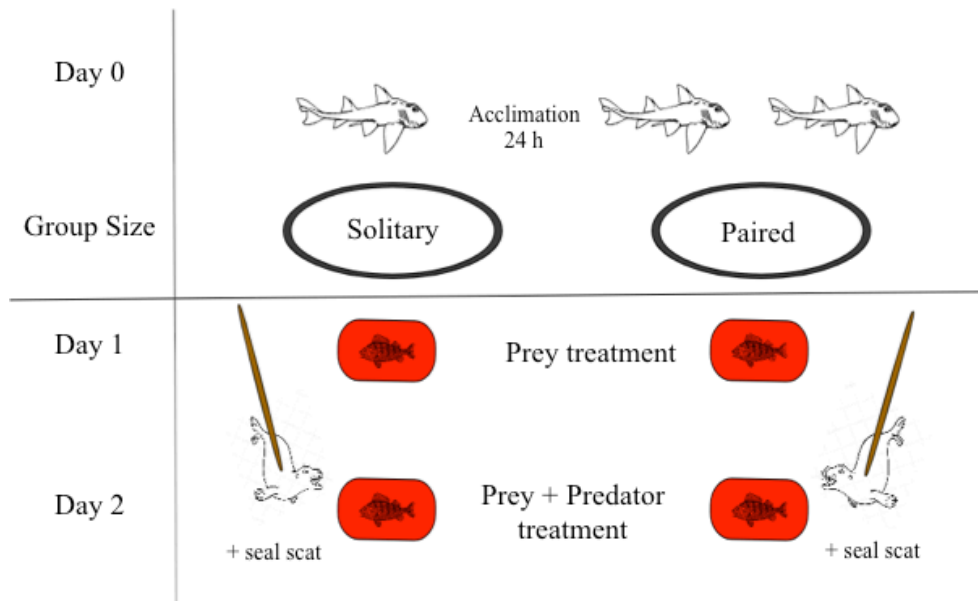
Figure 3.3: Schematic illustration of Experiment 2 layout.
On Day 0 sharks were assigned to a group size maintained for the entire duration of experiment 2. On Day 1, shark behaviour was observed when exposed to the prey stimulus (sardine head and live klipfish in perforated container). On day 2, shark behavioural responses were recorded when exposed to the prey stimulus, as well as being exposed to cues of predation risk (model predator i.e. inflatable seal; olfactory cues i.e. 2ml of seal scat diluted in sea water).

On day 1 (Figure 3.3), the sharks were exposed to the prey treatment as in Experiment 1. Once the plastic container was secured in the tank, shark foraging behaviour was filmed for 20 min with the GoPro mounted over the tank. The behavioural responses were subsequently quantified from video recordings (Table 3.1). At the end of the trial, the sharks were left overnight.

On day 2 (Figure 3.3), the sharks were exposed to the predator treatment as in Experiment 1, but with the prey stimuli also present. Once a shark approached the prey item for the first time, the predator model was presented, and 2 ml of diluted seal scat was added to the tank. For the next 20 min, each time a shark approached the prey item (within the 2 x 2 grids surrounding prey) it was presented with the predator stimulus. If a shark no longer showed a response to the model predator after 15 presentations, the predator stimulus ceased. The 20 min trial was filmed with the GoPro mounted above the tanks, and the behavioural responses were subsequently quantified from video recordings (Table 3.1).

3.3.3 Measures of behavioural responses

We compiled the behavioural responses of interest from video recordings using the event-logging software BORIS (Behavioural Observation Research Interactive Software) allowing user-specific coding of behaviours (Friard and Gamba, 2016). Behavioural responses were logged according to the ethogram established in Table 3.1. In addition, time of first emergence (ToE) from the shelter during or after predator threat treatments was recorded as a proxy of boldness because it describes the inclination of an individual to explore a familiar yet potentially dangerous environment (Byrnes and Brown, 2016). Failure to emerge from the shelter was assessed from the video recordings and occurred when an individual was not seen swimming or resting around the tank at any point during the trial. Two different individuals of 15 sharks failed to emerge from the shelter before the end of one of the 2 trials of Experiment 1, but never in both trials. Similarly, one of 33 sharks failed to emerge from the shelter before the end of on of the trials of Experiment 2. In these three occasions, the individuals were recorded as the final point of observation. The total time spent by the sharks resting together in Experiment 1 was also recorded as a measure of their

sociability. All behaviours were scored by the same observer in each experiment for consistency.

Anti-predator behaviour was characterized according to a standardized score to test whether sharks showed inter-individual differences in their response to the simulated predator threat. Ignore, swim away and escape (Table 3.1) were assigned a value of 1 to 3 respectively (1 = passive response; 3 = active response), with the mean value for each shark in each trial then being calculated.

3.3.4 Statistical analyses

All statistical analyses were performed in IBM SPSS Statistical version 25 (IBM Corporation, New York, USA) and R v3.5.1 (R Core Team, 2018).

3.3.4.1 Experiment 1: Do shysharks show personality?

We used mixed effects models to establish whether shysharks displayed significant inter-individual variability in their anti-predator behaviour. Generalized mixed effects models (GLMM) with negative binomial distribution were used on continuous responses that showed heteroscedasticity of the residuals, on sociability, and on responses that were count data using the glmmTMB function of the glmmTMB package (Brooks et al., 2017). Linear mixed effects models (LMM) were used to test mean speed and the anti-predator behavioural score using the lme4 package for mixed model analysis (Bates et al., 2015). The latter were square root-transformed to achieve homoscedasticity of the residuals. Shark ID was included as the random factor in all models. Trial number was included as a fixed factor, while total length, sex and whether the sharks were in single- or mixed-sex group were included as covariates (Kimber et al., 2009). Glucose levels in the blood samples taken on the previous day were also

included as a covariate to control for possible differences in shark appetite. Minimum adequate models (MAM) were obtained by stepwise deletion of non-significant terms from full factorial models. The significance of the fixed factors was calculated with the package lmerTest based on Satterthwaite's approximations (Kuznetsova et al., 2015). In LMM, the significance of the random factor was calculated using the function exactLRT from the package RLRsim comparing the MAM's with and without shark ID as random factor over 10000 permutations (Scheipl et al., 2008). In GLMM, the significance of the random term was calculated from the MAM using LRT comparing the models with and without shark ID as random factor.

Repeatability of the behavioural responses was calculated with the rptR package over 10000 permutations (Nakagawa and Schielzeth, 2010; Stoffel et al., 2017). To detect habituation, we performed the same analysis using a subset of the data: (i) the 1st and the 2nd trial, (ii) the 1st and the last trial, and (iii) the 9th and 10th trial. We calculated habituation rate by extracting the slope of the linear regression between assay number and the anti-predator score for that assay (Finger et al., 2016). To investigate individual variability in habituation, habituation rate was linearly regressed against the anti-predator score calculated for each shark in assay 1.

3.3.4.2 Experiment 1: Does individual variation in physiology predict variation in personality?

Principal component analysis (PCA) with varimax rotation (Tabachnick and Fidell, 2001; Dingemanse et al., 2007) was used to collapse behavioural responses into behavioural axes. The analysis was run separately for each treatment in Experiment 1, and with pooled data for solitary and paired individuals in Experiment 2, but separately for each treatment. Each shark was included only once in each PCA analysis to avoid

pseudoreplication. The calculated values for the first principal component (PC1) were used for further testing. Values for Kaiser-Meyer-Olkin (KMO) test and statistics for Bartlett's test of sphericity are given as a reference for sampling adequacy and validity of the overall analysis (Table 3.3). General linear models were used to determine if sex, total length, and group sex explained any of the variability in the first principal components calculated for Activity, Foraging, ToE, sociability, and anti-predator behavioural score for each treatment (Table S3.1).

The relationship in Experiment 1 between plasma glucose and Foraging measured after the prey treatment was investigated with Pearson correlation tests to determine if differences in hunger were the underlying drivers of shark foraging behaviour. In the predator treatment of Experiment 1, Pearson Correlation tests were used to investigate how the variation in shark secondary response to predation stress was associated with inter-individual variability in anti-predator behavioural score and Activity after the stressor is perceived. The secondary response to stress was represented by the concentration of the four biomarkers measured at the end of the 30 min predator treatment.

3.3.4.4. Experiment 2: behavioural syndromes and social environment

Principal component analysis (PCA) with varimax rotation (Tabachnick and Fidell, 2001; Dingemanse et al., 2007) was used to collapse behavioural responses collected in Experiment 2 as in Experiment 1. Values for Kaiser-Meyer-Olkin (KMO) test and statistics for Bartlett's test of sphericity are given as a reference for sampling adequacy and validity of the overall analysis (Table 3.3). We used Pearson correlation tests to determine if sharks showed syndromes in their behavioural axis within the same context (prey stimulus) and in between the two situations (i.e. with or without predation risk). General linear models were used to test whether the nature and occurrence of

behavioural syndromes varied with a shark's social environment. Activity in the prey only treatment was included as the dependent variable, while the interaction between social environment (solitary vs. paired) and Foraging was coded as the independent variable. A significant interaction is expected if the social environment has an effect on the correlation. The same analysis was performed for time of exit (ToE) and Activity in the prey only treatment, and then for ToE and Foraging, Activity and Foraging, Activity and anti-predator behavioural score, Foraging and anti-predator behavioural score, ToE and Activity, and ToE and Foraging in the prey and predatory treatment.

3.4 Results

3.4.1 Experiment 1: Do shysharks show personality?

Shysharks showed highly significant inter-individual differences in their behavioural responses when controlling for glucose concentrations, size, sex and group sex (Table 3.2).

Table 3.2: Results from linear mixed effects models and repeatability analysis (rpt) of shark behavioural responses. χ2 were calculated with LRT tests on GLMM with and without ID as a random factor, while LRT values were obtained with the exactLRT function on LMM. Rpt values were obtained with rptr packages. Data are for the 15 dark shysharks housed in pairs in the lab when presented with the model predator (inflatable seal) in each of 10 consecutive 10 min Assays.

Behaviour	All Assays	Assay 1 vs 2	Assay 9 vs 10	Assay 1 vs 10
Rest	χ^2 = 66.71, p < 0.001, dF = 6 rpt =0.61, **p = 0.001**	rpt = 0.72 **p = 0.005**	rpt = 0.87 **p = 0.001**	rpt = 0.71 **p = 0.002**
Locomotion	χ^2 = 127.33, p < 0.001, dF = 7 rpt = 0.62, **p = 0.01**	rpt = 0.725 **p = 0.006**	rpt = 0.87 **p = 0.001**	rpt = 0.71 **p = 0.002**
Rate of Movement	χ^2 = 121.32, p<0.001, dF = 7 rpt = 0.49, **p = 0.02**	rpt = 0.61 **p < 0.001**	rpt = 0.32 p = 0.24	rpt = 0.39 p = 0.36
Speed	LRT = 43.79, p < 0.001, dF = 4 rpt =0.75, **p = 0.001**	rpt = 0. 72 **p = 0.001**	rpt = 0. 76 **p = 0.001**	rpt = 0. 557 **p = 0.03**
Bottom swim	χ^2 = 119.7, p < 0.001, dF = 7 rpt = 0.568, **p = 0.001**	rpt = 0.65 **p = 0.007**	rpt = 0.85 **p = 0.001**	rpt = 0.625 **p = 0.008**
Ignore	χ^2 = 14.7, p<0.001, dF = 5 rpt = NA, p = 1	rpt = 0 p = 1	rpt = 0.05 p = 0.3	rpt = 0 p = 1
Swim Away	χ^2 = 24.65, p<0.001, dF = 6 rpt = 0.35, **p = 0.01**	rpt = 0 p = 1	rpt = 0.23 p = 0.18	rpt = 0 p = 1
Escape	χ^2 = 0.56, p = 0.41, dF = 6 rpt = 0.041, p = 0.2	rpt = 0.2 p = 0.14	rpt = 0 p = 1	rpt = 0.19 p = 0.12
Score	LRT = 45.15, p<0.001, dF = 4 rpt = 0.43, **p=0.001**	rpt = NA p = 1	rpt = 0.8 **p = 0.003**	rpt = 0.39 p = 0.12
Rest Paired	LRT = 46.9, p<0.001, dF = 5 rpt = 0.45, **p=0.001**	rpt = 0.54 **p = 0.03**	rpt = 0.51 **p = 0.039**	rpt = 0.038 p = 0.5

Shark activity in terms of total locomotion, time spent bottom swimming, mean speed of swimming, and time spent resting varied consistently between individuals and were significantly repeatable in all cases (Table 3.2, Figure 3.2).
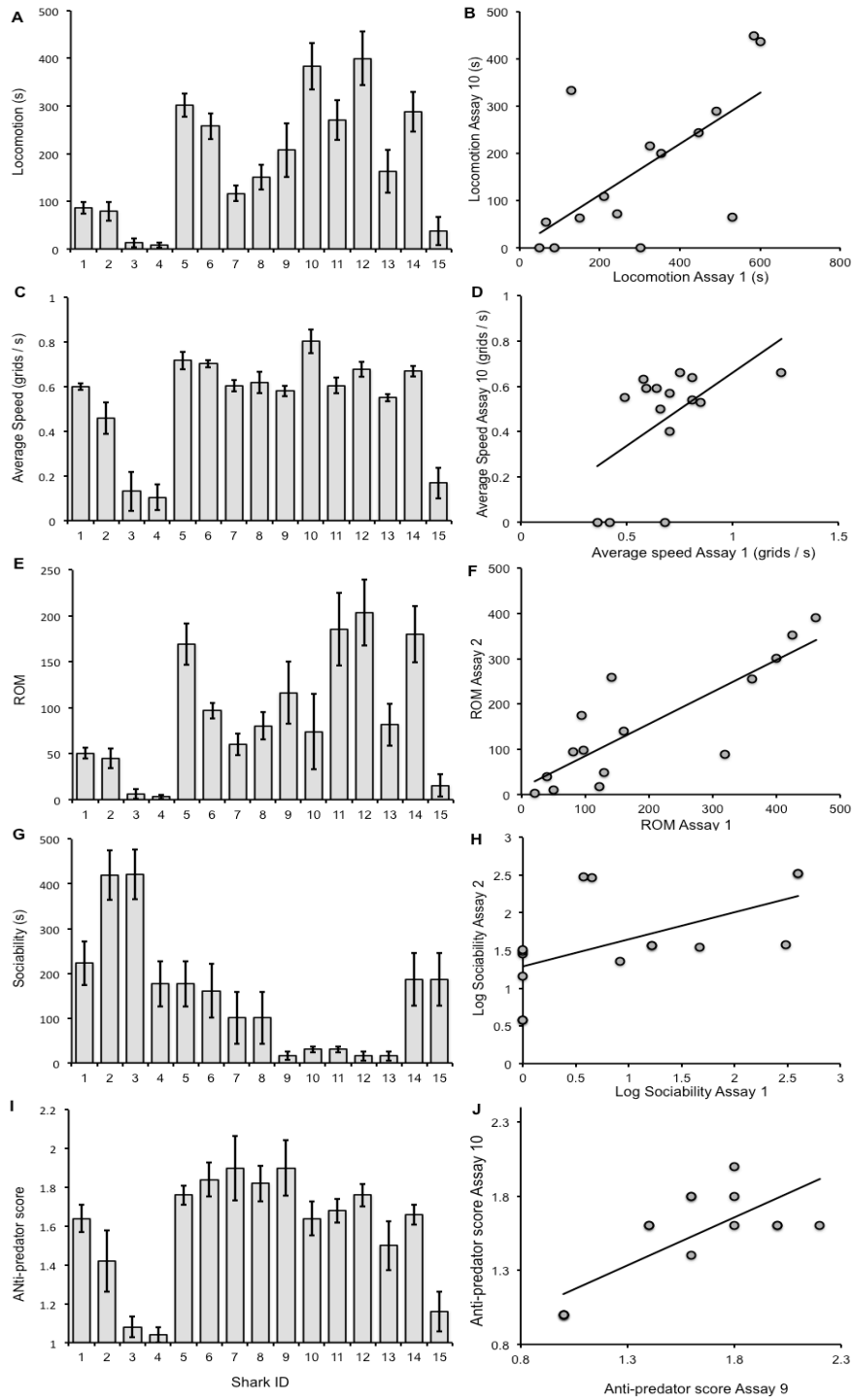
Figure 3.4: Individual variability and repeatability in dark shyshark anti-predator response. Panels in the left column show mean ± s.e. of locomotion, speed, rate of movement (ROM), sociability and anti-predator score. Panels in the right show significant correlations between two sequential trials (which indicate repeatability) for locomotion and average speed between Assays 1 and 10 (B and D), rate of movement, and sociability between Assays 1 and 2 (F and H), anti-predator behavioural score between Assays 9 and 10 (J). Repeatability values are reported in Table 3.2. Behavioural responses were collected in the lab for 15 shysharks, tested as pairs, when presented with the model predator (inflatable seal) in each of 10 consecutive 10 min Assays.

Rate of movement (ROM) was highly variable between individuals, and was significantly repeatable both when all assays were included in the analysis and when assays 1 and 2 were compared, but not when assay 1 and 10, and assay 9 and 10 were compared. Individual sharks also differed significantly in the time they spent resting in a pair. This measure of sociability was highly repeatable across all assays, and between assays 1 and 2, and assays 9 and 10, but not between assays 1 and 10 (Figure 3.2).

Sharks consistently differed in their anti-predator behavioural score, which was highly repeatable when all assays were included in the analysis, and when assays 9 and 10 where compared (Table 3.2, Figure 3.2). No significant relationship was found between the anti-predator score calculated for each individual in assay 1 and individual habituation rate (Adj R^2 = -0.061, p = 0.67).

3.4.2 Experiment 1: prey and predator treatments

The first principal components for Activity in both treatments explained the highest percentage of the variability in behaviour and accurately described the responses of interest (Table 3.3). Positive loading values reflected prolonged swimming and negative loadings represented sharks resting for long periods of time. The first principal component for Foraging in the prey treatment also captured the greatest proportion of variability in the behavioural responses. Sharks that interacted more with the prey and for longer scored higher positive values. Sex, size and group sex did not have any effect on PC1 values calculated, on sociability, ToE or anti-predator behavioural scores. These variables were excluded from any further analysis (Table S3.1).

Table 3.3: Principal component analyses used to collapse behavioural responses collected for all the dark shysharks into behavioural axes. The values reported are for PC1 Activity (KMO = 0.86; Bartlett's test: χ^2 = 75.8, p < 0.001) and PC1 Foraging (KMO = 0.68; Bartlett's test: χ^2 = 89.9, p < 0.001) for 15 sharks exposed to a confined prey stimulus (sardine head and live klipfish) in Experiment 1; PC1 Activity (KMO = 0.75; Bartlett's test: χ^2 = 155.3, p < 0.001) for the same 15 sharks exposed to predation risk (inflatable seal + diluted seal scat) in Experiment 1; PC1 Activity (KMO = 0.72; Bartlett's test: χ^2 = 248.6, p < 0.001) and PC1 Foraging (KMO = 0.86; Bartlett's test: χ^2 = 185.9, p < 0.001) for solitary (11 individuals) and paired (22 individuals) sharks exposed to the same confined prey stimulus in Experiment 2, and PC1 Activity (KMO = 0.702; Bartlett's test: χ^2 = 182.6, p < 0.001) and PC1 Foraging (KMO = 0.77; Bartlett's test: χ^2 = 116.9, p < 0.001) for the same solitary and paired individuals exposed to the presence of the confined prey stimulus but in the presence of predation risk in Experiment 2.

Category	Behaviour	Prey, Exp 1		Predator, Exp 1		Prey, Exp 2		Prey + Predator, Exp 2	
		Loadings	Variance explained	Loadings	Variance explained	Loadings	Variance explained	Loadings	Variance explained
Activity	Rest	-0.92	79.5%	-0.93	85%	-0.77	80.0%	-0.88	79.0%
	Locomotion	0.95		0.93		0.96		0.9	
	Rate of movement	0.94		0.84		0.9		0.93	
	Speed	0.23		0.33		0.27		0.25	
	Bottom swim	0.95		0.91		0.97		0.94	
Foraging	Nosing	0.73	92.6%	NA	NA	0.88	79.8%	0.84	78.4%
	Total duration of interactions	0.82				0.85		0.73	
	Average duration of interactions	0.46				0.97		0.95	
	Number of interactions	0.89				0.23		0.24	

3.4.3 Experiment 1: physiology (Figure 3.3)

Foraging did not correlate significantly with plasma glucose concentrations in the prey only treatment (t_{13} = - 0.24, p = 0.81, r = - 0.067). In the predator only treatment, sharks that were more active after being presented with the model predator also showed a trend toward lower glucose (t_{13} = - 1.81, p = 0.09, r = - 0.45) and higher pH concentrations (t_{13} = 1.95, p = 0.073, r = 0.47). Levels of pCO_2 were significantly lower (t_{13} = - 2.48, p =

0.03, r = - 0.57) in more active individuals, while K^+ concentrations did not correlate

with Activity (t_{13} = - 0.30, p = 0.77, r = - 0.08). Individuals who engaged in active anti-

predator behavioural responses had significantly lower levels of glucose (t_{13} = - 3.25, p

= 0.006, r = - 0.67) in their peripheral circulation. No correlations were found between

anti-predator score and pH (t_{13} = 0.65, p = 0.53, r = 0.18), pCO_2 (t_{13} = - 0.97, p = 0.35, r

= - 0.26), or K^+ concentrations (t_{13} = 1.53, p = 0.15, r = 0.39).
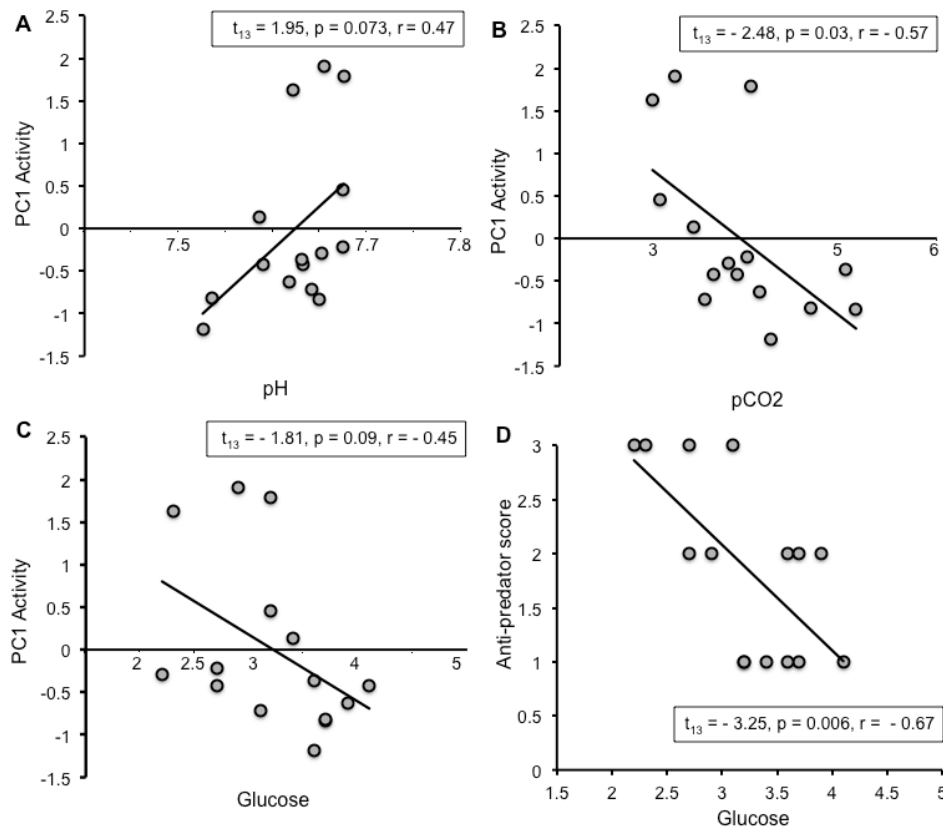
Figure 3.5: Relationship between shark behaviour and the physiological response to predation risk.
Panels show the correlation between some of the physiological indicators of stress and either the first principal component for Activity (panels A, B, and C) or anti-predator behavioural score (panel D). Data are for 15 dark shysharks tested in the lab in pairs after being presented with the model predator (inflatable seal and diluted seal scat), with blood samples taken at the end of the 30 min experiment.

3.4.4 Experiment 2: behavioural syndromes and social environment

The first principal component for both Activity and Foraging calculated in both

treatments yielded similar results found in Experiment 1. In the prey only treatment,

sharks that were overall more active spent significantly less time interacting with prey (t_{31} = -2.11, p = 0.04, r = - 0.35). The social environment had a strong effect on Activity ($F_{3,29}$ = 10.01, p = 0.004) as solitary individuals swam for longer than paired individuals. The social environment had no effect on the correlation between Activity and Foraging ($F_{3,29}$ = 0.69, p = 0.41), despite paired individuals showing a strong negative correlation (t_{20} = -2.06, p = 0.05, r = - 0.42) while no correlation was found for solitary individuals (t_9 = - 0.23, p = 0.82, r = - 0.07). Bolder sharks that left the shelter faster were also significantly more active (t_{31} = -3.25, p = 0.002, r = - 0.50), irrespectively of the social environment ($F_{3,29}$ = 1.39, p = 0.25). No correlation was found between ToE and Foraging (t_{31} = - 0.91, p = 0.3, r = - 0.16); (Figure 3.4).

In the prey and predator treatment, we found a significant effect of social environment on Activity ($F_{3,29}$ = 16.01, p < 0.001), with solitary sharks swimming for longer than paired individuals. Individuals who showed a weaker anti-predator response spent a significantly longer time interacting with the prey (t_{31} = - 2.15, p = 0.04, r = - 0.36), irrespectively of the social environment ($F_{3,29}$ = 2.49, p = 0.13), despite the correlation being significantly negative in paired individuals (t_{20} = - 2.33, p = 0.03, r = - 0.47) and non significant in solitary individuals (t_9 = 0.27, p = 0.78, r = 0.09). A non-significant correlation was found between Foraging and ToE (t_{31} = - 0.77, p = 0.44, r = - 0.14) and between activity and anti-predator behavioural score (t_{31} = 0.44, p = 0.66, r = 0.08); (Figure 3.4). No correlation was found between Activity and Foraging (t_{31} = -1.79, p = 0.08, r = - 0.31), between Activity and ToE for solitary individuals (t_9 = - 1.9, p = 0.08, r = - 0.54) and for paired individuals (t_{20} = - 0.75, p = 0.46, r = - 0.17).
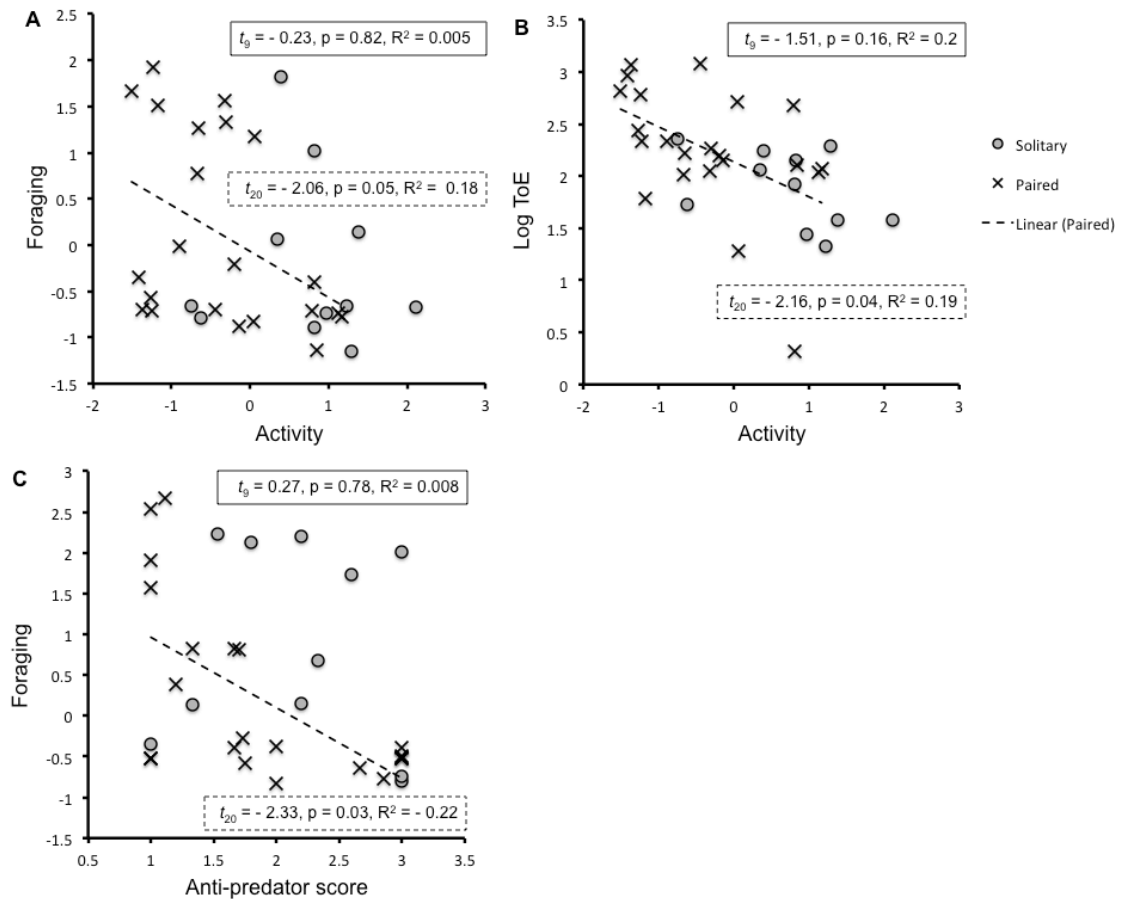
Figure 3.6: Behavioural syndromes for solitary and paired shysharks in the presence and absence of predation risk.

Panels A and B show the correlation between Activity and Foraging, and between activity and log time of exit, respectively. Panel C shows the correlation between Foraging and anti-predator behavioural score. Data in panels A and B are for each individual exposed to a confined prey stimuli (sardine head and live klipfish) for 20 min when in a pair (22 individuals) or when solitary (11 individuals). Data for panel C are for the same 22 paired and 11 solitary individuals when exposed to the prey stimuli in the presence of predation threat (inflatable seal and diluted seal scat) for 20 min. Grey circles and a solid line represent solitary individuals and the respective Pearson statistics, while crosses and a dotted line represent paired individuals, the respective line of best fit and Pearson statistics.

3.5 Discussion

Our results show that a significant portion of behavioural variability in activity, foraging, anti-predator behaviour and sociability can be attributed to individuality when controlling for size, sex, group sex, and hunger, and that individual differences in behaviour are highly repeatable. We also found evidence of individuality in shark stress

response to predation risk, and of the effect of social environment on behavioural syndromes within the predator-prey context.

There was evidence of habituation was detected in anti-predator behaviour in Experiment 1, but this was highly variable between individuals, suggesting that shysharks show personality related difference in habituation, similarly to lemon sharks in a semi-captive environment (Finger et al., 2016). The significant individual variability found in activity and anti-predator behaviour could not be attributed to differences in appetite, because there was a strong effect of shark ID on all behavioural responses when controlling for plasma glucose concentration. Considering that blood glucose relates to appetite and satiation in sharks and teleost fish and that it can directly influence feeding rate in mammals (Smith and Epstein, 1969; Colgan, 1973; Dill, 1983; Fletcher, 1984; Sims, 1994; Boujard et al., 1993; Le Bail and Bœuf, 1997), the absence of a relationship between plasma glucose and the Foraging axis in shysharks tested in the prey only treatment of Experiment 1 further supports our evidence that inter-individual variability in hunting behaviour could not be simply explained by differences in their hunger, but rather to personality.

Interestingly, Activity, Foraging, ToE, anti-predator behaviour and sociability in dark shysharks did not vary with size, sex or group sex irrespectively of the social environment or predation risk. Studies on small-spotted catsharks and Port Jackson sharks found evidence of differences in boldness, foraging and sociability according to size, sex and group sex (Kimber et al., 2009; Byrnes and Brown, 2016; Finger et al., 2016), suggesting that plasticity in the expression of personality traits might vary across species and emphasising the need for species-specific studies in elasmobranchs (Sih et al., 2004b).

Our results provide some evidence of the presence of coping styles in dark shysharks. As expected, reactive individuals showing lower activity and passive anti-predator behaviour experienced a more acute response to predation stress, as indicated by high pCO_2 in their blood and lower pH, although the last relationship was not significant, possibly because of a low sample size (Øverli et al., 2007; Skomal and Mandelman, 2012). Proactive sharks showing active "flight" responses to the model predator showed a weaker response to stress based on their low plasma pCO_2. Low glucose concentrations were detected in the blood of individuals that engaged in higher activity levels. Dark shysharks are slower than other species at mobilizing their glycogen reserves, hence individuals that engaged in active anti-predator responses likely depleted their plasma glucose faster to fuel the "flight" response (Skomal and Mandelman, 2012; Chapter 2). The absence of a strong correlation between both behavioural axes and K^+ is likely due to the longer time required for changes in this bioindicatior to be reflected in the response to stress (Mandelman and Farrington, 2007; Chapter 2). Our results therefore suggest that individuality in the stress response, known as coping styles, might be one of the mechanisms underlying consistent individual variability in shark behavioural responses within the predator-prey context, but this matter warrants further investigation.

Fish often face situations that favour high activity and increased foraging, despite a potential decrease in survival, hence the strong behavioural syndromes observed between activity and foraging, and between boldness and activity in many species (Sih et al., 2004b; Conrad et al., 2011; Mittelbach et al., 2014). Paired sharks, but not solitary individuals, that showed a weaker anti-predator response spent longer foraging and interacting with the prey item. Dark shysharks seem to benefit from group living as

an anti-predation defence to balance the high costs that active foragers might suffer under higher predation in order to encounter more prey (Sih et al., 2004a,b). Group living will also increase competition for food resources, potentially creating mutual interference while foraging (DeLong and Vasseur, 2011). This might be why, in the absence of predation risk, paired sharks that were foraging more spent less time being active, while solitary individuals did not show the same pattern. In the presence of conspecifics, foraging tactics can change and the amount of time invested in foraging often increases (Vardi et al., 2017).

In agreement with previous studies, predation risk decoupled the correlation between ToE and Activity in paired sharks. Experiencing predation risk often alters or decouples correlations between personality traits (Bell and Sih, 2007; Réale et al., 2007). Syndromes within the predator-prey context therefore have important ecological and fitness consequences, and are fundamentally centred on the trade-offs introduced by activity (Sih et al., 2004a). Dark shysharks also showed the same correlations, as active individuals were faster to exit the shelter (i.e. bolder) and were more likely to engage in foraging behaviour. Unexpectedly, we did not find evidence of a syndrome between time of exit from the shelter as a measure of boldness and shark foraging behaviour, despite there being evidence of a syndrome between boldness and foraging in many other animal species (Coleman and Wilson, 1998; Kurvers et al., 2010; Toscano and Griffen, 2014; Toscano et al., 2016). Boldness in animals has been measured in different ways in addition to the latency to exit a shelter, such as reaction to novel objects, novel environments or propensity to leave shoal mates (Coleman and Wilson, 1998; Brown et al., 2007; Kurvers et al., 2010; Carter et al., 2013; Toscano and Griffen, 2014; Toscano et al., 2016). It is possible that time of exit in shysharks is not be an accurate reflection of an individual's boldness, which would explain the absence of a

syndrome between ToE and foraging behaviour. It is also possible that our sample size was not large enough to capture the syndrome, especially if shysharks were to show more than one hunting strategy similarly to other apex predators (Huey and Pianka, 1981; O'Brien et al., 1990; Towner et al., 2016).

Our results add to recent studies showing that sharks exhibit personality and that these affect shark behaviour within the predator-prey context. Foraging and antipredator behaviour are critical factors determining animal fitness and survival, therefore inter-individual differences in hunting strategies, boldness and activity in the presence and absence of predators will likely influence how sharks adapt and survive any change in their environments. As sharks play an important ecological role in marine ecosystems thanks to their top down control of prey populations, consideration of the effects of personality on their foraging behaviour in fisheries management and conservation policy may be warranted.

4.   Personality and its effects on hunting behaviour and spatial use of the foraging landscape in a marine apex predator

4.1 Abstract

Personality differences have been observed in a wide range of animal species, and are likely to be of important ecological and conservation significance. Despite the increase in research aimed to determine the effects of personality on complex ecological processes such as habitat use and foraging strategies, our understanding of the occurrence and consequences of personalities in marine apex predators remains limited. Here, we investigated individuality and repeatability of hunting behaviour and habitat use in white sharks (*Carcharodon carcharias*) in Mossel Bay, South Africa. We obtained behavioural measurements from 28 individuals interacting with two controlled food stimuli (fish and seal silhouette), and information on the movement and residency of 12 of these sharks using acoustic tags. White sharks showed consistent inter-individual variability in their interactions with the fish, but not with the seal silhouette, and in the duration of residency and distance of movement events. We found a significant relationship between the principal component of shark behaviour representing the interactions of the 12 tagged sharks with the seal silhouette and the average distance of their movement events, suggesting the presence of behavioural syndromes in this species. Our results demonstrate individuality and consistency in hunting behaviour and habitat use of white sharks, which has important implications for public perception and the development of conservation strategies for this species.

4.2 Introduction

Personality has been observed in more than 200 animal species and influences many aspects of individual fitness such as movement patterns and habitat use, feeding strategies and how individuals interact with other species within the predator-prey context (Gosling, 2001, Smith and Blumstein, 2008; Carere and Locurto, 2011; Carter et al., 2013; Finger et al., 2017). This phenomenon is of great ecological, evolutionary and conservation significance (Dall et al., 2004; Dingemanse and Réale, 2005; Carter et al., 2013; van Oers and Sinn, 2013). Despite the rapid increase in research efforts, our current knowledge is largely limited to studies in captivity, causing a significant gap in our understanding of the ability of wild populations to respond to habitat degradation and global climate change (Archard and Braithwaite, 2010; Jacoby et al., 2014; Mittelbach et al., 2014; Byrnes and Brown, 2016; Finger et al., 2016; Finger et al., 2017).

The development of appropriate personality tests that provide multiple data points for individuals in the field and are able to disentangle personality from population differences or from individual responses to the environment is challenging. This has greatly constrained our ability to answer complex ecological questions related to habitat use and dietary preferences, especially for marine apex predators (Carter et al., 2013; DiRienzo et al., 2013; Jacoby et al., 2014; Byrnes and Brown, 2016; Finger et al., 2017). These aspects of the ecology of marine apex predators have mainly been modelled according to prey availability, and the sex, age- or size-classes of the consumers, irrespective of other sources of intraspecific variability like personality. However, the recent development of novel technologies such as acoustic biotelemetry provides a potential way to investigate individuality within the spatial ecology of wild marine predators (e.g. Simpfendorfer et al., 2010; Jewell et al., 2013; Jacoby et al.,

2016; Towner et al., 2016; rev. in Finger et al., 2017). Acoustic telemetry has proven useful in describing migration patterns, habitat use and foraging behaviour in some shark species including tiger sharks, ragged-tooth sharks, and white sharks, and could therefore become a valuable tool to investigate the presence of personality-related differences in marine predators (e.g. Jewell et al., 2013; Kock et al., 2013; Towner et al., 2016; Hammerschlag et al., 2017; rev. in Finger et al., 2017),

Marine apex predators are of great importance to ecosystem processes because of their high trophic position, but are simultaneously vulnerable to anthropogenic impacts because of their K-selected life-history strategy (Stevens et al., 2000; Myers et al., 2007; Afonso and Hazin, 2014; Danylchuk et al., 2014; Finger et al., 2016; Musyl and Gilman, 2018). Understanding how individuals use their habitat, and whether they show specialization in their feeding habits is paramount for the development of successful management and conservation plans, particularly for critical areas such as feeding grounds where sharks might be even more vulnerable to exploitation (Bolnick et al., 2003; Simpfendorfer et al., 2010; Hammerschlag et al., 2017; Kock et al., 2018). Personality in sharks has been investigated for a few species and behavioural traits: boldness, stress reactivity and laterality in Port Jackson sharks (Byrnes and Brown 2016; Byrnes et al., 2016); social network positions in spotted catsharks (Jacoby et al., 2014); social axis in lemon sharks (Wilson et al., 2015; Finger et al., 2016; Finger et al., 2018); foraging and anti-predator behaviour in dark shysharks (Chapter 3). Although personality related differences in migratory behaviour have been detected in some taxa including fish (Chapman et al., 2011), few studies have investigated inter-individual differences in habitat use and movement patterns in sharks: vertical movement in tiger sharks (Vaudo et al., 2014); activity and diel movement patterns in lemon sharks (Wilson et al., 2015; Finger et al., 2016); habitat use in bull sharks (Matich and

Heithaus, 2015); movements in blue sharks (Vandeperre et al., 2014). Within the predator-prey context, individuality in diet and ontogenetic trophic shifts have been inferred using isotope analysis in juvenile lemon sharks (Hussey et al., 2017), spurdogs, bullsharks and Caribbean reef sharks (Matich and Heithaus, 2015; Matich et al., 2019), and white sharks (Kim et al., 2012), although little is known of other important sources of variability including behaviour and personality. Inter-individual differences in foraging behaviour have been found in dark shysharks in the lab (Chapter 3), and some evidence has been found of short-term individuality in hunting mode in white sharks in the field (Towner et al., 2016). Through predation, sharks mediate many aspects of marine ecosystem dynamics including prey population size, foraging behaviour and habitat use, but many species are of significant conservation concern due to fishing pressure and pollution (Heithaus and Dill, 2006; Martin and Hammerschlag, 2012; Byrnes and Brown, 2016). Information on the limits imposed by personality on shark hunting behaviour and use of the foraging landscape is therefore key to understanding the ecological role of these predators in marine ecosystems, and informing conservation and fisheries management programs (Bolnick et al., 2003; Myers et al., 2007; Worm et al., 2013; Conrad et al., 2011; Mittelbach et al., 2014; Davidson et al., 2015, Finger et al., 2016; Finger et al., 2017).

Here, we use the white shark (*Carcharodon carcharias*) to investigate the occurrence of consistent inter-individual differences in habitat use and hunting strategies in a marine apex predator. White sharks protected by local legislations in seven countries including South Africa, and by the international agreements of CITIES, i.e. the Convention on International Trade in Endangered Species of Wild Fauna and Flora. Despite being protected, this apex predator is listed as Vulnerable on the IUCN red list as their population numbers have been declining, being threatened by targeted and by-catch

fisheries, culling programs, pollution and environmental change (Dudley and Simpfendorfer, 2006; Mull et al., 2012; Mull et al., 2013; Hammerschlag et al., 2019). White sharks are an excellent study system to investigate animal personalities in elasmobranchs because they show seasonal residency at pinniped colonies, therefore increasing the chance of multiple sightings of the same individuals within and between seasons, which is necessary for the characterization of personality (Laroche et al., 2008; Towner et al., 2016; Finger et al., 2016; Finger et al., 2017). We aim to determine (i) if white sharks show consistent inter-individual differences in their behavioural responses to controlled prey stimuli, (ii) if habituation occurs due to the repeated exposure to the same stimuli and whether the rate of habituation is consistently different between individuals, and (iii) if white shark show individuality in their use of the foraging landscape in terms residency and movement behaviour.

4.3 Methods

All research was carried out under research permit RES2017-14 issued by the South African Department of Agriculture, Forestry and Fisheries.

Between the 16th of May and the 7th of September 2017, we collected behavioural measurements for 33 white sharks (*Carcharodon carcharias*) interacting with a tuna head (fish) or rubber seal silhouette around our research vessel anchored at Seal Island in Mossel Bay, South Africa (34.1508^o S 22.1202^o E). Mossel Bay is located on the south-western coast of South Africa and it provides an ideal site for behavioural and acoustic studies as it is protected from most of the prevailing winds by the overall topography of the Cape St. Blaze peninsula. Seal Island is a rocky outcrop located 750 m from the shores of Mossel Bay and represents a prime residency and foraging area for white shark because it hosts a breeding colony of Cape fur seals (*Arctocephalus pusillus*

pusillus; Johnson et al., 2009; Ryklief et al., 2014). Most of the predatory events occur within a cone of area on the south east end of Seal Island, used by seals to reach their feeding grounds (Jewell et al., 2013).

4.3.1 Behavioural responses to stimuli

After the boat anchored within area on the southeast end of Seal Island, sharks were lured using a combination of chum and bait (Johnson et al., 2009; Jewell et al., 2013; Towner et al., 2016). We collected information on wind direction and speed (measured with a mechanical anemometer), SST, weather conditions, water visibility (measured with a Sacchi disk), and the presence/absence of a cage diving tourism boat. Each trial commenced as the fish head and the rubber silhouette of a seal were placed in the water on the port side of the vessel, and lasted for 3 h. Sharks were identified using photo ID of the dorsal fin and according to other markings such as scars, white and black pigmentation on the dorsal and caudal fins and on the rest of the body (Figure 4.1).
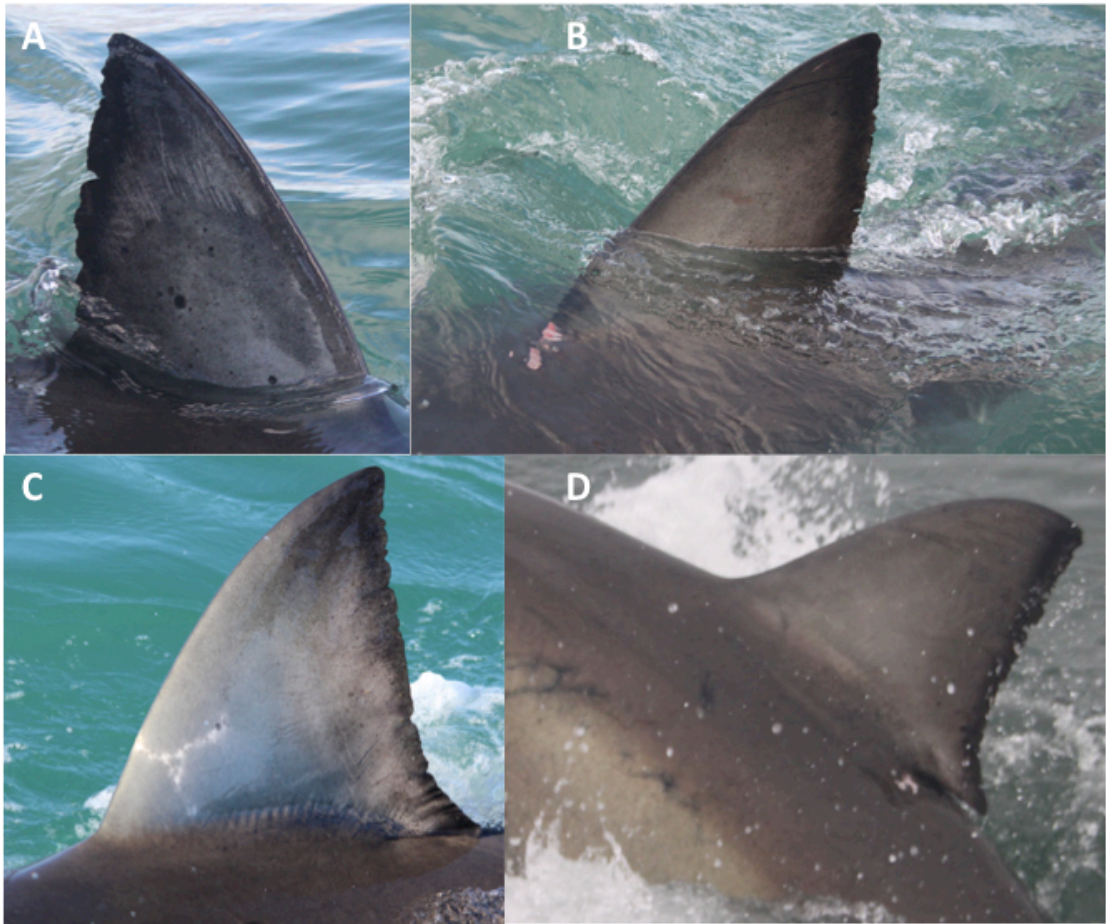
Figure 4.1: Shark ID based on different phenotypic traits.
Black (A) and white (C) pigmentation on the dorsal fin and on other markings such as scars (B, D) on the rest of the body.

Of 33 identified individuals, we used 28 sharks (4 males, 20 females, 4 unidentified sex) for which we observed behavioural responses on at least three occasions (mean ± s.e. = 6 ± 0.5). Total length was estimated in relation to the length of the research vessel and recorded according to size class: 275 cm (5 individuals); 300 cm (4 individuals); 325 cm (8 individuals); 350 cm (8 individuals); 375 cm (3 individuals). Individual sex was determined from the research vessel, or from recordings of the ventral side of the sharks obtained with a GoPro (Hero 3, 1080p; GoPro inc.) mounted on a metal pole submerged at 1.5 m from the surface. The time focal sharks first appeared was noted, and their behavioural responses were filmed for the duration of their stay with a GoPro

(Hero 6 black, 1080 p; GoPro Inc.) mounted on the crows nest of the vessel.

Behavioural responses were quantified from video recordings (Table 4.1, Figure 4.2).
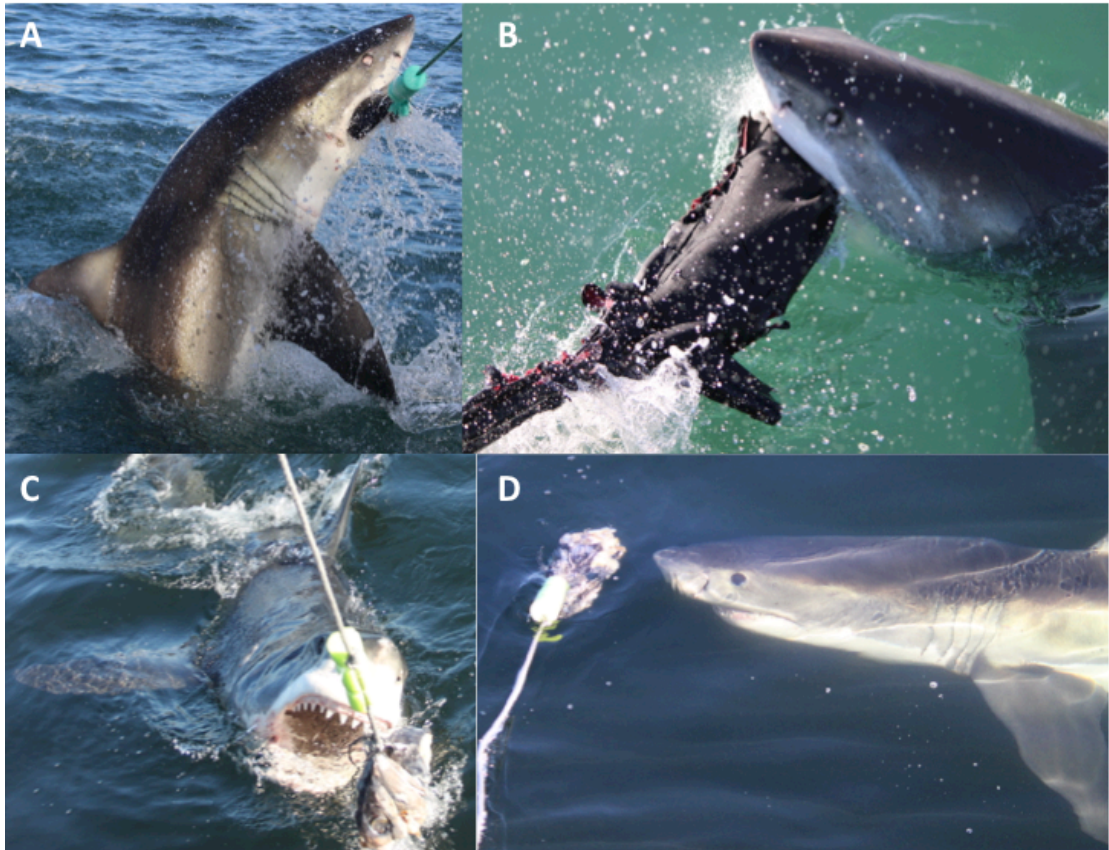
Figure 4.2: Shark interactions with the stimuli.
Examples of shark successful bites towards the fish (A) and the seal silhouette (B), of an attempted bite (C) and in investigation (D) towards the fish.

Behavioural scores were calculated to categorize and compare the sharks over a behavioural scale that could expresses their level of interest towards the stimuli. Far passes, close passes, investigations, attempted bites and successful bites (Table 4.1) were given a value of 1-5 respectively (1 = weak interest; 5 = strong interest), with the average value for each shark in each trial being calculated.

Table 4.1: Ethogram of behavioural responses quantified from the video recordings of the 28 white sharks interacting with the fish and the seal silhouette around the research boat in Mossel Bay during the study period. These responses were used to assess the presence of personality in the focus sharks.

Behavioural response	Definition	Category	Test	Transform
Time of arrival (s)	Delay between time of anchoring and first appearance of focus animal	Continuous	LMM	log
Average time of visit (s)	Average time spent by focal animal within the field of view of the camera between engines and baw			
Total time of stay (s)	Sum of the duration of each visit			
Average decision (s)	Average time before the focus animal engages in a preference interaction with a stimulus			sqrt
Behavioural score total	See methods for calculations			
Behavioural score bait				
Total number of visits	Number of times the focal animal enters the field of view of the camera	Count	GLMM	
Successful Bite	Focus animal opens mouth at stimulus and has it in its mouth			
Attempted bite	Focus animal opens mouth at stimulus but doesn't make contact			
Predatory attempted bite	Focus animal increases speed for attempted bite			
Investigatory attempted bite	Focus animal does not increase speed for attempted bite			
Investigation	Focus animal has clear direction towards stimulus, approaches within 2 meters			
Preference interactions	Bites + investigations			
Non-preference interactions	Focus animal does not have clear direction towards stimulus, but approaches it further (far pass), or within (close pass) 3 meters			
Total number of interactions	Total number of preference and non-preference interactions			

4.3.2 Movement data from acoustic telemetry

Between the 26th June and the 11th of July 2017, we equipped 12 of the 28 sharks (1 male, 11 females; 1 individual 275 cm, 2 individuals 300 cm, 7 individuals 325 cm, 2 individuals 350 cm) with VEMCO V16 frequency-specific continuous transmitters (emission interval = 30-90 s, mean = 60 s) using a modified spear gun (Figure 4.3).

Figure 4.3: VEMCO V16 frequency-specific continuous transmitters deployed on one of the 12 tagged sharks.

On the 13th July 2017, a VR2W-69 kHz acoustic monitoring receiver (loaned by the Acoustic Tracking Array Platform, ATAP) was deployed 350 m from seal island (34.4067^o S 22.195^o E) on a fixed mooring at 14.7 m depth within the cone of area used by seals. The presence/absence data downloaded from the receiver provided information on when and how long the tagged sharks were patrolling Seal Island. Data on the tagged sharks from receivers deployed along the coast of South Africa were provided by ATAP (Figure 4.4). Data on the tagged sharks was obtained from 1 receiver deployed at The Point in Mossel Bay (34.1822^o S 22.1754^o E), 4 receivers deployed at Terginet (34.0806^o S 22.1905^o E), 2 at Groot Brakriver (34.0665^o S 22.2421^o E), (Figure 4.4 A), and 4 in Plettemberg Bay (34.0871^o S 23.4011^o E), (Figure 4.4 B).
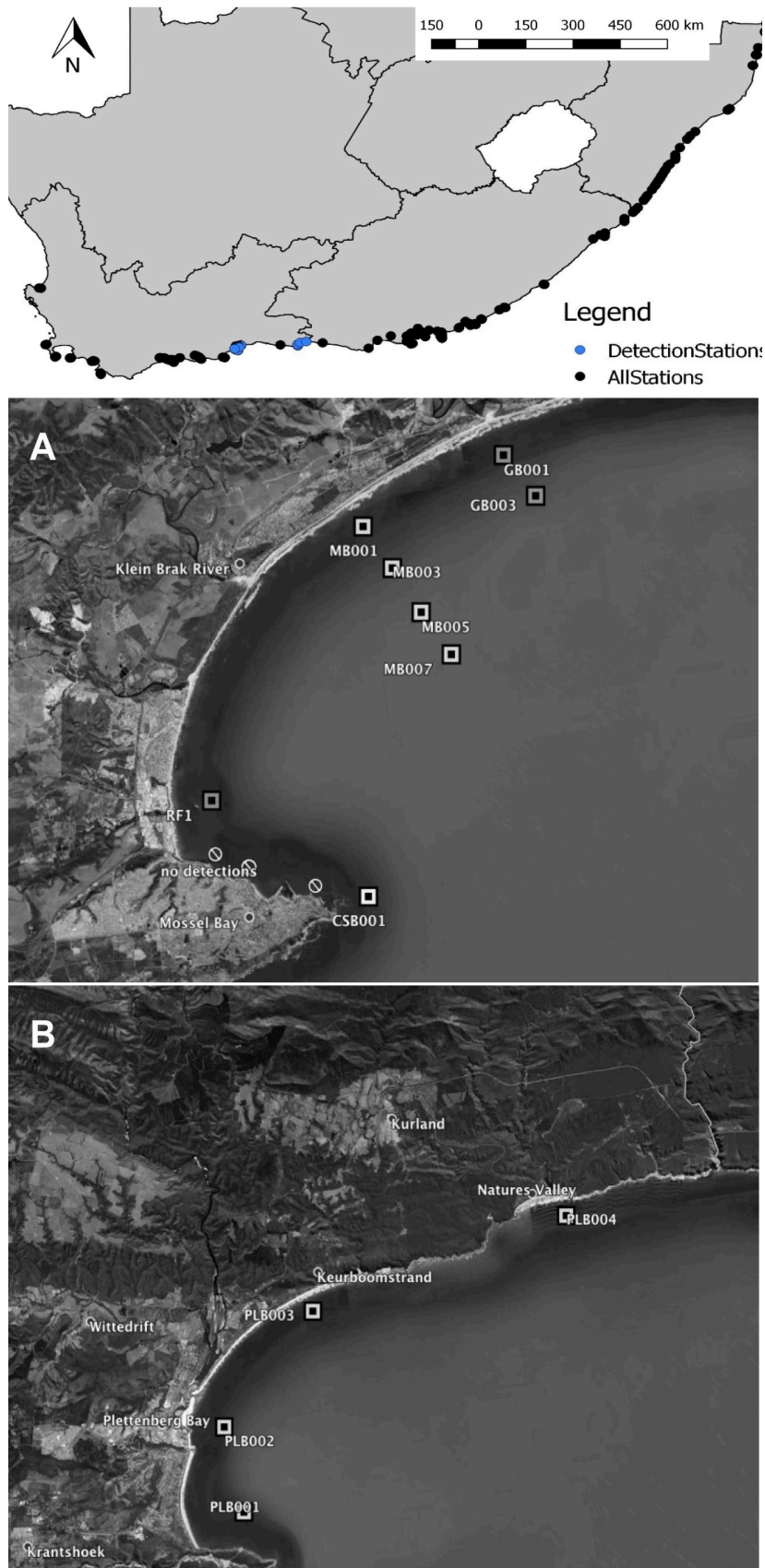
Figure 4.4: Location of the acoustic receivers on the South African coastline.
The top panel shows the map of all the VR2W-69 kHz acoustic monitoring receivers
managed by ATAP on the South African coastline.

Figure 4.4 continued: Blue stations represent receivers that detected the tagged sharks from this study, while black stations represent the receivers that did not detected the tagged sharks. The other two panels show a zoomed view of the exact locations of the VR2W-69 kHz acoustic monitoring receivers that detected the tagged sharks in Mossel Bay (A) and Plettemberg Bay (B). We deployed VEMCO V16 frequency-specific continuous transmitters (emission interval = 30-90 s, mean = 60 s) on 12 of the 28 sharks observed around the research vessel using a modified spear gun.

We filtered our acoustic data with VTrack Package (R v3.5.1; R Core Team, 2018) to remove false detections caused by collisions of acoustic signals or interference from background noise (Campbell et al., 2012; Becker et al., 2016; Hammerschlag et al., 2017; Dwyer et al., 2018). Detection data was removed (i) if the given tag wasn't recorded at least two consecutive times at any given receiver, (ii) if two consecutive detections occurred less than 25 s apart since the nominal delay of the transmitters was set between 30 s and 90 s; and (iii) for any shark that was detected at the same receiver for more than 5 days without being recorded at any other receiver as it was presumed to have lost its tag in the proximity of that receiver. Using functions within the VTrack package and for each tagged shark, we then calculated (i) the duration of every residency event using the time when the tagged animal arrived and departed the receiver's detection field (previously determined to be of 500 m in radius), and (ii) the distance of any movement event between the receivers that detected the individual tag over a 24 h period (Campbell et al., 2012; Dwyer et al., 2018).

4.3.3 Statistical analyses

All statistical analyses were performed in IBM SPSS Statistical version 25 (IBM Corporation, New York, USA) and R, v 3.5.1 (R Core Team, 2018).

We used mixed effects models to investigate whether white sharks display significant inter-individual variability in their behavioural responses to the stimuli. LMM were

used on continuous response variables (lme4 package for mixed model analysis; Bates et al., 2015). All response variables were transformed to respect the assumption of homoscedasticity of the residuals (Table 4.1). As successful bites (Table 4.1) towards the fish and towards the seal silhouette only accounted for 5 % and 6 % respectively, their total number were summed to attempted bites for analysis, and referred to as total bites. GLMM with negative binomial distribution were used on the response variables in the form of count data (glmer.nb function of lme4 package); (Table 4.1). Shark ID was always included as the random factor. In the models investigating the effect of shark ID on shark foraging behaviour, trial number, sex, size category, time of the day (morning or afternoon), environmental variables (wind direction and speed, SST, weather conditions, visibility, presence/absence of the cage diving company) were included as fixed covariates. In the models investigating the effect of shark ID on the movement variables, the duration of residence events was square-root transformed, while the duration of movement events was log transformed to respect the assumption of homoscedasticity of the residuals. Event number, sex, size category, whether the event happened during the day or at night, and moon cycle (e.g. full moon) were included as fixed covariates.

Minimum adequate models (MAM) were obtained by stepwise deletion of non-significant terms from the full factorial models. Significance of the fixed covariates was derived using the package lmerTest based on Satterthwaite's approximations (Kuznetsova et al., 2015). Likelihood ratio chi-square tests (LRT) and AIC values were used to select the best models. In LMM, the significance of the random term was calculated using the function exactLRT from the package RLRsim comparing the MAM's with and without shark ID as random factor over 10000 permutations (Scheipl et al., 2008). In GLMM, the significance of the random term was calculated from the

MAM using likelihood ratio tests comparing the models with and without shark ID as random factor.

Repeatability of each behavioural measure and for residency/movement behaviour was calculated with the rptR package to determine its significance over 10000 permutations (Nakagawa and Schielzeth, 2010; Stoffel et al., 2017).

To assess whether white sharks display any personality related differences in habituation, another set of mixed effect models were generated to include the interaction between trial and shark ID as a random slope model (Finger et al., 2016). We used the function exactRLRT from the package RLRsim (Scheipl et al., 2008) to compare LMM models including the random slope and models including only shark ID as a random intercept. In GLMM, we compared the models including the random slope and the models including only shark ID as a random intercept using likelihood ratio tests and AIC.

Principal component analysis (PCA) with varimax rotation (Tabachnick and Fidell, 2001; Dingemanse et al., 2007) was used to collapse the behavioural responses of the sharks to the fish and the seal silouhette into two principal components. Each shark was included only once in each PCA analysis to avoid pseudoreplication. We used regression analysis and curve estimation to determine if there was any relationship between the calculated values for the first principal component (PC1) and the second principal component (PC2) for each shark and their respective mean duration of residency events and distance of movement events obtained from the movement data. The same analysis was run on the data set including and excluding the individual who travelled to Plettemberg Bay.

4.4 Results

4.4.1 Behavioural responses to stimuli

The 28 sharks interacted with either stimulus at least once and up to a maximum of 140 times (19 ± 2 interactions) per trial, for a total of 3369 interactions. Sharks engaged more in left than right interactions (57.3 ± 0.2 %), and in more preference than non-preference interactions (69.5 ± 1.4 %), mainly towards the fish rather than the seal silhouette (78.6 ± 1.6 %). Of the preference interactions towards the fish, sharks engaged in more (total) bites (58.5 ± 0.03 %) than investigations (32.1 ± 0.03 %), and most attempted bites towards the fish were predatory (78.3 ± 0.03 %) rather than investigatory. When showing an interest towards the seal silhouette, sharks engaged in more investigations (63.8 ± 0.03 %) than (total) bites (32.5 ± 0.03 %). On average, sharks engaged in 46.1 ± 0.05 % predatory attempted bites and 41 ± 0.05 % investigatory attempted bites.

Table 4.2: Results from linear mixed effects models and repeatability analysis (rpt) on the behavioural responses recorded for the 28 white sharks when interacting with the stimuli around the research vessel during each three-hour trial. χ2 were calculated with LRT tests on GLMM with and without ID as a random factor. LRT and RLRT values were obtained with the exactLRT and exactRLRT functions on LMM; Rpt values were obtained with rptr packages.

Behavioural response	Stimulus	Individual variability	Repeatability	Habituation
Time of arrival (s)		LRT = 0.43, p = 0.17	rpt =0.067, p = 0.14	**RLRT = 4.18, p = 0.015**
Average time of visit (s)		**LRT = 1005.5, p < 0.001**	**rpt =0.19, p = 0.002**	**RLRT = 3.01, p = 0.029**
Total time of stay (s)		**LRT = 38.2 p < 0.001**	**rpt = 0.36, p < 0.001**	**RLRT = 3.54, p = 0.022**
Average decision (s)		**LRT = 4.89, p = 0.002**	**rpt =0.2, p = 0.001**	RLRT = 0.003, p = 0.42
Total N of visits		χ^2 **= 34.9, p < 0.001**	**rpt = 0.26, p = 0.02**	χ^2 **= 11.16, p < 0.001**
Total N of interactions	Both	χ^2 **= 17.9, p < 0.001**	rpt = 0.19, p = 0.06	χ^2 = 1.59, p = 0.2
Preference interactions	Bait	χ^2 **= 22.4, p < 0.001**	**rpt = 0.24, p = 0.03**	χ^2 **= 4.05, p = 0.04**
	Decoy	χ^2 = 0.1, p = 0.87	rpt = 0.024, p = 0.39	χ^2 = 0.05, p = 0.93
Investigations	Bait	χ^2 **= 23.5, p < 0.001**	**rpt = 0.32, p = 0.01**	χ^2 **= 9.3, p = 0.002**
	Decoy	χ^2 = 10.2, p = 0.74	rpt = 0.007, p = 0.28	χ^2 < 0.001 p = 0.98
Total bites	Bait	χ^2 **= 15.8, p = 0.003**	**rpt = 0.18, p = 0.02**	χ^2 = 2.85, p = 0.09
	Decoy	χ^2 = 1.06, p = 0.3	**rpt = 0.16, p = 0.01**	χ^2 = 0.001, p = 0.97
Attempted bites	Bait	χ^2 **= 6.49, p = 0.01**	**rpt = 0.20, p = 0.04**	χ^2 = 0.2, p = 0.65
	Decoy	χ^2 = 1.19, p = 0.27	**rpt = 0.2, p = 0.04**	χ^2 < 0.001, p = 0.98
Predatory att. bites	Bait	χ^2 **= 9.33, p = 0.002**	**rpt = 0.18, p = 0.04**	χ^2 = 0.08, p = 0.78
	Decoy	χ^2 = 0.009, p = 0.92	rpt = 0.05, p = 0.4	χ^2 < 0.001, p = 0.99
Investigatory att. bites	Bait	χ^2 = 0.27, p = 0.6	rpt = 0.03, p = 0.27	χ^2 < 0.001, p = 0.98
	Decoy	χ^2 = 0.64, p = 0.42	rpt = 0.07, p = 0.1	χ^2 = 0.86, p = 0.35
Behavioural score	Both	LRT = 0.25, p = 0.21	rpt = 0.036, p = 0.25	RLRT = 0.44, p = 0.21
	Bait	LRT = 0.62, p = 0.14	rpt = 0.043, p = 0.2	RLRT = 0.55, p = 0.18

We observed significant inter-individual variability and repeatability across trials for

shark total time of stay, mean time of visit and total number of visits per trial, but not

for their time of arrival after the onset of chumming procedures (Table 4.2, Figure 4.5).

Personality related differences in habituation were observed for time of arrival, mean time of visit, total time of stay and total number of visits (Table 4.2; Figure 4.5). Individuals showed a highly significant variability in their overall number of interactions and decision time, although only the latter was consistent across trials (Table 4.2; Figure 4.5). A strong individuality was observed for preference interactions towards both stimuli, which was also significantly repeatable during the study period (Table 4.2). Individual sharks showed significant variability in their investigations, total bites and attempted bites towards the fish, all also significantly repeatable across trials (Table 4.2; Figure 4.6). Individual sharks showed significant variability and repeatability in predatory but not investigatory attempted bites (Table 4.2, Figure 4.6). Sharks showed personality related habituation only for the total number of preference interactions and investigations towards the fish (Table 4.2). No significant inter-individual difference was observed for shark behavioural responses towards the seal silhouette, and only the numbers of total bites and of attempted bites towards the seal silhouette was repeatable across trials (Table 4.2, Figure 4.6). Sharks did not show personality related habituation for any of the behavioural responses towards the seal silhouette (Table 4.2).
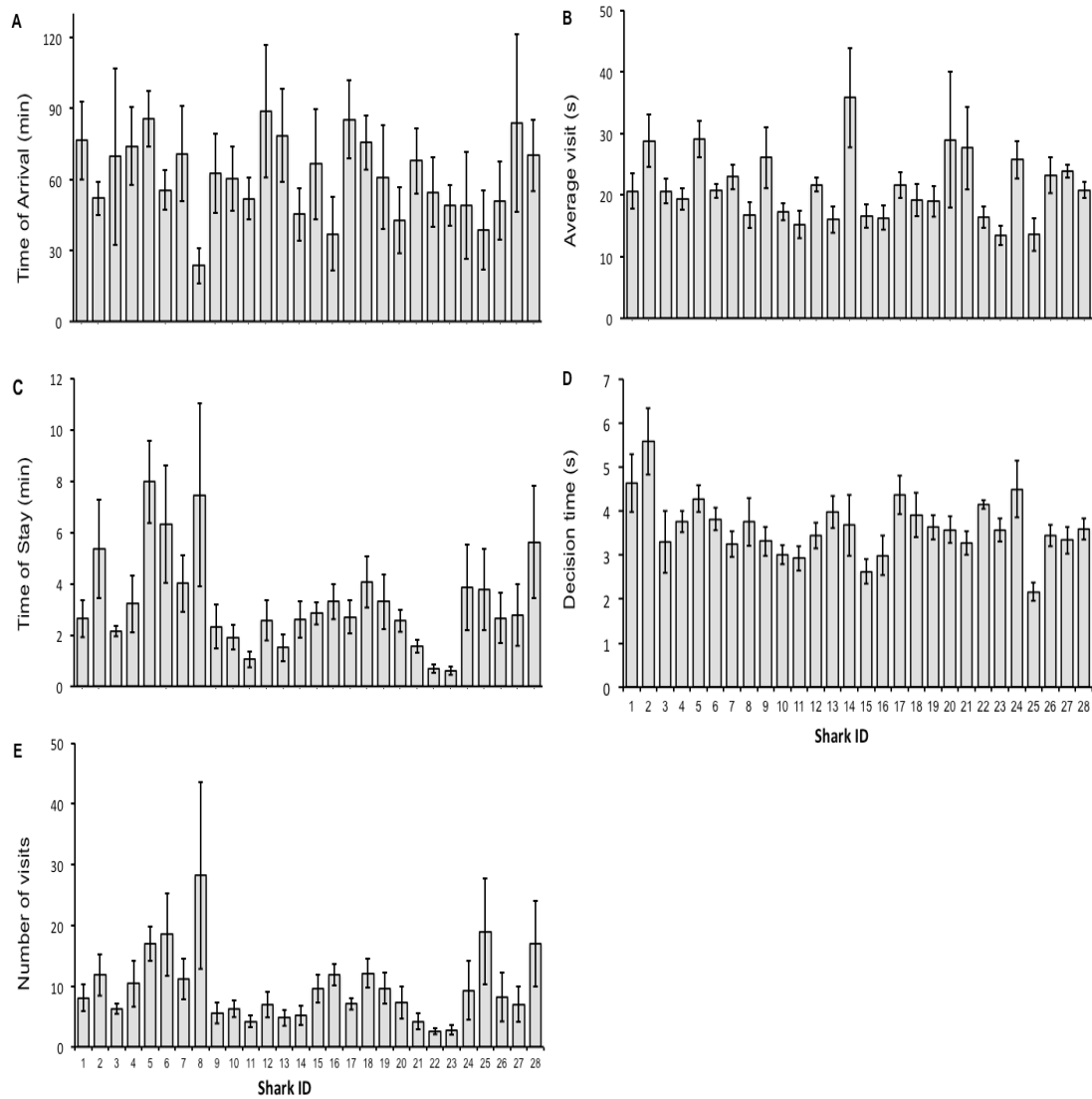
Figure 4.5: Inter-individual variability in shark activity around the research vessel. Mean ± s.e. of time of arrival (A), duration of visits (B), total time of stay (C), decision time (D) and number of visits (E) for the 28 white sharks (4 males, 20 females, 4 unidentified sex) for which we observed behavioural responses on at least three occasions (mean ± s.e. = 6 ± 0.5). We calculated the represented values across each three-hour trial when the sharks were observed interacting with the fish or with the seal silhouette around the research vessel in Mossel Bay after the onset of chumming procedures.
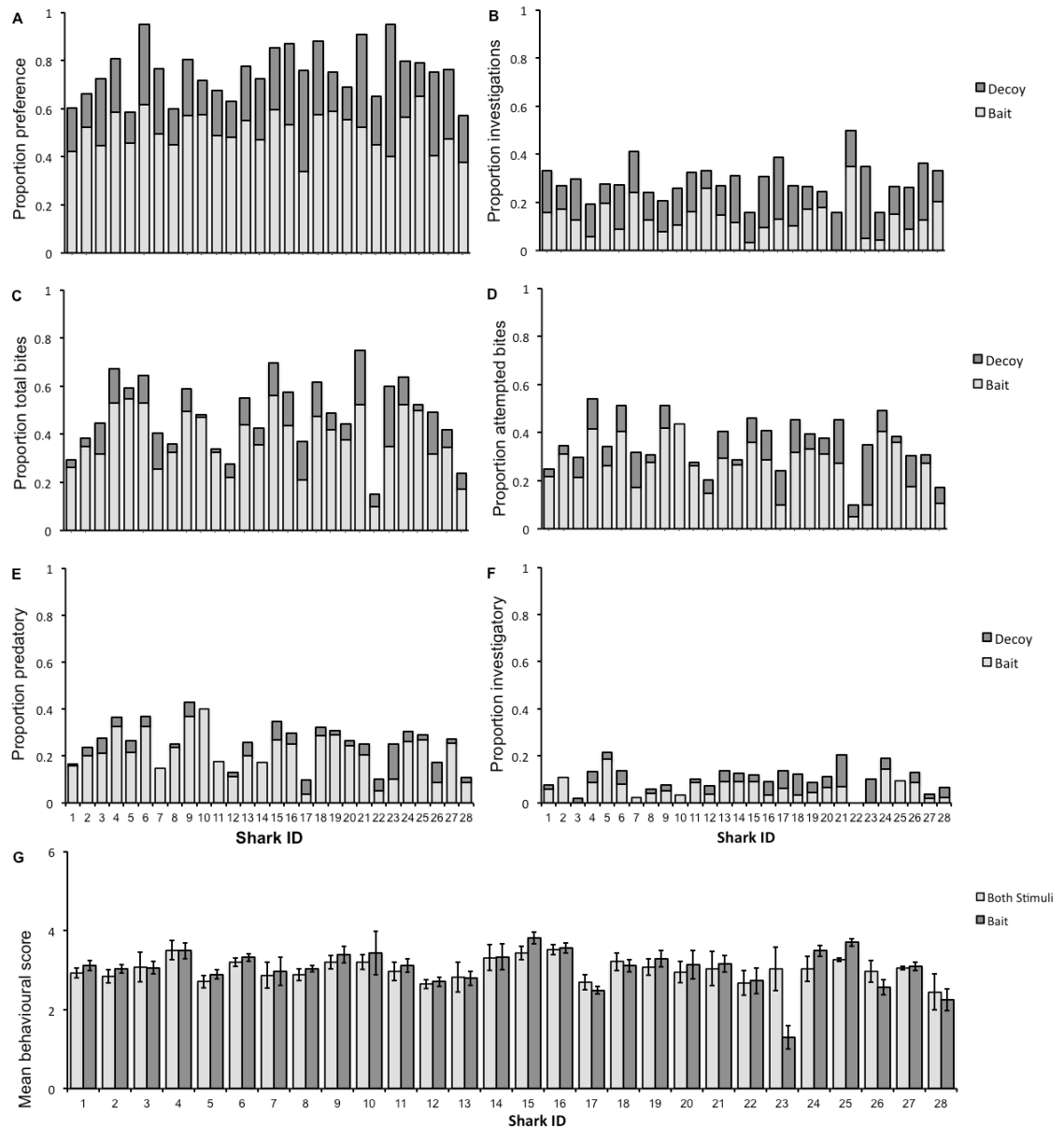
Figure 4.6: Inter-individual variability in shark behavioural responses to the stimuli. Proportion of preference interactions (A); investigations (B); total bites (attempted + predatory C); attempted bites (D); predatory attempted bites (E); investigatory attempted bites (F); calculated in respect of the mean number of total interactions across all three-hour trials when the 28 sharks were observed interacting with the fish (light grey) and the seal silhouette (dark grey) around the research vessel in Mossel Bay during the study period. Panel G displays the mean behavioural score for the 28 sharks calculated across the trials towards the fish and towards the seal silhouette.

4.4.2 Movement data from acoustic telemetry

Data was available for the 12 sharks for 6-70 days (27.6 ± 6.23 days). Within Mossel Bay, the mean movement event recorded was of 5.5 ± 0.25 km (min = 0.6 km, max = 13.5 km). Only one female shark was recorded at the receivers located in Plettenberg Bay. Its longest movement event was of 121.1 km over a period of 8 days and 14 h, and it stayed in Plettemberg Bay for 10 days and 10 hours before returning to Mossel Bay. Significant inter individual variability and repeatability were found for both the duration of resident events (LRT = 22.2, $p < 0.001$; rpt = 0.14, $p = 0.002$) and the distance of the movement event (LRT = 12.5, $p < 0.001$; rpt = 0.21, $p = 0.03$), (Figure 4.7).

4.4.3 Correlations between behavioural responses and movement data

When including all 12 tagged sharks in the analysis, the first principal component (PC1) explained 58.4% of the variability, and included the highest loading values for shark activity around the research vessel and for their interactions with the fish. The second principal component (PC2) added a further 14.8% to the total variance explained by the PCA analysis, and its highest loadings represented the interactions of the sharks with the seal silhouette (Table 4.3). No significant relationships (Figure 4.7) were found between PC1 and residency ($F_{1,10} = 0.48$, $p = 0.51$, $R^2 = 0.046$) and movement ($F_{1,10} = 0.31$, $p = 0.59$, $R^2 = 0.03$), or between PC2 and residency ($F_{1,10} = 1.19$, $p = 0.3$, $R^2 = 0.11$) and movement ($F_{1,10} = 85$, $p = 0.38$, $R^2 = 0.08$).

When the shark that travelled to Plettemberg Bay was excluded from the analysis as the outlier, the first principal component (PC1) explained 58.5% of the variability, and included the highest loading values for shark activity around the research vessel and for their interactions with the fish. The second principal component (PC2) added a further 15.5% to the total variance explained by the PCA analysis, and its highest loadings

represented the interactions of the sharks with the seal silhouette (Table 4.3). A

significant correlation was found between PC2 and mean distance of movement events

($F_{1,10}$ = 3.47, p = 0.05; R^2 = 0.53), while no relationship was found between PC2 and

residency ($F_{1,10}$ = 0.93, p = 0.36, R^2 = 0.094), or between PC1 and residency ($F_{1,10}$ =

0.72, p = 0.42, R^2 = 0.074) or movement ($F_{1,10}$ = 0.07, p = 0.79, R^2 = 0.008), (Figure

4.7).

Table 4.3: Results of the principal component analyses on behavioural responses towards the fish and towards seal silhouette collected from the tagged sharks around the research vessel during each three-hour trial. We ran the analysis on all 12 tagged sharks, and then on 11 tagged sharks after excluding the only individual that travelled to Plettemberg Bay. In both cases, PC1 included the highest loading values for shark activity around the research vessel and for their interactions with the fish, while the highest loadings for PC2 represented the interactions of the sharks with the seal silhouette.

Behavioural response		Stimulus	Principal component	Loadings		Principal component	Loadings
Time of arrival (s)			PC1	-0.08		PC1	-0.174
Average time of visit (s)			PC1	0.205		PC1	0.326
Total time of stay (s)			PC1	0.777		PC1	0.773
Average decision (s)			PC1	0.363		PC1	0.435
Total N of visits			PC1	0.689		PC1	0.625
Total N of interactions		Both	PC1	0.778		PC1	0.754
Preference interactions		Bait	PC1	0.272		PC1	0.343
		Decoy	PC2	0.777		PC2	0.792
Investigations	All 12 sharks	Bait	PC1	0.167	11 sharks	PC1	0.092
		Decoy	PC2	0.539		PC2	0.587
Total bites		Bait	PC1	0.9		PC1	0.89
		Decoy	PC2	0.901		PC2	0.897
Attempted bites		Bait	PC1	0.824		PC1	0.783
		Decoy	PC2	0.869		PC2	0.868
Predatory Att. bites		Bait	PC1	0.819		PC1	0.765
		Decoy	PC2	0.817		PC2	0.771
Investigatory Att. bites		Bait	PC1	0.835		PC1	0.886
		Decoy	PC2	0.821		PC2	0.878
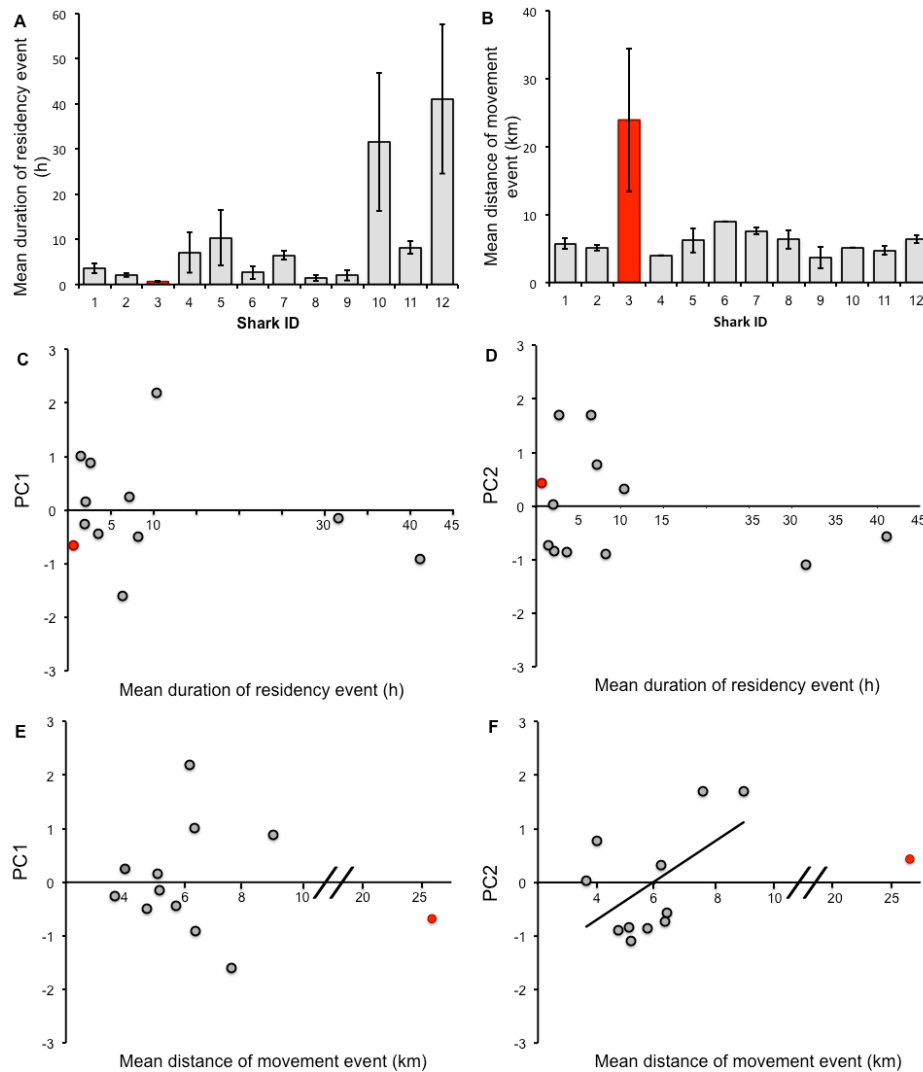
Figure 4.7: Inter-individual variability in the use of the foraging landscape in white sharks and its correlation to their behavioural responses to stimuli.

The mean ± s.e. of the duration of the residency events and the distance of movement events are shown in panels A and B, respectively. Panels C) and D) show the relationship between the first and second principal components of the PCA analysis of the mean of shark behavioural responses across all the three-hour trials when they interacted with the stimuli, and the mean duration of their residency events obtained from the movement data. Panels E) and F) show the relationship between the same first and second principal components for each individual, and the mean distance of their movement events from the movement data. Data and statistics are for the 11 tagged sharks (minus the outlier) while their VEMCO V16 frequency-specific continuous were active (minimum of 6 days to a maximum of 70 days; mean ± s.e = 27.6 ± 6.23 days). The red data point refers to the outlier (i.e. shark that travelled the furthest) removed from the part of the analysis reported in this graph. We obtained the duration and distance of the events by analysing movement data with VTrack package in R after removing false detections caused by collisions of acoustic signals or interference from background noise. The curves of best fit in the figure are calculated for the data when the only shark that travelled to Plettemberg Bay (represented by the red data point) was excluded.

4.5 Discussion

When observed from the research vessel, sharks exhibited more preference interactions, total bites and attempted bites towards the fish, while being more investigative towards the seal silhouette. Most attempted bites were predatory towards both controlled stimuli. We found significant and repeatable inter-individual variability in shark activity around the research vessel and in all preference interactions towards the seal silhouette, when sex, size, and other environmental variables were controlled for. These have been shown to affect hunting behaviour in this species (Hammerschlag et al., 2006; Towner et al., 2016). No individuality was detected in any of the interactions with the seal silhouette. This is possibly because most interactions with the seal silhouette were investigatory. Investigatory behaviour in animals often results from the uncertainty between approach and withdrawal (Hammerschlag et al., 2012), and might therefore lack the consistency over time that characterises personality related differences among individuals. Personality related habituation was observed in the total number of preference interactions and investigations of the fish. We found initial evidence of the presence of a behavioural syndrome between shark hunting behaviour and movement within their foraging grounds.

Our results are in agreement with previous reports of personality related traits in sharks that were tested in captive or semi-captive conditions (Jacoby et al., 2014; Wilson et al., 2015; Byrnes and Brown 2016; Byrnes et al., 2016; Finger et al., 2016; Finger et al., 2017; Finger et al., 2018), but show that this phenomenon also occurs when sharks are observed in their natural environment and for their natural prey-capture behaviours, which had only been tested once in captivity (Chapter 3). Similarly to lemon sharks in a semi-captive environment (Finger et al., 2016) and to dark shysharks in the laboratory (Chapter 3), white sharks in the wild showed habituation when repeatedly exposed to a

stimulus, but their rate of change in behavioural responses could also be attributed to personality, since it was highly variable between individuals. Our conclusions that individuality in hunting white sharks should be attributed to personality rather than differences in their appetite is reinforced by the significant repeatability across trials found in their responses to the fish, and is further supported by the lack of a connection between variability in foraging behaviour and possible differences in hunger in captive dark shysharks (Chapter 3).

In this study, we made novel use of movement data collected from sharks and found strong statistical evidence of individuality and consistency in residency and movement behaviours within the foraging landscape. Although some white sharks move along the South African coast, and between Mossel Bay and other well-known aggregation sites like False Bay and Gansbaai, our tagged sharks were not recorded at any of the receivers in these areas during the period of the study (Johnson, 2003; Kock and Johnson, 2006; Wcisel et al., 2010). Our findings confirm that white sharks exhibit high residency in Mossel Bay during the winter months to feed on Cape fur seals young of the year, and suggest that the absence of long movement events in this timeframe may be, in part, a consequence of the limits in behavioural plasticity imposed by personality. Only one of our tagged sharks was found to patrol Plettemberg bay, which suggests that some individuals may display higher plasticity in their movement within the foraging landscape by travelling between foraging areas. Obtaining movement data for a longer than 70 days and for a larger sample size could provide clearer patterns of individual differences in seasonal residency, and in the frequency of shark migrations between pinniped colonies (Stewardson, 2010). Our results provide evidence that personality directly influences shark movement within the foraging landscape, an idea suggested in other shark species (Vandeperre et al., 2014; Matich and Heithaus, 2015) but previously

shown in teleost fish like Atlantic cod (Villegas-Ríos et al., 2017) and mammals (Boon et al., 2008; Santicchia et al., 2018). Personality related differences in habitat use have been linked to fitness and survival in mammals and teleost (Villegas-Ríos et al., 2017; Santicchia et al., 2018), and should therefore be integrated to conservation related measures for high-risk taxa like elasmobranchs (Conrad et al., 2011; Mittelbach et al., 2014)

The relationship found between PC2 and movement behaviour in 11 of the 12 sharks suggests that white sharks are likely characterised by a behavioural syndrome between their hunting behaviour and their use of the foraging landscape, while the absence of such correlation when all 12 sharks were included in the analysis highlights the atypical behaviour displayed by the only shark that travelled the furthest but interacted very little with the stimuli. White sharks are known to engage in two different foraging strategies, patrolling or "sit-and-wait" (Towner et al., 2016). The atypical behaviour of the migratory individual suggests that some individuals in the Mossel Bay area might be displaying an alternative foraging strategies when compared to others. Migratory individuals might be more difficult to observe and acoustically tag if they spend little time around the research vessel or if they tend to interact with the stimuli far less than more resident sharks. Increasing the sample size of the study would maximise the chance of tagging more migratory individuals, and provide important information on their atypical behaviours. The absence of any other relationship between shark foraging behaviour and their residency behaviour further strengthens the need for a larger sample size to be able to determine with more confidence whether white sharks show strong syndromes between their hunting strategies and use of the foraging landscape.

Our study provides novel evidence for strong and consistent individuality in foraging behaviour of white sharks and in their movement within their foraging landscape. If personality reduces the ability of this species to adjust their behaviour in terms of movement and foraging strategy, this might impact the response of white sharks to rapid environmental changes caused by human activities (Dall et al., 2004; Sih et al., 2004a; Dudley and Simpfendorfer, 2006; Conrad et al., 2011; Mull et al., 2012; Mull et al., 2013; Mittelbach et al., 2014; Hammerschlag et al., 2019). Another important implication of our results is that white sharks, as individuals differing in their foraging strategies and in their behavioural correlations depending on the prey stimulus, will likely vary in their propensity to engage in human-animal conflict. This therefore has the potential to contribute to changing the public perception of this species, and to encourage managers and conservation authorities to develop better human protection strategies that can successfully mitigate the risk of shark encounters without causing harm to sharks and other marine species.

5.   General Discussion

"I am convinced that the most experienced naturalist would be surprised at the number of the cases of variability, even in important parts of structure…" (Darwin 1859).

Throughout the history of community ecology, researchers have focused on species as the principal units participating in predator-prey interactions and mediating the flow of energy within and between ecosystems (Layman et al., 2015). Any observed intraspecific variability in niche and habitat use has tended to be attributed to ontogenetic shifts or sexual dimorphism, with minimal consideration of how inter-individual variability (i.e. personality) mediates such processes (Araújo et al., 2011; Layman et al., 2015). Despite the recent research efforts focusing on the role of personality in ecology (Gosling, 2001; Sih et al., 2004a,b; Dingemanse et al., 2007; Herborn et al., 2010; Matich et al., 20115; Byrnes and Brown, 2016; Finger et al., 2016; Finger et al., 2017), there remains a significant gap in our general understanding on how individuals use resources and how population dynamics and community structures are affected by individual specialization and variability (Araújo et al., 2011; Finger et al., 2017). This holds particularly true for large vertebrates that enhance biodiversity and influence many aspects of marine ecosystem dynamics thanks to their top-down trophic effects (Byrnes and Brown, 2016, Finger et al., 2016; Finger et al., 2017), but are highly vulnerable to anthropogenic threats because of their K-selected life-history strategies (Stevens et al., 2000; Myers et al., 2007; Afonso and Hazin, 2014; Danylchuk et al., 2014; Finger et al., 2016; Musyl and Gilman, 2018).

5.1 Personality and behavioural syndromes in sharks

In this thesis I show the presence of consistent inter-individual variability in activity, foraging and anti-predator behaviour in dark shysharks observed in captivity. These results add onto the recent body of literature investigating shark personality (e.g. Byrnes and Brown, 2016; Byrnes et al., 2016; Finger et al., 2016; Finger et al., 2018), but most if not all such studies are constrained by the limitations imposed by captivity. As studies in the laboratory have the risk of introducing animals into situations that may be different from their natural conditions, it is particularly important to answer complex ecological questions by observing sharks in their natural environment (Mittelbach et al., 2014; Finger et al., 2017). I therefore took a step further and investigated the occurrence of consistent inter-individual variability in white sharks in the field, and showed how their hunting behaviour towards controlled food stimuli and their use of their foraging landscape strongly varied between individuals and was highly repeatable over time. In addition, the results showing how white shark interactions with the fish and with the seal silhouette explained the variability of two different behavioural axes suggest that individuals might consistently differ in their foraging behaviour depending on the prey item they interact with. These results, in addition to previous research on other taxa illustrating how some personality types have a greater chance of being captured (Finger et al., 2017), further reinforce doubts on the efficacy of current fishery management policies and of human protection strategies like drum lines and inshore exclusion nets, which are not only harmful for most large marine organisms, but also assume that all individuals from the target will show similar use of their habitats, and be equally likely to investigate a baited hook or a human swimming on the surface.

Dark shysharks exhibited a significant behavioural syndrome between the behavioural axes of activity and foraging. Such behavioural correlation seemed stronger in this

mesopredator shark than in white sharks, as there was a detectable relationship between the movement behaviour in this apex predator and only one aspect of its hunting preference. This might be because mesopredators are influenced by predation risk, which imposes opportunity costs for animals within the food web arena (Sih et al., 2004a,b; Bell and Sih, 2007; Dingemanse et al., 2009). Active individuals that spend more time foraging are, in fact, more vulnerable to predation, despite potentially being more successful at finding resources to allocate towards growth and reproduction (Sih et al., 2004a,b; Dingemanse et al., 2009; Conrad et al., 2011; Sommer-Trembo et al., 2016). The increase in predation risk by seven-gill sharks (*Notorynchus cepedianus*) and cape fur seals resulting from the loss of apex predatory sharks in some areas (Condir and Le Boeuf, 1984; Fallows et al., 2015; Hammerschlag et al., 2019) might therefore have important effects on hunting behaviour and fitness of shysharks and other mesopredators. Mesopredators are likely an important link between upper and lower trophic levels, as they mediate the changes in the structure and function of marine communities cause by the loss of apex predatory sharks as a result of fishing pressure and environmental changes (Dudley and Simpfendorfer, 2006; Vaudo and Heithaus, 2011; Mull et al., 2012; Mull et al., 2013; Worm et al., 2013; Hammerschlag et al., 2019). Information on the effect of relevant contextual experience (e.g. predation risk) on behavioural correlations can provide valuable insight into the ability of individuals to adapt their behaviour in response to changes in predation pressure or abundance of resources (Stamps and Groothuis, 2010). The effects of predation pressure on shyshark hunting behaviour may not only be the result of direct predation, but also from behavioural modification elicited by the presence of more predators in their environment, as hypothesized by the 'landscape of fear' framework (Gallagher et al., 2017a), but this needs further investigation.

5.2 The individuality of coping styles

When observing shysharks in the laboratory, I observed the relationship between shark physiological response to stress and their consistent variability in Foraging behaviour and Activity physiology (i.e. the individuality of coping styles). This shows that inter-individual differences in physiological profiles are likely one of the underlying causes of animal personality (Øverli et al., 2007), and also suggests that there might be personality related differences in how individuals respond to stressors such as capture-and-release. The acute stress caused by capture and handling practices elicit substantial changes in the physiological profile of many shark species, which likely have sublethal effects on their reproductive output and survival that go beyond the immediate mortality caused by fishing activities (Skomal and Mandelman, 2012; Wilson et al., 2014; Hammerschlag et al., 2019). Even a short 3 min capture event elicited an acute stress response in the peripheral circulation of individuals of three species of the Scyliorhinidae family of catsharks endemic to Southern Africa (pyjama catsharks, leopard catsharks, and *Haploblepharus spp.*). These findings further strengthen the concerns for the survival of these shark populations when high numbers are caught by both recreational fisheries and commercial fisheries but are generally discarded due their low commercial value, and for the changes that are likely to result from the loss in top-down trophic control of their prey species (Fowler et al., 2005; DAFF, 2013; da Silva et al., 2018; Barragán-Méndez et al., 2019; Silva and Ellis, 2019).

5.3 Implications for conservation

The limited behavioural variability imposed by personality and behavioural syndromes is likely to have important conservation consequences for shark species despite their positions within the trophic cascade. Both catsharks and white sharks will likely be

particularly vulnerable to human activities as they frequently use coastal areas that are usually highly threatened by anthropogenic impact, habitat loss and pollution (Dudley and Simpfendorfer, 2006; Mull et al., 2012; Mull et al., 2013; Hammerschlag et al., 2019). If these species will not be able to adjust their behaviour or migrate to other foraging areas due to the limits in behavioural plasticity imposed by personality, they might experience declines in population numbers due to the rapid environmental changes caused by human activities in addition to fishing pressure (Dall et al., 2004; Sih et al., 2004a; Conrad et al., 2011; Mittelbach et al., 2014). Considering that social environment had an effect on shysharks hunting behaviour and activity, changes in community composition and individual density as a result of population declines might further strengthen the effects of personality on the fitness and survival of this species and of other elasmobranchs that show the tendency to aggregate, like juvenile lemon sharks in nursery areas or hammerhead and zebra sharks during their reproductive stages (Jacoby et al., 2012). If social environment plays a significant role in shaping behaviour and personality, then consideration in conservation plans of its effects on the individuality of shark behaviour may be beneficial, as some of these aggregating species are considered endangered in the IUCN Red List of endangered species.

The recent increase in research efforts on the effects of personality on complex ecological processes like habitat use and predator-prey interactions has broadened our understanding of the consequences that limited behavioural plasticity can have on species fitness and on the survival of communities and ecosystems as a whole. Yet, large marine vertebrates remain underrepresented in this growing body of research because of the difficulties of obtaining replicated behavioural measures for the same individuals in the field. My findings are an important first step in the study of the effects of personality and behavioural syndromes on shark behaviour within the complex

framework of predator-prey interactions and habitat use of the foraging landscape, which is critical for a more complete appreciation of the role of mesopredators and apex predators in marine ecosystems. The loss of both mesopredators and apex predators as a result of fishing pressure and, possibly, of the limits imposed by personality on their ability to adapt to environmental changes will likely to have cascading effects on the community structure of entire ecosystems (Ritchie and Johnson, 2009; Vaudo and Heithaus, 2011), and should therefore be considered in conservation plans to better protect vulnerable taxa.

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Supplementary tables

Table S2.1: List of total lengths (TL) measured for each shark captured during the study.

Species	Sex	Total Length (cm)
Pyjama	F	67
	F	75
	F	88.5
	F	94.5
	M	57.5
	M	66
	M	67.5
	M	68
	M	78
	M	80.5
	M	84
	M	86.5
	M	92.5
	M	94
	M	95
Leopard	F	59
	F	60
	M	52.5
	M	61
	M	64
	M	67
	M	67.5
	M	68
	M	69
Shysharks	M	71
	F	61
	F	62
	F	58.5
	M	63
	M	66.5
	M	56
	M	55
	F	62

Table S3.1: Results from general linear models used to investigate the effect of sex, size and group sex on all PC1, on sociability and anti-predator behavioural score calculated for the 48 dark shysharks. In Experiment 1, 15 individuals tested in the lab as pairs were exposed to the presence of a confined prey stimulus (sardine head and live klipfish) for 30 min and, after 24 hours, to predation risk (inflatable seal and diluted seal scat) for 30 min. All F values for the behavioural responses in Experiment 1 are calculated on 3 and 11 dF. In Experiment 2, 11 solitary and 22 paired individuals were exposed to the confined prey stimulus for 20 min and, after 24 hours, to the confined prey stimulus again but in the presence of predation risk. All F values for the behavioural responses in Experiment 2 are calculated on 3 and 18 dF for paired individuals, and on 2 and 8 dF for solitary individuals.

	Treatment	Sex	Size	Group sex
Activity	prey, Exp 1	F = 0.23 p = 0.63	F = 3.15 p = 0.11	F = 1.27 p = 0.28
	predator, Exp 1	F = 1.98 p = 0.18	F = 0.39 p = 0.55	F = 2.02 p = 0.18
	prey, Exp 2	F = 1.21 p = 0.28	F = 0.85 p = 0.36	NA
	prey + predator Exp 2	F = 0.71 p = 0.41	F = 0.38 p = 0.54	NA
Foraging	prey, Exp 1	F = 0.87 p = 0.37	F = 3.46 p = 0.09	F = 0.34 p = 0.57
	prey, Exp 2	F = 2.25 p = 0.14	F = 0.073 p = 0.79	NA
	prey + predator Exp 2	F = 0.36 p = 0.55	F = 0.36 p = 0.56	NA
Sociability	prey, Exp 1	F = 0.41 p = 0.54	F = 0.03 p = 0.86	F = 0.87 p = 0.37
	predator, Exp 1	F = 0.05 p = 0.82	F = 1.28 p = 0.28	F = 0.17 p = 0.69
ToE	prey, Exp 1	F = 0.12 p = 0.73	F = 0.18 p = 0.68	F = 1.47 p = 0.25
	prey, Exp 2	F = 0.029 p = 0.87	F = 0.81 p = 0.38	NA
	prey + predator Exp 2	F = 0.011 p = 0.92	F = 0.005 p = 0.94	NA
Score	predator, Exp 1	F = 0.04 p = 0.84	F = 0.028 p = 0.87	F = 1.04 p = 0.33
	prey + predator Exp 2	F = 0.008 p = 0.93	F = 0.56 p = 0.45	NA