

# **Predicting shark behaviour: the influence of species, size and brain organisation**

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**ABSTRACT:** Understanding the behaviour of sharks and how it varies may have multiple benefits in informing conservation and bycatch mitigation efforts, fisheries management and efforts to decrease the risk of negative interactions between sharks and humans. However, shark behaviour is difficult to study in a natural setting given their size, predatory nature and environment. Novel techniques are therefore required to acquire *in situ* observations of shark behaviour. This study used baited remote underwater stereo-video systems (stereo-BRUVS) in conjunction with an ethogram, to investigate the effect of species and size on behavioural diversity, frequency and timing in eight species of shark, based on imagery collected in the Chagos Archipelago. Additionally the effect of brain organisation on behaviour was investigated through consideration of the relative sizes of major brain regions among species. Species was found to be the primary determinant of behavioural variation with size playing a secondary role. Variation in diversity, frequency and timing of behaviours among species was partially explained by known physiological and ecological differences. Brain organisation was moderately correlated with behavioural composition indicating support for the theory of the neural basis of behaviour. Additionally, this study, as a proof of concept, shows that valuable behavioural data can be derived from the novel application of stereo-BRUVS, representing a viable means of generating *in situ* behavioural observations of marine apex predators.

**KEYWORDS:** Behavioural variation · Phylogeny · Ontogeny · Chagos Archipelago · stereo-BRUVS · Brain organisation · Neural basis of behaviour

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# RESEARCH THESIS

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## INTRODUCTION

Understanding animal behaviour is fundamental to understanding an animal's ecological role and how it interacts with the environment around it (Bres 1993, Pickett et al. 2010). The links between behaviour and ecology have long been a subject of interest (Cullen 1957, Winn 1958, Crook 1964), with studies not limited to the effect of phylogeny, but also including size, physiology, learning, and neural systems, among other factors (Krebs & Davies 2009, Lucifora et al. 2009). A comparative approach that uses quantitative data to investigate behavioural variation among groups (e.g. species or size) can illuminate the links between behaviour and ecology, and the factors influencing their variation (Crook 1964, Crook & Gartlan 1966, Lucifora et al. 2009). This approach may also allow broader questions of behavioural origin to be investigated (Lorenz 1950). As behaviour is theorised to have a neurological basis (Delcomyn 1980), differences in behaviour may be attributable to differences in neural composition. A better understanding of animal behaviour may have important implications for conservation by improving understanding of habitat use and movements (Vianna et al. 2013), contributing to an understanding of roles in ecosystem functioning (Bres 1993) and informing conservation strategies such as bycatch mitigation. For instance, some recent studies have trialled methods to reduce the incidental capture of sharks in fisheries (Hutchinson et al 2012), however, differences in foraging ecology and feeding behaviour among species and among size classes introduces complications in the deployment of these techniques (Hobson 1963, Lucifora et al. 2009). Behavioural understanding also contributes to fisheries management by, for example, providing insights into catchability and gear selectivity (Spaet et al. 2010). Finally, understanding shark behaviour relating to foraging, defence, aggression and competitive interactions may also help reduce negative shark-human interactions, by providing insights on the motivation behind shark bites with respect to feeding, non-foraging aggression, anti-predatory response or investigatory behaviour (Caldicott et al. 2001, Nelson et al. 1986, Baldrige 1988).

As apex predators, sharks play a significant ecological role in modifying ecosystem structure through top down control (Friedlander & DeMartini 2002, Schindler et al. 2002, Worm & Myers 2003, Myers et al. 2007, Baum & Worm 2009). As a result, their removal through fishing has been associated with trophic cascades, mesopredator release, and changes in prey life history parameters and behaviour (Worm & Myers 2003, DeMartini et al. 2005, Myers et

al. 2007, Madin et al. 2010a, Madin et al. 2010b, Klages et al. 2013, Ruppert et al. 2013). Sharks are particularly susceptible to overfishing due to their late age at maturity, low fecundity and large body size (Schindler et al. 2002, Cortés et al. 2010, Graham et al. 2010, Dulvy et al. 2014). Due to their key ecological role, shark population declines are of concern not only from a conservation perspective (Graham et al. 2010), but also economically. A key example was the removal of large sharks in North Carolina due to overfishing which enabled an explosion of the cownose ray population, which in turn decimated the local scallop fishery leading to significant financial losses (Myers et al. 2007).

There are a number of factors which act in concert to determine the behaviour of individual animals. In sharks, the most prominent predictors of behaviour appear to be species and size (Lowe et al. 1996, Lucifora et al. 2009, Wueringer et al. 2012, Oliver et al. 2013). Species and broader taxonomic classes vary widely in physiology, size, dominant prey species, sensory ecology, brain morphology and other anatomical adaptations (Carrier et al. 2012). It follows that, due to these differences, species perceive and interact with their environment in different ways and therefore display different suites of behaviour. For example, the foraging behaviour of thresher sharks (*Alopiidae*) and hammerhead sharks (*Sphyrnidae*) differs markedly and involves major anatomical differences: thresher sharks have greatly elongated tails which are used in a whipping motion to stun fish (Oliver et al. 2013), whereas hammerheads have an increased cephalofoil related to increased use of electroreception and therefore an increased ability to detect prey cryptic to other senses (Kajiura & Holland 2002). Within species, variation in size has also been linked with behavioural differences. These include changes in diet (Lowe et al. 1996, Lucifora et al. 2009), social preferences (Springer 1967, Guttridge et al. 2009, Mourier et al. 2012), dominance (Myrberg and Gruber 1974) and other behaviours (Sperone et al. 2010). These ontogenetic changes may have their bases in physiological, neural, energetic and anatomical changes as the animal develops, or in learning (Engen & Stenseth 1989, Kardong et al. 1996, Lisney et al. 2007, Punzo & Chavez 2003).

The theory of the neurological basis of behaviour suggests that the behaviour of animals can be predicted in part, based on a comparison of peripheral sense organs and the relative size of brain regions (Delcomyn 1980). This has led to the study of cerebrotypes, or similar patterns of brain organisation in species which share ecological similarities such as feeding strategies, habitat occupation or cognitive ability (Iwaniuk & Hurd 2005). With both brain



organisation and relative brain size showing strong correlations to ecology in all vertebrate groups (Yopak & Montgomery 2008), differences in behaviour among species and size classes may be due to associated variation in brain size and composition. There is widespread variation in both brain size and complexity among shark species (Northcutt 1977, Yopak et al. 2007), and ontogenetic shifts are evident in both peripheral sense organs and relative sizes of brain regions within species (Lisney et al. 2007, Litherland et al. 2009). Some of this variation has been linked to differences in sensory ability, ecology and habitat use, in addition to phylogeny (Lisney & Collin 2007, Schleussel et al. 2008, Yopak & Lisney 2012). However, no observational behavioural studies have attempted to directly link brain organisation with behavioural variation in sharks in order to provide support for this theory.

Previous studies of shark behaviour have investigated a wide array of aspects, ranging from specialised feeding behaviours, agonistic displays and dominance to social aggregations and larger scale movement and migration patterns (Tricas 1985, Martin 2007, Guttridge et al. 2009, Mourier et al. 2012, Oliver et al. 2013, Vianna et al. 2013). The majority of these studies are based on observations from in-lab studies, divers or tagging and telemetry data (Nelson 1976, Tricas 1985, Sperone et al. 2010, Vianna et al. 2013). There have, however, been calls for more *in situ* behavioural studies based on more defined, quantitative techniques (Gruber & Myrberg 1977, Martin 2007). However, studying sharks in their natural setting is challenging given that they are large predators and inhabit relatively remote environments that can be difficult to sample directly (Nelson 1977, Bres 1993). Diver based studies present a number of limitations, with sharks often avoiding divers and undertaking vertical migrations below SCUBA depths for extended periods (Nelson 1976, Bres 1993). Additionally diver number and proximity has been shown to have a significant effect on shark behaviour and there is the risk of aggressive interaction (Johnson & Nelson 1973, Graham et al. 2010, Smith et al. 2010). Moreover telemetry can be limited in terms of its provision of behavioural detail (e.g. display behaviours such as pectoral fin depression) therefore innovative methods for subsurface sampling are required. Using remote video to make behavioural observations is one method suggested to avoid observer effects (Watters et al. (Watters et al. 2009).

Baited remote underwater stereo-video systems (stereo-BRUVS) are a cost-effective sub-surface sampling method used primarily for abundance and size structure studies (Cappo et al. 2006). They offer a range of advantages, allowing species identifications, length and distance measurements, visual habitat sampling, and provide footage of a range of species interacting with each other in a reproducible and controlled manner (Cappo et al. 2004, Harvey et al. 2007). They also have the distinct advantage of the option of deployment in waters inaccessible to divers in depths of up to 1200m (Zintzen et al. 2012). However, they have been utilised for relatively few behavioural studies (Ebner et al. 2009, Oliver et al. 2011, Renchen et al. 2012, Ryan et al. *subm*) and few of these have incorporated the stereo component. Stereo-BRUVS have some limitations in that they rely on good visibility and may induce inquisitive behaviour (Watson et al. 2005), however, these issues are also common to diver based studies and are difficult to overcome in underwater studies. Their limited field of view is also a limitation in that only a subset of the behaviour exhibited will be observed, however, with adequate sample sizes, the behaviour exhibited within the field of view is more likely to be a representative subset of overall behaviour exhibited. Finally, the use of bait does not mimic a natural situation, however, it aids in ensuring adequate sample sizes are achieved and may induce exhibition of behaviours of interest, for example agonistic displays among individuals and behaviours related to foraging. As such, these systems, combined with classical techniques used for the study of behaviour such as ethograms (Shleidt 1984), may be a valuable source of data for the field of behavioural research.

This study used previously collected stereo-BRUVS footage from the Chagos Archipelago (central Indian Ocean), coupled with a behavioural catalogue (ethogram) to quantify the behaviour of sharks, thus testing a novel approach to the study of apex predator behaviour in the marine environment. This study aimed to determine the relative influence of species and size on the behaviour exhibited by sharks as recorded by stereo-BRUVS. It further aimed to determine whether these differences in behaviour have a neurological basis through the consideration of the relative sizes of major brain regions among species. It was hypothesised that as differences in brain composition and physiology are generally larger among species than among size classes within species, that species would be the major determinant of behavioural repertoire, frequency and timing and that size would play a secondary role in moderating behaviour.

## METHODS

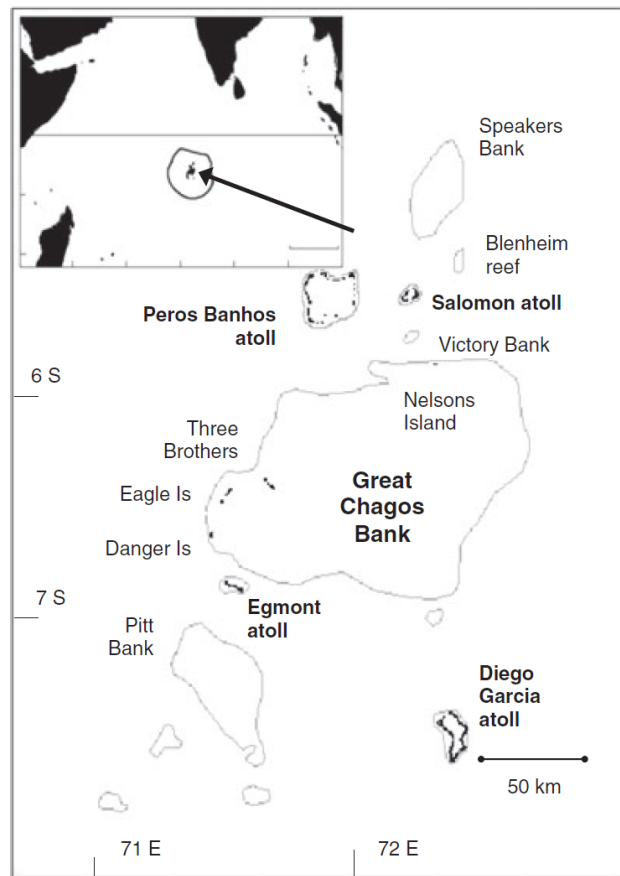
### *Study site*

Video imagery used in this study was collected in 2012 as part of a coral reef-focused scientific expedition to the Chagos Archipelago, British Indian Ocean Territory (BIOT). Located in the central Indian Ocean, approximately 500km south of the Maldives between 04°50' to 07°40' S and 70°10' to 72°40' E, the Chagos Archipelago is comprised of seven major atoll structures, 55 islands and 86 seamounts (Graham et al. 2010) (Figure 1). Diego Garcia, the largest of the islands in the Archipelago, hosts a US naval base and the British administration for the territory. The remaining islands are uninhabited and have been since the early 1970s (Graham et al., 2010). In 2010, the Chagos Marine Reserve (hereafter referred to as the Chagos) was established as the world's largest no-take marine protected area, covering over 544,000 square kilometres (Koldewey et al. 2010). Its relative remoteness and reserve status make the Chagos an ideal site for ecological and behavioural studies (Graham et al. 2010, Sheppard et al. 2012). Reef and lagoon habitats were sampled at 35 sites within the Chagos, ranging in depth from 5.4 – 82.2 metres, around the two northernmost atolls, Salomon and Peros Banhos, at the submerged shoal at Victory Bank, at Brothers and Eagle Islands and Danger Bank along the western edge of the Grand Chagos Bank, and at a deeper seamount south of the Grand Chagos Bank (Figure 1).

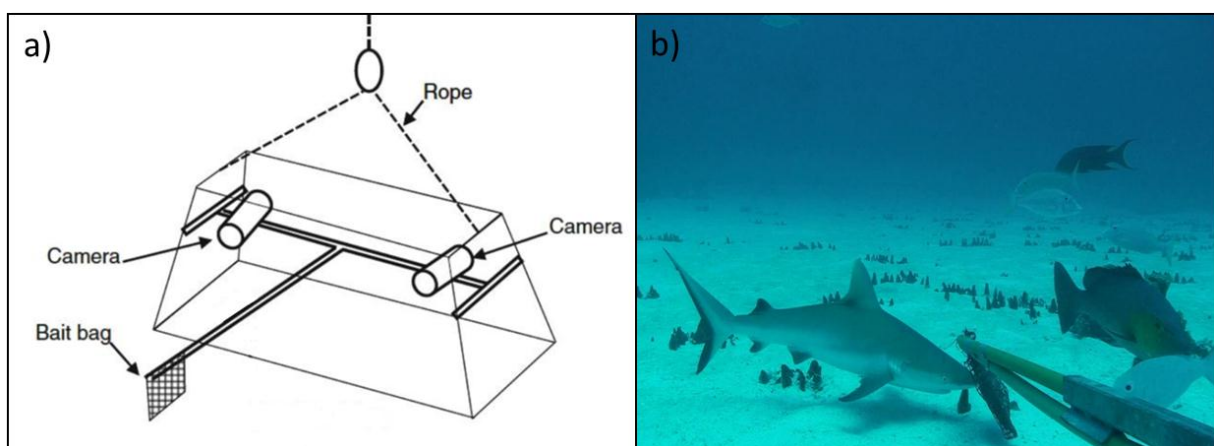
### *Collection of video imagery*

Video imagery was collected by a team from UWA using stereo-BRUVS (Cappo 2006) during a three week expedition in February and March 2012. This imagery was originally collected to establish a baseline for fish and shark assemblages across the archipelago. Following previous stereo-BRUVS studies (Cappo 2006, Watson et al. 2010; Harvey et al. 2007), the sampling rigs consisted of two HD digital video cameras (Sony Handycam™ models HX7 or HX12), housed in pressure resistant housings and fixed to a rigid bar held within a steel frame that provides both stability and protection on the seabed (Figure 2). A flexible bait arm, made from 15mm plastic pipe and 1.5m long, was fixed between the cameras. A plastic mesh bait-bag was attached to the end of the bait arm and baited with 0.5kg of pilchards (*Sardinops* spp., sourced frozen in Singapore), roughly chopped to release blood and oil. Stereo-BRUVS were deployed for a minimum of one hour to allow for post-processing to be standardised to 60 minutes. Rigs were deployed at 138 sampling stations from the BIOT Patrol Vessel Pacific Marlin's fast rescue craft. In this study, this footage was reanalysed for the collection of behavioural data. All data collection in the Chagos was

carried out under UWA ethical guidelines and was approved by the BIOT Administration and Scientific Advisory Group.



*Figure 1:* Map of the Chagos Archipelago, the inset shows the position of the archipelago within the Indian Ocean and the reserve boundary. The main map shows; the major atolls with land (in bold), the islands of the Great Chagos Bank and submerged reef atolls (not in bold) (from Sheppard et al. 2012).



*Figure 2:* a) Schematic of a stereo-BRUVS (baited remote underwater stereo-video system), showing the metal frame, two video cameras in waterproof housings, bait arm, bait bag and rope (Adapted image from T. Letissier). b) Example image from the left camera of a deployed stereo-BRUVS in the Chagos, with the bait arm projecting forward.

### ***Ethogram***

I developed an ethogram, creating a defined catalogue of behaviours and actions exhibited by sharks to allow the consistent, semi-quantitative analysis of behaviour (Shleidt et al 1984). The specific behaviours included in the ethogram (Table 1) were based on a preliminary review of the imagery to determine which behaviours could be observed; focusing primarily on behaviours referred to in the primary literature, which were selected based on a number of criteria (Myrberg & Gruber 1974, Martin 2007, Smith et al. 2010, Sperone et al. 2010). These criteria included whether the behaviour: (1) could be defined objectively; (2) was observed across more than one species; (3), occurred sub-surface; and (4) was ecologically relevant. Consequently, the ethogram included a range of agonistic displays and movements which may be related to dominance or communication between individuals, a number of behaviours involving the bait bag (approach, nudging, biting and bait bag removal), and a record of the passive behaviours of entering and exiting the frame in order to give an indication of activity level and allow the derivation of more descriptive information relating to timing (Table 1).

### ***Processing stereo-BRUVS imagery for behavioural analysis***

Behavioural observations of sharks were recorded from a total of 33 of the stereo-BRUVS samples. Only samples of adequate visibility, and for which imagery was available for 60 minutes were included. Additionally, deployments were subsampled to acquire an adequate sample size for each species present: all possible footage of rare species was analysed and some videos containing only footage of *C. amblyrhynchos* (the most common species) were not analysed.

Video imagery was converted to AVI format using Xilisoft™ video conversion software and analysed using EventMeasure™ ([www.seagis.com.au/event.html](http://www.seagis.com.au/event.html)). Imagery was analysed for one hour following deployment (from when the rig came to a rest on the seabed) or until no bait was present. Stereo analysis used the synchronised footage from both cameras to record lengths of individual sharks by reference to a measurement calibration file created for each camera rig ([www.seagis.com.au/bundle.html](http://www.seagis.com.au/bundle.html)) and log behavioural observations. Individuals were identified first by their species then by a combination of their size, sex and individual identifying characters (such as scars, fin tears, fin shape and pigment patterns) (Myrberg & Gruber 1974, Nelson 1977). Each time a new individual entered the field of view a fork length measurement of the individual was taken (from the tip of the snout to the fork of the tail), and their species and identification information was noted. When sharks exited the field

*Table 1:* Ethogram used to define behaviours recorded in this study. Definitions were derived or adapted from previous literature where possible, new definitions were determined for behaviours of interest not previously cited. BL/s = body lengths, Hz = frequency per second

<b>Class</b>	<b>Behaviour</b>	<b>Definition</b>
Entries/Exits	Enter	The first point at which the shark appears in both camera frames
	Exit	The last point at which the shark is visible in both camera frames
Displays and other behaviours	Charging (Chase) <sup>a</sup>	Rapid (2 to 3+ BL/s) approach toward a receiver, often terminated by veering away on a perpendicular course within a distance of 1–2 BL
	Ramming with snout <sup>a</sup>	A signaller using its rostrum to forcefully strike a receiver, often causing it to recoil or retreat
	Jaw gaping <sup>a</sup>	A slow, exaggerated opening of the jaws ( $\pm 30 - 90^\circ$ , measured or estimated as the angle formed at the mouth commissure), conspicuously wider than during ram ventilation and in an agonistic context, resembling a yawn
	Jaw Snap <sup>a,b</sup>	Rhythmic ( $\pm 1$ Hz), exaggerated opening and closing of the jaws, once or twice in rapid succession ( $\pm 30-90^\circ$ , measured or estimated as the angle formed at the mouth commissure)
	Rapid withdrawal <sup>a,b,c</sup>	Rapid swimming movement away from a stimulus (at $\geq 3$ BL/s) initiated by several strong tail-beats followed by a long glide covering a distance of 5 m or more
	Pectoral Fin Depression <sup>a</sup>	A sustained ( $>5$ s), bilateral lowering of the pectoral fins from their usual position during swimming
	Head Shake	Rhythmic, exaggerated lateral shaking of the head along a horizontal plane, usually rapid ( $>2$ Hz) and through an arc of $>30^\circ$
Nictitating	Closing of the nictitating membrane over 50% or more of the eye	
Bait associated behaviours	Approach	Approaching the bait bag within 0.5 m as if to make contact before veering away.
	Nudge	Contacting an object with the snout or head
	Bite	Jaw closure around an object/animal and contact with teeth
	Remove Bait Bag	Removal of the bait bag from the bait arm, usually through continued biting, head shaking or body rolling

Sources: a)Martin 2007; b)Myrberg & Gruber 1974; c) Smith et al. 2010

of view and there was reasonable doubt that it was the same individual returning, these characters were used to distinguish between individuals where possible. However, if animals could not be identified as “new” or an earlier identified animal, no measurement was taken in order to avoid pseudoreplication (Hurlbert 1984). Each time a behaviour identified in the ethogram was exhibited by an individual shark, a point was placed on the animal’s snout, and the behaviour was recorded in the comment field, resulting in a record of the individual’s position in three-dimensional space in front of the camera and the time of occurrence.

***Metrics: frequency and timing***

The frequency of behaviours was summed across the number of animals of each species or size class and then divided by the number of individuals in that group. This generated a mean frequency value that controlled for differing numbers of animals in different species and size classes. Timing metrics were calculated to determine whether species and or size classes varied in the timing of their behavioural responses. Timing metrics included: (1) time of first arrival, (2) time of first observation of a behaviour from time from first arrival; (3) total time spent in frame, (4) mean duration in frame and (5) total time from first entry to last exit (Table 2). All were again calculated as mean values across the individuals within a given species or size class.

*Table 2:* Derivation of timing and duration metrics from raw timing data

<b>Metric</b>	<b>Definition</b>	<b>Calculation</b>
Time of first arrival	Time from deployment of stereo-BRUVS to first observation of an individual	Time of first observation – time of deployment
Time from first arrival to behaviour x	Time from the arrival of an individual to the first observation of a given behaviour	Time of first occurrence of behaviour x – Time of first arrival
Mean duration of pass	Mean time spent within frame from entry to exit	Total duration in frame ÷ number of passes
Total duration in frame	Total time spent within the field of view	$\sum(\text{Time of exit of a given pass} - \text{time of entry of that pass})$
Time from first entry to last exit	Time from the first time an animal entered the field of view to the last time it exited the field of view	Time of last exit – time of first entry

### ***Brain organisation***

Morphometric data on brain organisation were compiled from previous (Bauchot et al. 1995, Yopak 2007, Yopak et al. 2010, Yopak & Lisney 2012); and ongoing research (Yopak unpublished data). Total body mass, total brain mass, and mass of each of the major brain regions (olfactory bulbs, telencephalon, diencephalon, mesencephalon, cerebellum and medulla oblongata) were extracted. From these, the brain to body ratio was calculated and relative proportions of the major brain regions as a proportion of the total brain mass were calculated for each species.

### ***Statistical analysis***

Statistical analyses were carried out using the software PRIMER 6 as well as Microsoft Excel with the add-in Statisti-XL. Mean length was compared among species using a full-factorial ANOVA with eight levels and a Tukey test for pairwise comparisons of species. The frequency of behaviours was compared among species and among size classes using chi-squared contingency tests (Zar 1999). The composition of behaviours was compared among species based on a Bray-Curtis resemblance matrix of square root transformed frequencies for individual sharks, including log transformed length as a covariate in a permutational ANCOVA (Anderson et al. 2008). To investigate whether size affected frequency of behaviours, a distance based linear model (distLM; Anderson 2004) based on a Bray-Curtis resemblance matrix of square root transformed frequencies for grey reef shark was completed. The time metrics (time of first arrival, total time in frame, mean duration of pass and time from first entry to last exit), were compared among species using full-factorial ANOVAs with eight levels and pairwise Tukey tests, and among species with length as a covariate using an ANCOVA in order to determine whether length was an important factor. The time of first observation from first entry was compared among behaviours, and among species using ANOVAs.

To investigate the patterns in brain composition among species a canonical analysis of principal coordinates grouped by family was run on a Euclidean resemblance matrix of log transformed and normalised values of major brain regions (olfactory bulbs, telencephalon, diencephalon, mesencephalon, cerebellum and medulla oblongata) as a proportion of total brain mass for each species (note: values were not corrected for phylogeny) (Yopak 2012). To investigate the relationship between brain composition and behavioural composition, a 2<sup>nd</sup> stage MDS was run on a Euclidean resemblance matrix of normalised proportions of major



brain regions by species and a Bray-Curtis resemblance matrix of square root transformed behavioural frequency by species (Clarke et al. 2006).

## RESULTS

Videos from 33 stereo-BRUVS deployments were analysed, generating observations of 86 sharks from eight species (*Carcharhinus albimarginatus*, *Carcharhinus amblyrhynchos*, *Carcharhinus melanopterus*, *Galeocerdo cuvier*, *Triaenodon obesus*, *Nebrius ferrugineus*, *Sphyrna lewini* and *Sphyrna mokarran*) and three families (*Carcharinidae*, *Ginglymostomatidae* and *Sphyrnidae*) (Table 3). These species are ecologically diverse in the habitats they occupy, ranging from primarily benthic and reef associated to semi-oceanic ecosystems, as well as in the variety of prey they consume, from predominantly benthic invertebrates to mid-water fishes and cephalopods, with trophic levels varying from 3.9-4.5 (Froese 2010). They also vary in their method of respiration with five species being obligate ram ventilators and the remaining three species able to use buccal pumping to force water over the gills whilst stationary (Table 3).

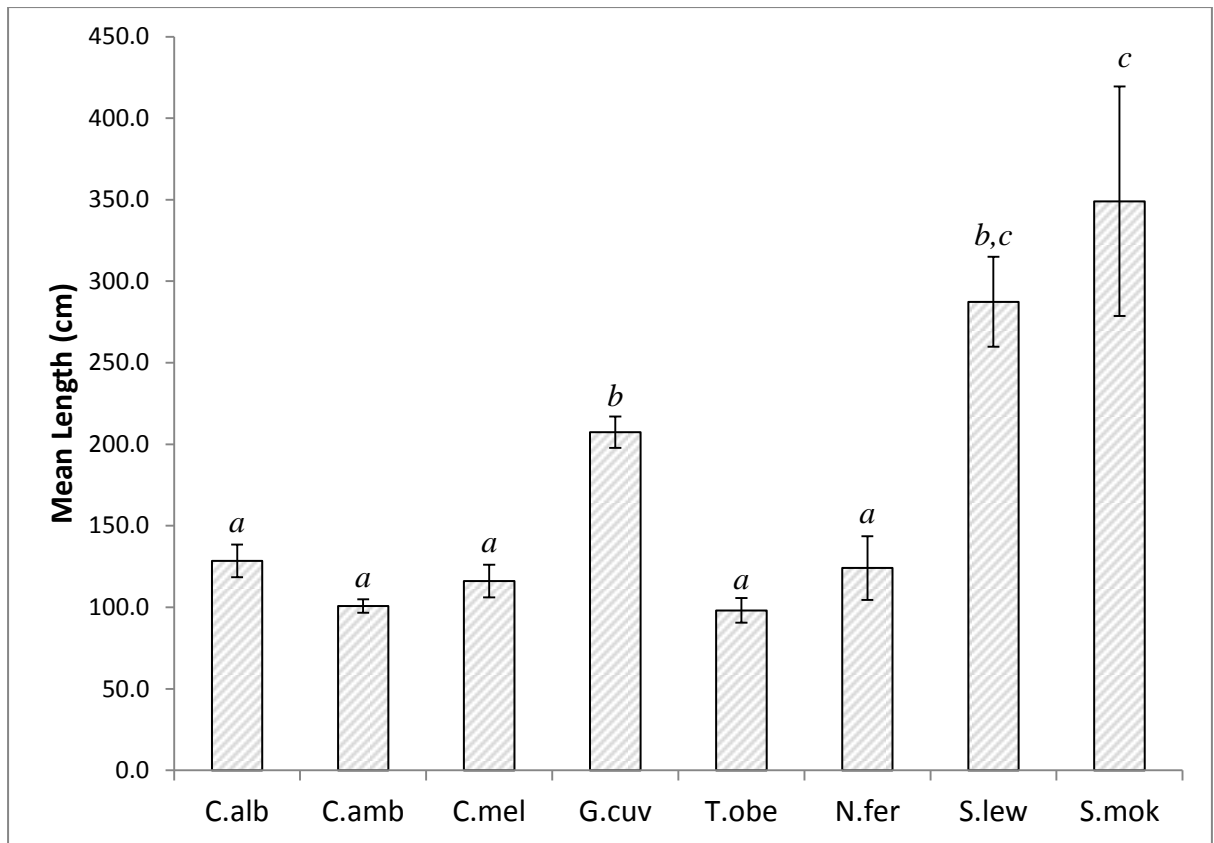
Of the 86 sharks for which behavioural observations were made, estimates of size were generated for 73 individuals (85%). Fork length varied from 55.1 cm to 4194 cm with a mean length of  $127.3 \text{ cm} \pm 7.7 \text{ SE}$ . Mean length varied among species ( $p=3.34 \times 10^{-19}$ ), with *S. lewini*, *S. mokarran* and *G. cuvier* significantly larger than all other species and *S. mokarran* significantly larger than *G. cuvier* (Figure 3). Individual sharks were classified as small (<1m; n=30 sharks), medium (1-1.5m; n=29) or large (>1.5m; n=14).

I recorded 4500 instances of 14 behaviours identified in our ethogram (Table 3). These represented three general groups of behaviours; entries and exits, displays and other behaviours which included nictitating, rapid withdrawal, pectoral fin depression, head shake and other active behaviours (other active behaviours was a grouping of the low frequency jaw snap, jaw gape, ramming with snout and chase behaviours), and direct interactions with the bait which included approaches, nudges, bites and bait bag removals. These behaviours varied in the frequency of their occurrence as well as their timing.

*Table 3:* Species information, number of observations is the total number of behavioural observations per species; number of behaviours is the number of different types of behaviour observed within a species; n individuals is the total number of individual animals observed; n lengths is the total number of length estimates derived; mean length, size range and maximum length are all stated as fork lengths and RAM/pump ventilation indicates whether a species is an obligate RAM ventilator or whether they can ventilate using buccal pumping

Common Name	Family	Species	Number of observations	Number of Behaviours	n Individuals	n Lengths	Mean Length $\pm$ SE (cm)	Size Range (cm)	Maximum Length <sup>a</sup> (cm)	RAM/pump ventilation <sup>b</sup>
Silvertip Shark	<i>Carcharinidae</i>	<i>Carcharhinus albimarginatus</i>	572	7	10	10	128.5 $\pm$ 10.0	88.6 - 203.6	242.4	Obligate
Grey Reef Shark	<i>Carcharinidae</i>	<i>Carcharhinus amblyrhynchos</i>	3352	10	44	37	100.8 $\pm$ 4.2	66.5 - 198.7	217.39	Obligate
Blacktip Reef Shark	<i>Carcharinidae</i>	<i>Carcharhinus melanopterus</i>	24	2	5	5	116.1 $\pm$ 10.1	98.4 - 152.6	163.8	Obligate
Tiger Shark	<i>Carcharinidae</i>	<i>Galeocerdo cuvier</i>	73	7	2	2	207.4 $\pm$ 9.6	197.9 - 217.0	617.25	Non-obligate
Whitetip Reef Shark	<i>Carcharinidae</i>	<i>Triaenodon obesus</i>	360	6	7	7	98.2 $\pm$ 7.6	82.6 - 141.7	175.512	Non-obligate
Tawny Nurse Shark	<i>Ginglymostomatidae</i>	<i>Nebrius ferrugineus</i>	12	2	10	6	124.1 $\pm$ 19.6	55.1 - 179.9	250.24	Non-obligate
Scalloped Hammerhead	<i>Sphyrnidae</i>	<i>Sphyrna lewini</i>	17	5	6	4	287.4 $\pm$ 27.5	209.1 - 337.8	333.25	Obligate
Great Hammerhead	<i>Sphyrnidae</i>	<i>Sphyrna mokarran</i>	90	8	2	2	349.0 $\pm$ 70.4	278.6 - 419.4	457.5	Obligate
Grand Total			4500	11	86	73	127.3 $\pm$ 7.7	55.1 - 419.4		

Sources: a) Froese 2010; b) Morgan & Burges 2006



*Figure 3: Mean fork length (cm) observed for each species observed during analysis of 33 videos collected in Chagos Archipelago, British Indian Ocean Territory 2012. Letters indicate the results of a post-hoc Tukey test based on a full factorial ANOVA. Species assigned different letters had significantly different mean observed lengths. C.alb (*Carcharhinus albimarginatus*, n=10), C.amb (*Carcharhinus amblyrhynchos*, n=37), C.mel (*Carcharhinus melanopterus*, n=5), G.cuv (*Galeocerdo cuvier*, n=2), T.obe (*Triaenodon obesus*, n=7), N.fer (*Nebrius ferrugineus*, n=6), S.lew (*Sphyrna lewini*, n=4) and S.mok (*Sphyrna mokarran*, n=2).*

### **Frequency**

Across all species, the most frequent behaviours were entry (35.8%) and exit (35.3%). The high proportion of these behaviours largely represents the repeated movements of individual animals in and out of the field of view. Exits were slightly fewer as some individuals were still on screen at the completion of 60 minutes of analysis. The next most common behaviours were generally those involving direct interaction with the bait bag (i.e. nudges, bites and approaches) along with the pre-interaction behaviour of nictitating (Figure 4).

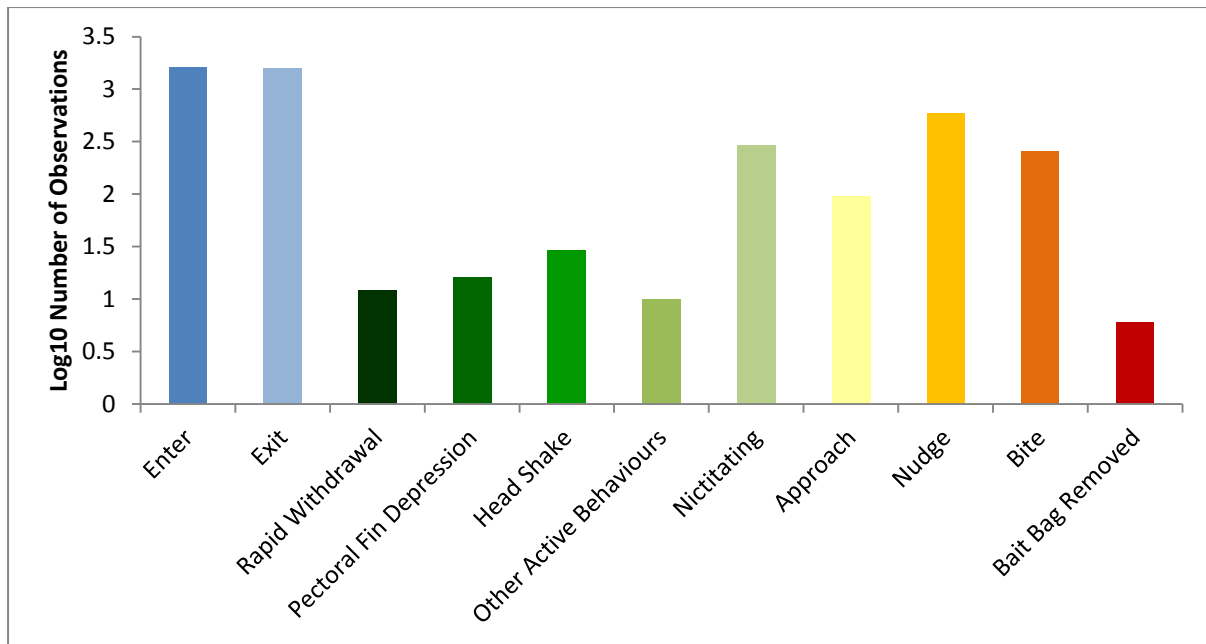


Figure 4: Log transformed total number of observations of each behaviour summed across all species observed during analysis of 33 videos collected in Chagos Archipelago, British Indian Ocean Territory 2012.

The frequency of behaviours varied significantly among species (chi-squared with 20 df = 377.3,  $p < 0.0001$ ) (Figure 5a) and among size classes (chi-squared with 20 df = 62.8,  $p < 0.0001$ ) (Figure 5b). In general, the most active species, in terms of the total number of behavioural observations and the different types of behaviours were *C. albimarginatus* and *C. amblyrhynchos* whereas species such as *C. melanopterus* and *S. lewini* effectively exhibited no behaviours other than a limited number of entries and exits. *Carcharhinus amblyrhynchos* showed the highest total number of behaviours (3352) followed by *C. albimarginatus* (572) as well as the highest number of different behaviours (10) followed by *T. obesus* (8) (Table 3; Fig 5a). Smaller sharks also generally had higher numbers of observed behaviours and greater diversity of behaviours than medium and large individuals (Fig 5b).

The permutational ANOVA indicated highly significant effects of species on frequency of behaviours, with size as a covariate (Table 4) with no interaction ( $p=0.45$ ). Most of the variance in the frequency of behaviours was explained by species rather than size (Table 4). Frequencies of entries and exits were highest in *C. amblyrhynchos* and *C. albimarginatus*, these species also had relatively high frequencies of bites, nudges and approaches (Figure 5a). *Nebrius ferrugineus* displayed the highest frequency of bites and head shakes and the equal highest frequency of bait bag removals with *G. cuvier* (Figure

5a). *Galeocerdo cuvier* also had relatively high frequencies of entries, exits, bites and nudges while *T. obesus* had the highest frequency of other active behaviours, and *S. lewini* and *C. melanopterus* displayed a low number of entries and exits and no other behaviours (Figure 5a).

As size varied significantly by species, I considered the effect of size on behaviour within *C. amblyrhynchos*, as they were the species most frequently observed (44 individuals; Table 3). *Carcharhinus amblyrhynchos* individuals are known to reach 217.4cm in length and animals observed in this study ranged in size from 66.5-198.7cm. However most of these animals were juveniles with only seven animals larger than the size at maturity for this species (113.cm; Froese 2010). Distance based linear modelling of frequency of the suite of behaviours of each individual grey reef shark as a function of length was not significant ( $p=0.38$ ), suggesting that, within the size range observed within this species, size does not play a significant role in determining the suite of behaviours exhibited by an individual and their frequency.

### ***Timing and Duration***

Time of first arrival varied between 0 and 59.6 mins minutes with an average of 25.5 mins ( $\pm 0.21$ ) post deployment (Table 5). There was no effect of species or size on time of first arrival ( $p=0.128$ ). Total time in frame varied between 0.02 and 32.5 mins with an average presence of 3.0 mins ( $\pm 0.52$ ), and varied significantly among species when length was controlled for ( $p=0.002$ ) (Table 6). This pattern was largely driven by *N. ferrugineus* which exhibited the longest mean sum of time spent in frame per individual, followed by *C. amblyrhynchos* and *C. albimarginatus* (Table 5). Mean duration of pass varied between 0.01 and 1.35 mins with an average of 0.12 mins ( $\pm 0.02$ ) and also varied significantly among species ( $p= 8.46E-10$ ) (Table 6), with this result driven primarily by *N. ferrugineus*, which had a significantly higher mean time spent in frame than all other species (Table 6; Table 5). Time from first entry to last exit varied between 0.02 and 56.52 mins with an average of 16.39 mins ( $\pm 1.92$ ). Time from first entry to last exit also was significantly different among species, with *C. amblyrhynchos* and *C. albimarginatus* typically spending the longest period of time in the area of the stereo-BRUVS ( $p=0.008$ ) (Table 6; Table 5).

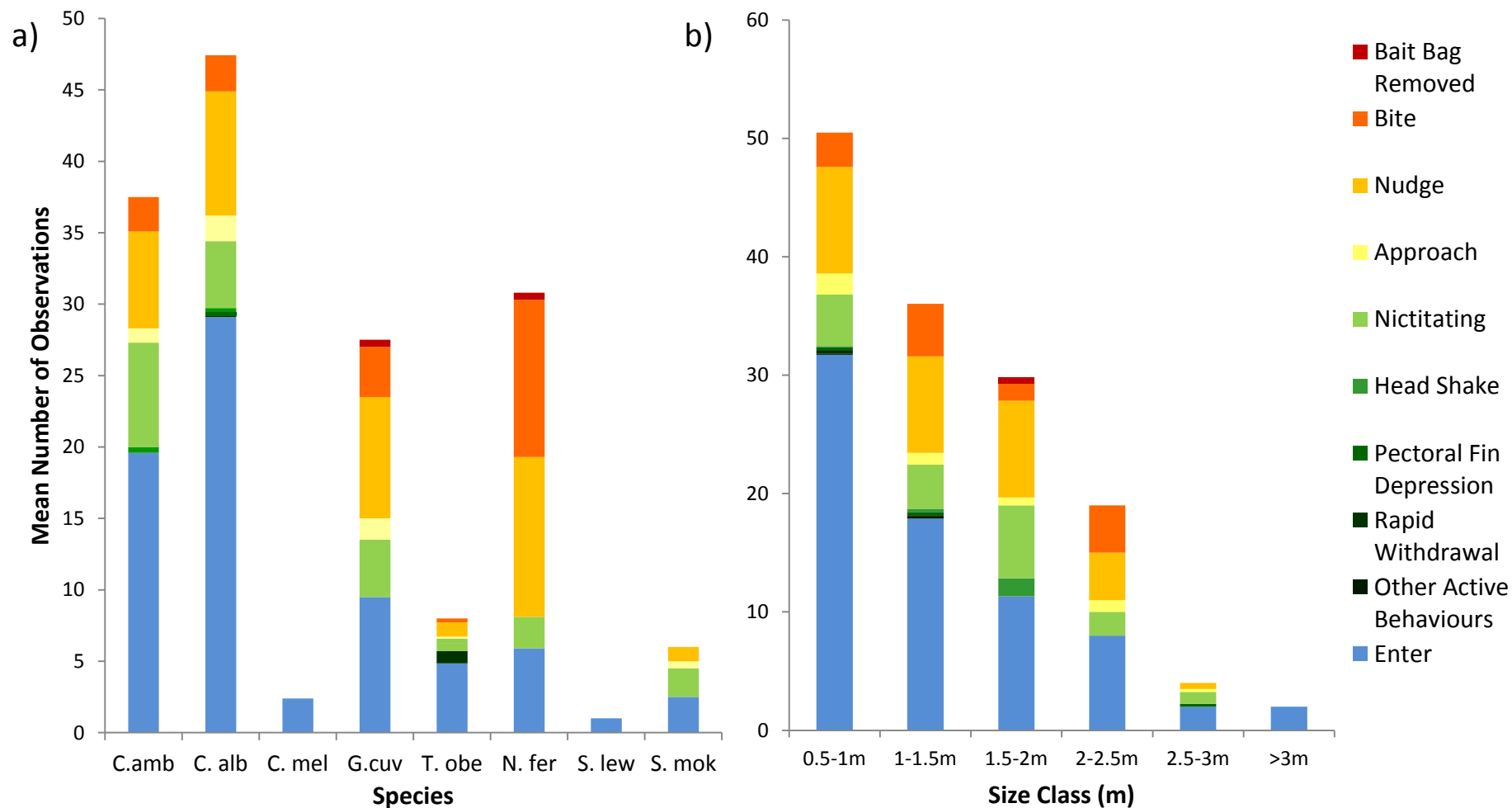


Figure 5: Mean number of observations per individual of each behaviour recorded, grouped by; a) Species (n = 86) C.alb (*Carcharhinus albimarginatus*), C.amb (*Carcharhinus amblyrhynchos*), C.mel (*Carcharhinus melanopterus*), G.cuv (*Galeocerdo cuvier*), T.obe (*Triaenodon obesus*), N.fer (*Nebrius ferrugineus*), S.lew (*Sphyrna lewini*) and S.mok (*Sphyrna mokarran*), and b) Size class (n = 73).

Table 4: Results of a PERMANOVA investigating the effect on behavioural frequency of species, with log transformed length included as a covariate

Source	df	SS	MS	Pseudo-F	p-value	Unique permutations
LogLength	1	9230.4	9230.4	6.8079	0.0017	9948
Species	7	36945	5277.9	3.8927	0.0001	9910
Residual	64	86773	1355.8			
Total	72	1.33E+05				

Timing of the first instance of behaviours did not generally vary ( $p=0.13$ , ANOVA), however, when viewed on a species basis some patterns became apparent in the timing of behaviours associated with the bait bag. Behaviours associated with the bait bag either showed no clear pattern (e.g. *C. albimarginatus*) or displayed a logical progression from investigatory behaviours of approaching and nudging to bites and finally removal of the bait bag in some species (Figure 6). However some patterns were noted when size of individuals was considered.

Table 5: Mean values with associated standard errors and ranges for derived timing metrics for all species included in the study, all values are presented in minutes

Species	Time of First Arrival		Sum of time spent in frame		Mean duration of pass		Time from first entry to last exit	
	Mean ( $\pm$ SE)	Range	Mean ( $\pm$ SE)	Range	Mean ( $\pm$ SE)	Range	Mean ( $\pm$ SE)	Range
<i>C.albimarginatus</i>	14.65 $\pm$	0 –	2.7 $\pm$	0.65 -	0.08 $\pm$	0.05 -	27.08 $\pm$	1.53 -
	5.45	47.5	0.53	6.04	0.01	0.16	6.43	56.52
<i>C.amblyrhynchos</i>	26.55 $\pm$	3.17 -	3.64 $\pm$	0.09 -	0.06 $\pm$	0.03 -	22.16 $\pm$	0.09 -
	2.67	59.6	0.61	15.33	0	0.11	2.72	55.46
<i>C.melanopterus</i>	30.41 $\pm$	0.885-	0.18 $\pm$	0.02 -	0.04 $\pm$	0.01 -	9.08 $\pm$	0.02 -
	8.34	52.3	0.08	0.48	0.01	0.08	5.92	29.4
<i>G.cuvier</i>	28.89 $\pm$	4.48-	1.49 $\pm$	0.06 -	0.05 $\pm$	0.03 -	5.02 $\pm$	0.06 -
	7.50	55.1	0.3	0.82	0.01	0.07	2.34	13.46
<i>T.obesus</i>	41.04 $\pm$	24.25 -	6.19 $\pm$	1.19 -	0.08 $\pm$	0.07 -	2.46 $\pm$	1.96 -
	16.79	57.8	3.3	1.79	0	0.08	0.5	2.97
<i>N.ferrugineus</i>	22.98 $\pm$	2.42 -	0.24 $\pm$	0.06 -	0.51 $\pm$	0.03 -	7.53 $\pm$	0.06 -
	6.49	57.3	0.04	32.54	0.14	1.35	3.82	37.82
<i>S.lewini</i>	21.71 $\pm$	21.6 -	0.24 $\pm$	0.05 -	0.12 $\pm$	0.03 -	0.24 $\pm$	0.05 -
	0.03	21.8	0.13	0.36	0.02	0.18	0.04	0.36
<i>S.mokarran</i>	40.79 $\pm$	30.3 -	0.47 $\pm$	0.11 -	0.05 $\pm$	0.05 -	0.88 $\pm$	0.11 -
	10.48	51.3	0.14	0.37	0.01	0.06	0.77	1.65
<b>Total</b>	25.5 $\pm$		3 $\pm$	0.02 -	0.12 $\pm$	0.01 -	16.39 $\pm$	0.02 -
	1.92	0-59.6	0.52	32.54	0.02	1.35	1.92	56.52

Table 6: Results of full factorial ANOVAs and ANCOVA investigating; the effect of species and length as a covariate on total time spent in frame by individual sharks, the effect of species on mean duration of pass and time from first entry to last exit

Source	Type III SS	df	MS	F	p-value
<b>Sum of time spent in frame</b>					
Model	589.69	8.00	73.71	3.46	0.002
LogLength	27.64	1.00	27.64	1.30	0.259
Species	562.74	7.00	80.39	3.78	0.002
Error	1277.72	60.00	21.30		
Total	1867.41	68.00			
<b>Mean duration of pass</b>					
Species	1.77	7.00	0.25	11.30	8.466E-10
Error	1.75	78.00	0.02		
Total	3.52	85.00			
<b>Time from first entry to last exit</b>					
Species	6363.13	7.00	909.02	2.98	0.008
Error	23791.09	78.00	305.01		
Total	30154.22	85.00			

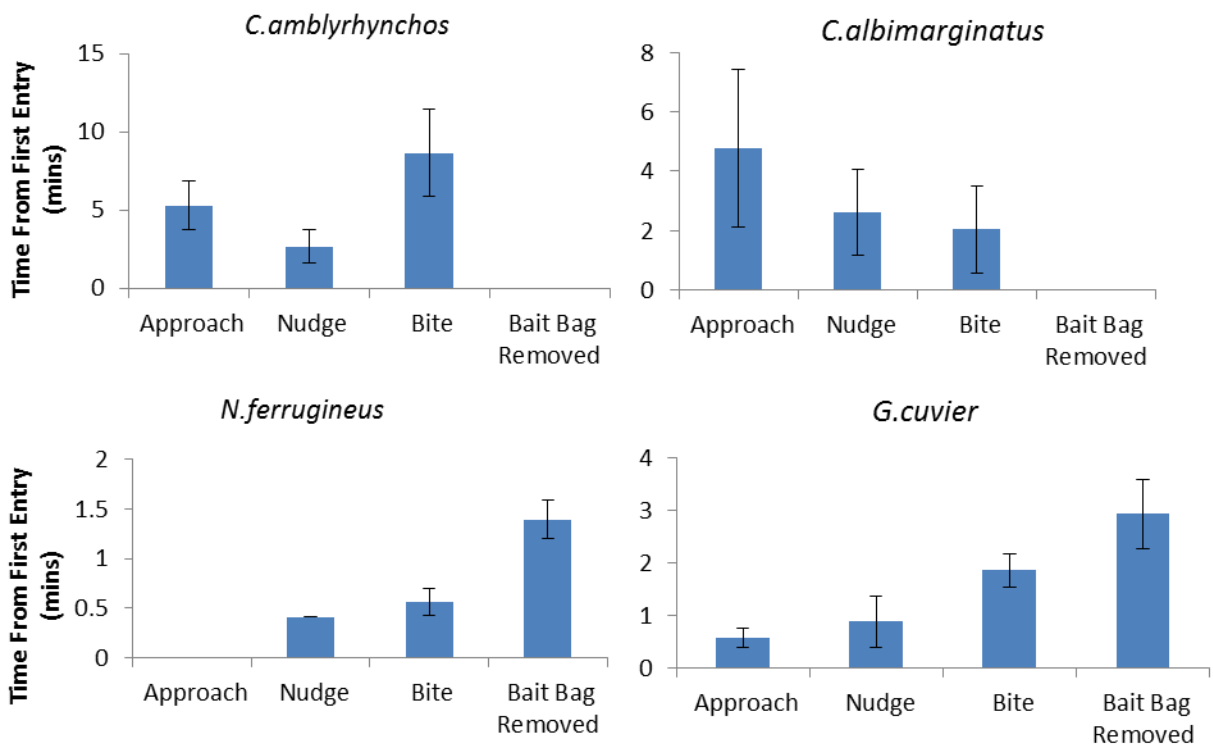


Figure 6: Time from first entry to first observation of behaviours associated with the bait bag, for the four species of shark which most commonly contacted the bait.



Although there was a high degree of variation between individual time from first entry to first observation of given behaviours, behaviours associated with feeding (nictitating, nudge and bite) took longer on average to be observed in small individuals than medium and large individuals.

Size did not consistently influence timing responses of *C. amblyrhynchos*. The sum of time spent in frame for grey reef sharks varied from 0.02 – 32.54 mins with a mean of 2.7 mins ( $\pm 0.53$ ) but did not vary with length. Mean duration of pass ranged from 0.01 - 1.35 mins in this species with a mean of 0.08 mins ( $\pm 0.01$ ) (Table 6) but also did not vary with length (Figure 9). Time of first arrival ranged from 0 – 47.49 mins with a mean of 14.65 ( $\pm 5.45$ ) and was positively correlated to size with larger individuals on average taking longer to enter the field of view (Figure 9). Total time spent in area varied from 1.53 – 56.52 mins with an average of 27.08 mins ( $\pm 6.43$ ) and decreased with increasing size, with total time spent in the area greatest for small animals (Figure 9).

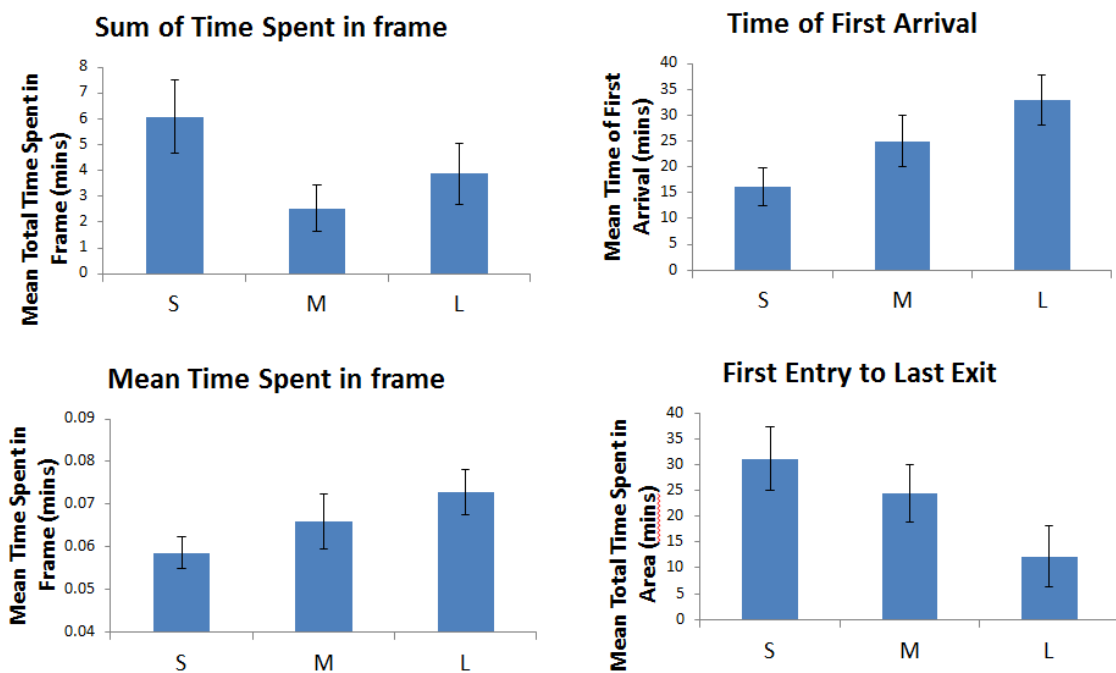
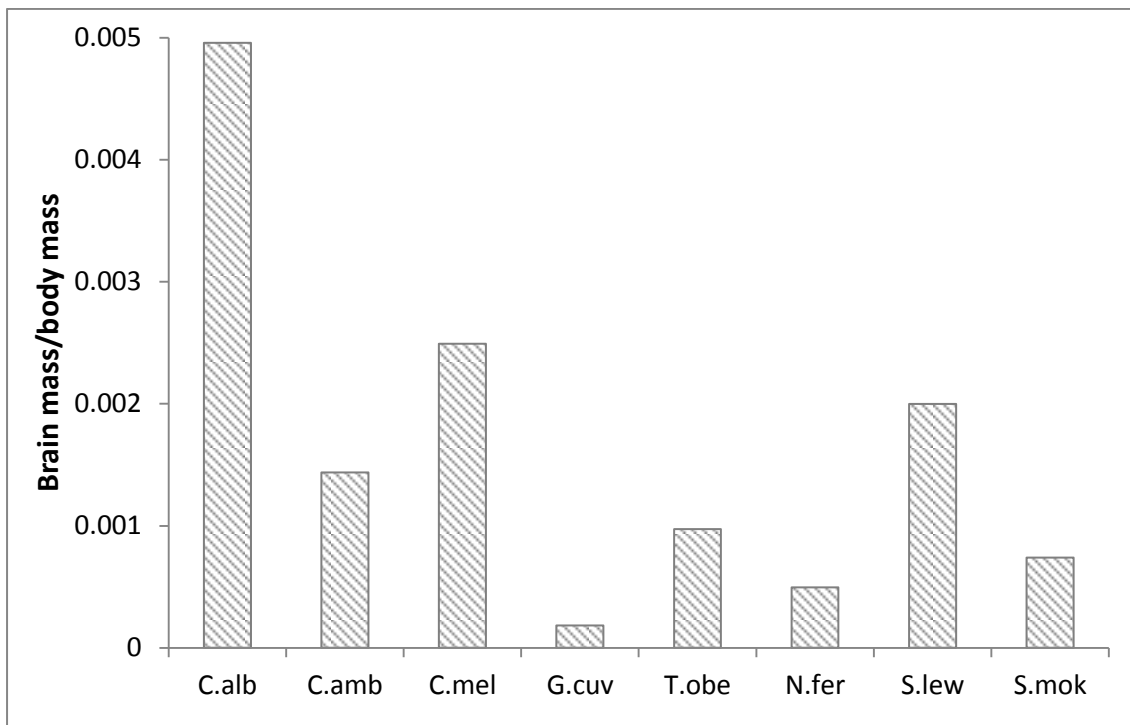


Figure 7: Duration metrics in relation to size classes for *C.amblyrhynchos* only (n=37). Size classes; S <89cm, M 89-105cm and L >105cm.

### ***Brain Organisation***

Brain to body ratio varied among species (Figure 8) (Appendix III). There were no data on the masses of the major brain regions for *C. albimarginatus* and therefore this species was excluded from comparisons of brain organisation and behaviours. Brain organisation among the remaining species was variable (Figure 9), the most noticeable differences being attributed to *G.cuvier* with enlarged olfactory bulbs and reduced telencephalon relative to other species. The 2<sup>nd</sup> stage MDS on behavioural frequency and brain organisation returned a correlation coefficient of 0.42.



*Figure 8:* Brain mass to Body mass ratio for each of the species included in the study. C.alb (*Carcharhinus albimarginatus*), C.amb (*Carcharhinus amblyrhynchos*), C.mel (*Carcharhinus melanopterus*), G.cuv (*Galeocerdo cuvier*), T.obe (*Triaenodon obesus*), N.fer (*Nebrius ferrugineus*), S.lew (*Sphyrna lewini*) and S.mok (*Sphyrna mokarran*) (Sources and raw values are included in Appendix III)

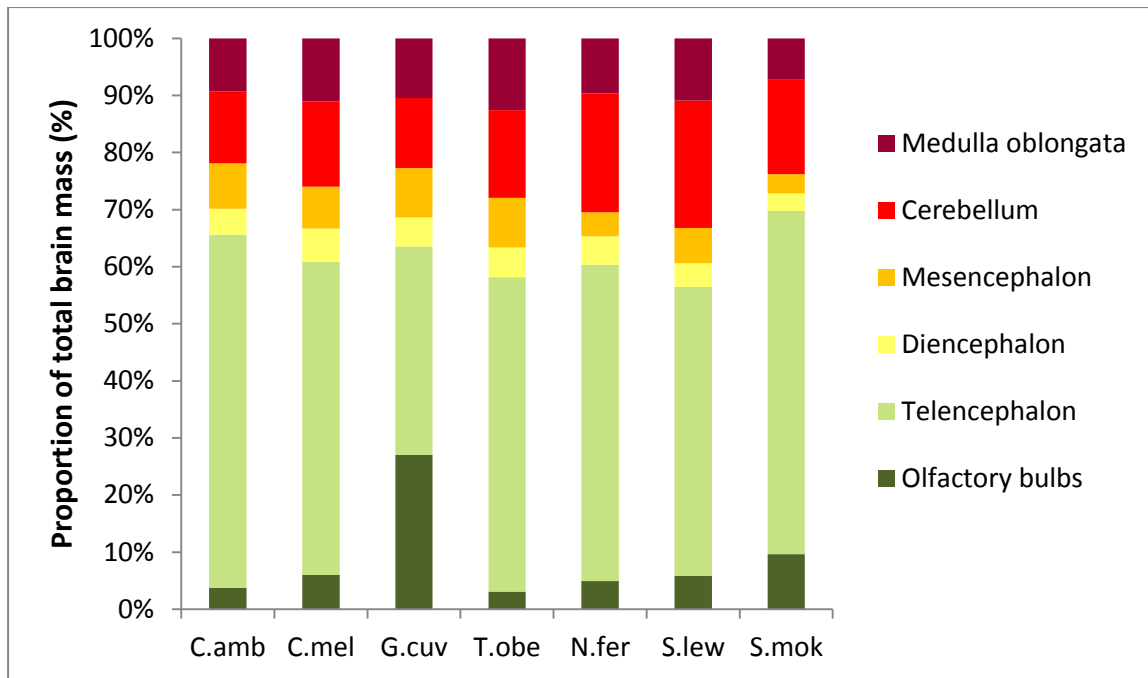


Figure 9: Proportional composition of the total brain of each species in terms relative mass of major brain regions. C.amb (*Carcharhinus amblyrhynchos*), C.mel (*Carcharhinus melanopterus*), G.cuv (*Galeocerdo cuvier*), T.obe (*Triaenodon obesus*), N.fer (*Nebrius ferrugineus*), S.lew (*Sphyrna lewini*) and S.mok (*Sphyrna mokarran*). No data was available for *C.albimarginatus* (Sources and raw data available in Appendix III).

## DISCUSSION

This study showed that large amounts of behavioural data can be derived from the novel analysis of footage collected by stereo-BRUVS. The results suggest that the primary determinant of behavioural variation with respect to its frequency and timing is phylogeny, with size having less influence on behaviour. This study also showed that brain organisation and behaviour are moderately correlated.

Overall behaviour consisted primarily of entries and exits, followed by behaviours associated with the bait bag and a lower number of displays and other behaviours. Frequency of nictitating was relatively high, and is often associated with feeding (Frazzetta & Prange 1987). Our results were consistent with this study as observations of nictitating primarily occurred in conjunction with other behaviours directly associated with the bait. Agonistic interactions and other displays such as pectoral fin depression and rapid withdrawals, although present, were relatively low in frequency compared to the other behaviours, suggesting that these animals for the most part coexist in the same environment with limited need for agonistic interaction even when a food resource is present. This coexistence with minimal aggression is observed in large aggregations of

conspecifics in various shark species (Guttridge et al. 2009, Mourier et al. 2012), and could be the result of size or sex based dominance hierarchies negating the need for aggressive interaction (Myrberg & Gruber 1974, Sperone et al. 2010). Although agonistic interactions among sharks are well documented (Johnson & Nelson 1973, Martin et al. 2007), aggressive interactions which actually result in fighting among individuals are not. As animals, especially large predators, often bear significant costs associated with fighting, displays and established dominance hierarchies are often evolutionarily favoured in order to settle disputes with relatively little energetic cost (Parker 1974, Neat et al. 1998, Lopez & Martin 2001). As sharks possess the ability to inflict serious damage in aggressive interactions, the formation of dominance hierarchies and agonistic displays should have a strong evolutionary pressure (Parker 1974). If dominance hierarchies were already established in the animals present in the footage, this may explain why agonistic displays were only occasionally required to avoid aggressive interaction, whether used in a defensive or offensive capacity.

Species was the primary driver in determining behavioural variation in the animals studied with the frequency and distribution of behaviours observed varying significantly among species. Although care must be taken in interpreting results, as some species have small sample sizes, many patterns of interest were evident. Requiem sharks (*Carcharinidae*) were generally very active around the stereo-BRUVS, observed making multiple entries and exits, and interacting with the bait on multiple occasions. Such observations support results of previous studies describing increased excitement in these animals during feeding, especially in groups (Hobson 1963, Motta 2001). However, this did not hold true for *C. melanopterus*, which had very low observed behavioural frequencies and showed no interactions with the bait, suggesting that perhaps this species is less inquisitive or more cautious of novel objects than its confamilials. In contrast to requiem sharks, both hammerhead (*Sphyrnidae*) species exhibited relatively low behavioural frequencies. It may be that the bait was of relatively low interest to hammerheads due to differences in sensory perception. Hammerhead sharks have a well-developed electroreceptive system used in prey location and capture (Karjiura & Holland 2002). As the bait was not alive and was therefore not emitting an electric field, hammerhead sharks may be less interested in the bait than other species which rely more on olfaction for example. Alternatively this difference could have a basis in optimal foraging decisions, as the hammerheads observed were large individuals, their cost of

movement is relatively smaller and the bait represents a relatively small reward, this could therefore lead to them moving on in search of other food sources therefore interacting with the bait and entering the field of view less (Pyke et al. 1977).

Behavioural repertoire also varied among species with *C. amblyrhynchos* exhibiting the highest number of different behaviours. This is consistent with previous studies showing a wide behavioural repertoire for this species with agonistic displays composed of multiple components described (Johnson & Nelson 1973, Martin 2007). Despite having a relatively low overall frequency of behaviours, *T. obesus* displayed the next most varied range of behaviours. This high behavioural variation relative to frequency in *T. obesus* can be attributed, in addition to interactions with the bait bag, to the exhibition of a number of display behaviours. This included a multi-faceted display observed in multiple individuals on multiple occasions, which involved a noticeable slowing in swimming speed to an almost stationary position, rolling of the body, head shaking and jaw snapping. This display has not been previously described in the literature for *T. obesus* and so its function is unknown, however, it bears similarities to agonistic displays observed in grey reef sharks (Johnson & Nelson 1973) and its components bear considerable comparison to the feeding mechanism (Motta & Wilga 2001). This mirroring of feeding behaviour in agonistic behaviour has been postulated in *C. amblyrhynchos* (Barlow 1974). Other species with less varied behaviour tended to exhibit fewer display behaviours and fewer behaviours associated with the bait bag and nictitating, the latter of which also often occurs during feeding in order to protect the eye (Frazzetta & Prange 1987). These comparatively low agonistic behavioural repertoires in the remaining species are consistent with those reported by Martin (2007) in a review of agonistic behaviour in sharks, who found grey reef sharks to have the highest number of agonistic display elements of the species in this study reviewed, although *N. ferrugineus* and *T. obesus* were not included.

Behaviours differed not only in their frequency and variety but also in their proportions. For instance, *C. amblyrhynchos* exhibited a higher proportion of entries and a lower proportion of interactions with the bait bag, whereas *N. ferrugineus* exhibited a much higher proportion of nudges and bites. This could be explained by a fundamental difference in their physiology as *C. amblyrhynchos* are obligate ram ventilators meaning that they must maintain forward motion through the water column in order to maintain water flow over their gills (Hughes 1963, Morgan & Burges 2006). In contrast, *N.*

*ferrugineus* is able to use buccal pumping to ventilate its gills when not swimming (Hughes 1963, Motta et al. 2002). Therefore, if *C. amblyrhynchos* are to interact with the bait for an extended period of time, they must continue to make multiple passes taking them into and out of the camera field of view, whereas if *N. ferrugineus* individuals are to interact with the bait for extended periods, they can approach once and nudge and bite multiple times with little adjustment of position relative to the camera field of view. This suggests that fundamental differences in the physiology of animals among species may be a driving force in moderating their behaviour.

A significant difference in behavioural frequency among size classes was also detected. However, as mean size of animals varied among species, it was unclear whether this difference was driven by differences in size or whether it was simply an artefact of the collinearity present in the data between species and size. The analysis of the effect of size on *C. amblyrhynchos* behavioural frequency suggested that size was not a significant factor in determining behavioural frequency and that this pattern most probably was due to an overriding effect of species. This is in contrast to previous studies which have found size to be a primary determinant in intraspecific behavioural changes (Springer 1967, Myrberg & Gruber 1974, Lowe et al. 1996, Guttridge et al. 2009, Lucifora et al. 2009, Sperone et al. 2010, Mourier et al. 2012). It may be that, although a representative size range was present in this species (66.5-199cm FL; max reported observed fork length is approximately 217 cm FL), there were too few animals in the larger size classes to confidently disentangle the effect of size on behaviour. Indeed, only seven individuals were larger than the stated age of maturity of the species (113cm FL) (Table 1). Thus the lack of a significant effect of size may be due to low representation of larger animals and the overriding effect of juvenile and sub-adult individuals acting in a similar way. Given this, care must be taken in making generalisations about the effect of size on behaviour and more robust data is required to confirm this finding.

Timing of animal behaviour varied with respect to the metrics considered. There was no significant effect of species on the time of first arrival of animals, suggesting that perhaps timing of first arrival is simply a function of where the stereo-BRUVS are deployed and which animals happen to be in the area at that time, rather than being representative of an active, directional response from the animals. The lack of variation in time of first arrival also suggests that the spread of bait plume, relative olfactory sensitivity and swimming speed of the species in question, highlighted as important in other studies (e.g. Ellis &

DeMartini 1995, Cappo et al. 2006) are relatively unimportant to time of first arrival. Total time in frame and mean duration in frame were highest in *N. ferrugineus*, which are likely attributable to its physiology and its method of ventilation that allows this species to maintain a relatively stationary body position for extended periods in comparison to other species (Hughes 1963, Motta et al. 2002). Time from first entry to last exit (a proxy for total time spent in the area) was highest in *C. amblyrhynchos* and *C. albimarginatus* ( $p = 0.008$ ; Table 14), this may be attributed to more inquisitive behaviour towards the stereo-BRUVS or stronger site fidelity (McKibben & Nelson 1986). Time from first arrival to the exhibition of certain behaviours did not vary significantly among behaviours or among species, however, when investigated on an individual species basis showed some interesting patterns. For example, interactions with the bait bag seemed to follow a logical temporal progression in *C. amblyrhynchos*, *N. ferrugineus* and *G. cuvier*, with approaches without contact and nudges tending to be performed earlier, indicating investigation of the bait, followed by bites and then possibly removal of the bait bag. The timing of these behaviours among species, although non-significant (most probably due to small sample sizes in this study), suggested patterns of variation among species, indicating that some species may be less cautious and feed more readily than others. Finally, the timing of behaviours within *C. amblyrhynchos* displayed a decreasing trend with length, indicating that larger individuals take less time exhibit behavioural responses to a baited scenario. This could be due simply to the greater abundance of small individuals, therefore being more likely to be present for a larger portion of video. Alternatively it could reflect optimal foraging decisions, with larger individuals having a lower cost of movement and a lower relative value of the bait, leading to different decisions about energetic investment (Werner & Hall 1974, Pyke et al. 1977).

Brain organisation was correlated with behavioural composition. However, this relationship was not as strong as was expected given the arguments supporting the neural basis of behaviour, such that the size of major brain regions would mirror variations in behaviour (Delcomyn 1980, Iwaniuk & Hurd 2005). Indeed, ecology, habitat usage, and rhythmic behaviours have been linked to variations in neural organisation and specific behaviours have been linked to particular brain regions (Brown 1973, Notebohm et al. 1976, Delcomyn 1980, Iwaniuk & Hurd 2005, Yopak et al. 2012). In this study, the strength of the correlation may have been reduced by an ontogenetic mismatch between the size of animals displaying behaviours and the size of animals from which the brain

data were derived. The relative size of brain regions changes with ontogeny (for example sizes of the optic tecta and olfactory lobes in some shark species) (Lisney et al. 2007) and this study used averaged brain composition data for each species (Appendix III). Moreover, the size of brain regions may not be wholly representative of their relative importance or functional power (Herculano-Houzel et al. 2007). For example, there could be other quantitative or qualitative differences in brain regions among species or size classes such as differences in neuron density, glial cell density or foliation as seen in the cerebellum of many chondrichthyans, which may have associated effects on cognition, information processing, perception or other functions and therefore have effects on behaviour not taken into account simply by considering the mass of a region (Herculano-Houzel et al. 2007, Lisney et al. 2007, Bandiera et al. 2009). More detailed data on brain organisation, for example on the relative sizes of sensory areas of the brain, may further elucidate the relationships between brain function and behaviour. Furthermore, consideration of other factors, for example peripheral sense organs, ventilation method and other physiological differences may also be taken to determine whether other neurological, physiological or anatomical differences have an effect on behavioural composition. Finally, the relatively sparse data across narrow size ranges in some species may have also influenced the strength of the relationship between behaviour and brain structure.

This study demonstrates that stereo-BRUVS represent an effective way of sampling behaviour of animals in the marine environment, and are a viable alternative to lab-and diver-based studies. High densities of semi-quantitative data can be extracted from footage allowing comparisons to be made across species and size classes. The large libraries of stereo-BRUVS imagery that already exist globally (>15,000 hours; Meeuwig pers. comm.), represent a valuable and untapped resource for behavioural studies. Imagery collected by stereo-BRUVS allow studies well below depths accessible to SCUBA divers (Zintzen et al. 2012) and studies could be conducted at night under blue light to investigate diurnal behavioural variations believed to be present in many shark species (Bres 1993, Sundstrom et al. 2001). Additional metrics such as swimming speed can also be derived, allowing studies of animal energetics to be conducted (Ryan et al. *subm.*). Stereo-BRUVS also allow behaviour to be observed that may be involved in foraging and aggression in a safe manner.



Future research on shark behaviour based on stereo-BRUVS imagery should focus on expanding the size ranges of the species included in this study to allow intraspecific investigations of behaviour, therefore reducing the influence of the existing collinearity between species and size. Additionally, including a broader range of species with variable ecologies and brain structures as well as adding any other behaviours of particular interest to the ethogram may allow broader trends in behavioural variation to be uncovered. The addition of more species would also allow the use of phylogenetically independent contrasts, thereby controlling for the effect of phylogeny when making comparisons (Garland et al. 1992, Abouheif 1999). This would allow deviations from the expected allometric relationships between total brain mass and brain region mass to be determined such that adaptations of particular species could be identified, possibly providing a clearer picture of the effects of changes in brain composition on behaviour (Yopak et al. 2012). There is also a need to characterise the effect of ontogenetic stage on major brain regions in the species studied, as well as making qualitative as well as quantitative considerations of brain morphology. This may allow more tangible conclusions to be made about the effect of brain organisation on behaviour and provide support for the neurological basis of behaviour. A possible solution to size mismatches between animals observed for behaviour and those from which brain data are collected would be to use brain organisation data from a range of different sized individuals within a species to derive curves predicting how brain structure varies with size, then fit the estimated sizes of regions to the respective individuals of given size in the data set.

Overall, this study found that phylogeny at the species level is most likely the major determinant of behavioural variation, although size may have some effect. Brain composition has some effect on behavioural composition; however, the effect detected was not as strong as expected. This may be the result of a number of factors which should be addressed in future research. Stereo-BRUVS provide a novel means of submarine behavioural research, representing a viable alternative to diver based observational studies with increased safety, greater depth ranges, a reduction of observer effect and the ability to derive additional observations such as length and swimming speed estimates (Watters et al. 2009). Studies of this nature can provide information on variation in the diversity, frequency and timing of behaviours, which is of significant value in multiple fields. For instance, investigations of foraging behaviour may inform on the relative impact of fishing techniques on particular species or size classes, which could inform not

only fisheries management but also conservation and bycatch mitigation efforts (Hutchinson et al. 2012). Investigations of agonistic and feeding behaviours may also expand knowledge on the basis of negative interactions between sharks on humans informing mitigation strategies (Nelson 1986). To these ends, there remains a significant need to improve our understanding of shark behaviour and its drivers.

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## **Effects of Taxonomy, Ontogeny and Sensory Ability on Shark Behaviour**

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### **Research Proposal**

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**ABSTRACT:** Many studies have investigated aspects of shark behaviour; however, the factors driving behavioural variation both within and between species remain largely unstudied. Variations in behaviour have been noted between species and within species between individuals of different sizes. Studies of brain morphology in sharks have suggested that brain morphology and sensory ability may also play a role in determining behaviour in sharks. An understanding of behavioural variations in sharks and the factors driving them may prove useful in informing conservation efforts and management decisions. Behavioural observations may also have implications for mitigating negative interactions of sharks with humans and informing the shark ecotourism industry. This study will investigate the behaviour of eight shark species at the Chagos Archipelago (British Indian Ocean Territory), focussing on interactions with the bait, interactions between individuals and other displays. Video footage collected with stereo-BRUVS (baited remote underwater video systems) will be analysed using the computer program Event Measure. This will allow size, distance from the bait and behaviours of individual sharks to be recorded at intervals throughout the footage. The aim of this project is to determine the primary drivers of behaviour in sharks and as such it will investigate the effects of taxonomy, ontogeny (based on body size) and sensory ability (based on total relative brain size, relative size of major brain regions and relative size of sensory brain regions) on behaviour.

## **INTRODUCTORY STATEMENT**

Sharks play an important role in controlling ecosystem structure through top down effects and as a result their removal can have significant implications for ecosystem functioning (Madin et al., 2010a; Myers et al., 2007; Worm and Myers, 2003). Behaviour forms a key part of the ecology of an organism and affects the way that individuals interact with their environment and other individuals (Bres, 1993). As a result, gaining a better understanding of the behaviour of sharks and how this behaviour varies among individuals and between species will provide information relevant to informing conservation efforts and management decisions and mitigating negative shark-human interactions.

Investigations in this study will be focussed on variation in a set of specific behaviours among and within eight species of reef and coastal sharks. These behaviours will include interactions between sharks, interactions with the bait and other behavioural displays. A number of possible factors affecting behaviours will be investigated including, but not limited to, taxonomy, ontogeny and neurobiology including the development of peripheral sense organs, brain size and morphology. These investigations will be based on behavioural data derived from existing videos collected with stereo baited remote underwater video systems (BRUVS) deployed in the Chagos Archipelago during 2012. As such, I am trialling a novel method of submarine behavioural observation that removes the influence of divers and allows

the logging of more detailed behaviours than can be derived from tagging studies. This study is also unique in that it will investigate the effects of taxonomic, ontogenetic and neurological factors on the behaviour of multiple species in a relatively natural setting.

The proposed study aims to determine the factors affecting the behaviour of sharks and how these behaviours vary, as well as providing evidence for theories regarding the neuroecological bases of behaviour. Based on the literature, it is hypothesised that shark behaviour around BRUVS in the Chagos Archipelago will vary according to taxonomy, ontogeny, relative brain size and sensory modality.

## **BACKGROUND**

### *Population Declines*

Sharks are particularly vulnerable to fishing pressure as they have a late age at maturity, large body size and low fecundity (Graham et al., 2010; Schindler et al., 2002). There is concern about the state of shark populations worldwide, including in the Indian Ocean, with reported declines in abundances and overfishing commonplace (Clarke et al., 2012; Graham et al., 2010). Shark populations even in remote, otherwise relatively natural marine areas are still vulnerable to distance fishing fleets (Clarke et al., 2012). For instance, in the Chagos Archipelago, numbers of sharks observed per scientific dive declined by 90% from 1975 to 2006, with poaching in the Archipelago highlighted as the most likely cause given the number of illegal vessels containing large numbers of sharks arrested since 1996 (Graham et al., 2010). Fishing also typically targets larger individuals, which often leads to a rapid drop in high order predators which has implications for ecosystem structure (Friedlander and DeMartini, 2002; Sibert et al., 2006; Worm and Myers, 2003). The global decline of sharks in recent history has been attributed primarily to an increase in the shark fin trade with increased consumer spending power in China resulting in fins selling for as much as US\$700 per kilogram (Graham et al., 2010; Verlecar et al., 2007). Bycatch of sharks by fishermen targeting other species is another factor contributing to their decline. This has led to studies attempting to find methods to mitigate the incidental capture of sharks in these fisheries (Hutchinson et al., 2012). However, differences in the feeding ecology between species introduce complications in applying these techniques. Observations of feeding behaviour such as those proposed in this study may aid in developing solutions.

### ***Ecological importance***

Many species of sharks occupy the role of apex predators in marine food webs (Myers et al., 2007; Schindler et al., 2002). Such apex predators are known in many cases to control ecosystem structure through top down control (Baum and Worm, 2009; Friedlander and DeMartini, 2002; Myers et al., 2007; Worm and Myers, 2003). The direct effect of predation on organisms of lower trophic levels has implications for the overall functioning and stability of ecosystems and thus the removal of apex predators can lead to trophic cascades and mesopredator release (Myers et al., 2007; Worm and Myers, 2003). In addition to these direct effects, sharks have been shown to have significant effects on observed fish assemblages (Klages et al., 2013), suggesting that their presence alters the behaviour and distribution of other species thereby indirectly modulating ecosystem dynamics through processes such as behaviourally mediated trophic cascades (Madin et al., 2010a; Madin et al., 2010b). The presence of predators has even been shown to alter the timing of life history events of prey such as time of sex change in reef fishes (DeMartini et al., 2005). The removal of apex predators therefore has been shown to have significant ramifications for ecosystem structure.

Such observed changes in ecosystem structure are important to consider not just in terms of their ecological implications, but also in their possible effects on commercially important fisheries. The classic example of impacts on commercial fisheries as a result of declines in apex predators is the decimation of the North Carolina bay scallop fishery by cownose rays, where the ray population exploded following widespread decline of sharks, their primary predators (Myers et al., 2007). Due to their ecological roles and the economic implications, the decline of large-bodied predatory species in the oceans is of concern from a sustainability and management perspective (Graham et al., 2010). A better understanding of the natural behaviour of these top order predators, including their feeding behaviour and interactions with one another, will provide insight into their ecological roles in altering ecosystem structure.

### ***Shark Behaviour***

Sharks display a wide array of behaviours, from specialised feeding behaviours and agonistic displays to social aggregations and interactions indicative of dominance hierarchies and social preferences (Guttridge et al., 2009; Martin, 2007; Mourier et al., 2012; Oliver et al., 2013). While sharks are often considered to be solitary hunters, grouping is relatively common across a range of species with varying ecologies (Springer, 1967), indicating that

these animals are more social than initially thought. Previous studies predominantly investigated agonistic behaviours of sharks both in relation to one another and to divers (Johnson and Nelson, 1973; Martin, 2007; Sperone et al., 2010). Other studies note observations of social preferences (Guttridge et al., 2009), dominance hierarchies (Myrberg and Gruber, 1974), behaviours believed to be involved in courtship (Sims et al., 2000) and foraging behaviours (Oliver et al., 2013; Wueringer et al., 2012). However, studies of natural feeding behaviour remain few, with many observations based on anecdotal reports (Motta and Wilga, 2001) and previously undescribed behaviours are still being observed (Oliver et al., 2013), indicating that there is still much to be gained from quantitative and controlled observations of shark behaviour, especially in a natural setting. Many previous studies used observations from divers or boats or looked at broader scale behavioural patterns through tagging studies (Nelson, 1976; Vianna et al., 2013).

The behaviour of animals varies at many different scales including, between species, between individuals and temporally within single individuals (Arnold and Bennett, 1984; Lowe et al., 1996; Lucifora et al., 2009; Sperone et al., 2010). The factors affecting these variations and at what scale they operate can provide useful insights into shark ecology and may be applicable to other taxa. Previous studies show evidence for, or hypothesise that; taxonomy, ontogeny, and the relative importance of both peripheral and central sensory input play a role in determining the suite of behaviours exhibited by individuals and their relative frequency. This section will outline the basis of these claims, the links between them and the supporting studies.

### ***What are the primary drivers of behavioural variation?***

#### ***- Taxonomy***

As would be intuitively expected, behaviour of sharks varies widely with taxonomy (Oliver et al., 2013; Wueringer et al., 2012). There are many differences between species and broader taxonomic classes such as differences in physiology, size, dominant prey species, dominant habitat, sensory ecology, brain morphology and other anatomical adaptations. It follows then that species would interact differentially with their environment and therefore display different suites of behaviours. For example, the foraging ecology of thresher sharks and hammerhead sharks would be expected to differ simply based on their anatomical adaptations, with thresher sharks having long tails which can be used to stun fish and hammerhead sharks having an increased cephalofoil that is related to an increased use of

electroreception and therefore an increased ability to detect prey that are cryptic to other senses (Kajiura and Holland, 2002; Oliver et al., 2013). Dominant period of activity, i.e. day or night, has also been found to vary between species (Klimley and Nelson, 1984) and may have an impact on the suite of exhibited behaviours. As inherent differences may be linked on evolutionary scales, phylogenetic closeness must be taken into account when considering behaviour (Abouheif, 1999).

#### - *Ontogeny*

Ontogenetic changes in behaviour have been observed in a many shark species using body size as an indicator of life stage. I suggest that shark size may be a useful indicator of behavioural variation, both in terms of social interactions and preferences and in feeding ecology. Strong correlations have been observed between behavioural patterns and size in great white sharks (Sperone et al., 2010). Ontogenetic diet shifts have been observed in tiger sharks (Lowe et al., 1996) and copper sharks (Lucifora et al., 2009) leading to changes in the size and type of prey taken, indicative of variation in foraging behaviour and optimal foraging decisions. This has implications for management as it indicates that predation by larger sharks provides a different ecological role to that by smaller sharks, suggesting that to maintain their ecological role, larger individuals must be present in the population. Lucifora et al. (2009) suggest introducing maximum size limits in addition to minimum size limits for effective ecosystem management. Sharks have also been shown to display social preferences based on size, sex and species, which influence assortment and aggregations at the individual, population and community levels (Guttridge et al., 2009; Mourier et al., 2012; Springer, 1967). Myrberg and Gruber (1974) reported size dependent dominance hierarchies and sexual divergence in the performance of certain behavioural patterns in captive bonnethead sharks. Gaining a clearer understanding of the role of ontogeny in behavioural changes may allow more targeted conservation efforts.

#### - *Brain Morphology and Sensory Perception*

Cerebrotypes are similar patterns of brain organisation found in groups of often phylogenetically distant species which share ecological similarities such as feeding strategies, cognitive ability, or habitat occupation (Iwaniuk and Hurd, 2005). It follows then that brain organisation should play a role in determining the ecology and therefore the behaviour of a species. Both brain organisation and relative brain size show strong correlations to ecology in all vertebrate groups (Yopak and Montgomery, 2008).

The sensory ability of animals affects the way they perceive the world around them and the way they interact with it. In general, the sensory perception of sharks is recognised as acute with chemical sensitivities recorded as low as one part per million, visual sensitivity exceeding that of man by a factor of 10, a hearing range extending below human sensitivity thresholds and an electroreceptive system in the nanovolt range (Gruber and Myrberg, 1977). However, the sensory abilities of sharks have been shown to vary substantially both with ontogeny and between species (Litherland et al., 2009). A number of recent morphometric studies have examined the relative size of sensory brain areas (olfactory bulbs, optic tecta, anterior and posterior lateral line lobes) in sharks and rays and suggested that the relative size of these areas could infer sensory dominance (Lisney et al., 2007; Lisney and Collin, 2006). This has been supported by studies comparing the relative sizes of these areas with the size of peripheral sense organs (Schluessel et al., 2008). This neurological mirroring of sensory dominance indicates that variation in brain organisation should affect the way an animal perceives and interacts with its environment. It follows then that neurological differences could form the basis of behavioural variation although empirical evidence is lacking.

The theory of the neurological basis of behaviour states that the behaviour of animals can in part be predicted based on a comparison of peripheral sense organs and the relative size of sensory brain areas (Delcomyn, 1980). Differences in the sizes of regions of the brain associated with different senses allow inferences to be drawn on the relative sensory dominance of animals (Lisney and Collin, 2006; Wagner, 2002). Information on how these differences translate into observed behaviours, for example in terms of foraging and interactions with other animals, could have broad implications and lead to further research in other species. As this is a multispecies study and sharks have a wide range of senses (including electroreception), this group of animals is ideal for investigating these relationships.

In recent years, morphological brain studies have linked the relative size of brain regions to the ecology of the species studied. The widespread variation in both brain size and complexity displayed in sharks has both a phylogenetic and an ecological basis, suggesting that brain development reflects the habitat and foraging ecology of animals in addition to phylogeny (Northcutt, 1977; Yopak et al., 2007). Variation in brain morphology between several species of sharks shows evidence of adaptive patterns which cannot be explained purely by phylogenetic closeness (Yopak and Montgomery, 2008). For example, a comparison of the brain size and organisation of the whale shark (*Rhincodon typus*) and the



basking shark (*Cetorhinus maximus*) shows evidence of convergent evolution suggesting that organisation of the brain is more similar in species with similar, but independently evolved lifestyles than those that simply share phylogenetic closeness (Yopak and Frank, 2009). These species are both large bodied and exhibit filter feeding behaviour indicating that brain organisation may be a good indicator of behavioural similarities and that brain structure in this group may have developed in concert with specific behaviours or enhanced cognitive abilities (Yopak and Lisney, 2012). The proposed study will use behavioural data to provide evidence for the theory that brain organisation and size play a significant role in determining behaviour.

### Vision

The visual abilities of sharks show substantial interspecific variability as well as varying ontogenetically (Litherland et al., 2009). Relative and absolute eye size varies considerably in elasmobranchs and these differences have been linked to differences in the ecology (prey type and habitat) of species (Lisney and Collin, 2007). Species-specific retinal specializations highlight differences in visually mediated behaviours and foraging strategies between the sandbar shark (*Carcharhinus plumbeus*) and the shortspine spurdog (*Squalus mitsukurii*) (Litherland et al., 2009). The dominant axis of high resolution vision differs between the two species.

Litherland et al. (2009) found that spatial resolving power (based on ganglion cell density) is higher in adults of some shark species than in juveniles. They suggested that this increase in spatial resolving power could confer a predatory advantage to adults allowing them to prey on more agile and reef associated species. This ontogenetic change in sensory perception could be a factor contributing to the ontogenetic dietary shifts reported in some shark species (Lowe et al., 1996; Lucifora et al., 2009). The relative size of the optic tectum has been proposed as a proxy for reliance on vision and has been shown to vary with species and ecological niche (Yopak and Lisney, 2012).

### Olfaction

The olfactory system of sharks appears to be fundamental for prey location and may aid in social interactions between conspecifics (Theiss et al., 2009). The ecological variation of elasmobranchs indicates that there may be significant variation in the olfactory sense between species. Theiss et al. (2009) found significant differences in the number of olfactory lamellae

in several species of wobbegong shark and a positive relationship between total body length and olfactory surface area. If the assumption is made that these morphological differences are translatable to true olfactory capability then this may indicate ecological differences.

However, other research on morphological indicators of sensory ability have been shown not to be translatable to true sensory capabilities (Higgs et al., 2002). A correlation between the size of the olfactory bulbs and the sensory (olfactory) epithelial surface area has been revealed in some species (Schluessel et al., 2008). However, the question remains whether these morphological differences translate into enhanced sensory sensitivity and whether this has any effect on how these animals interact with the environment. Behavioural studies taking into account these indicators of sensory power may provide support for these theories.

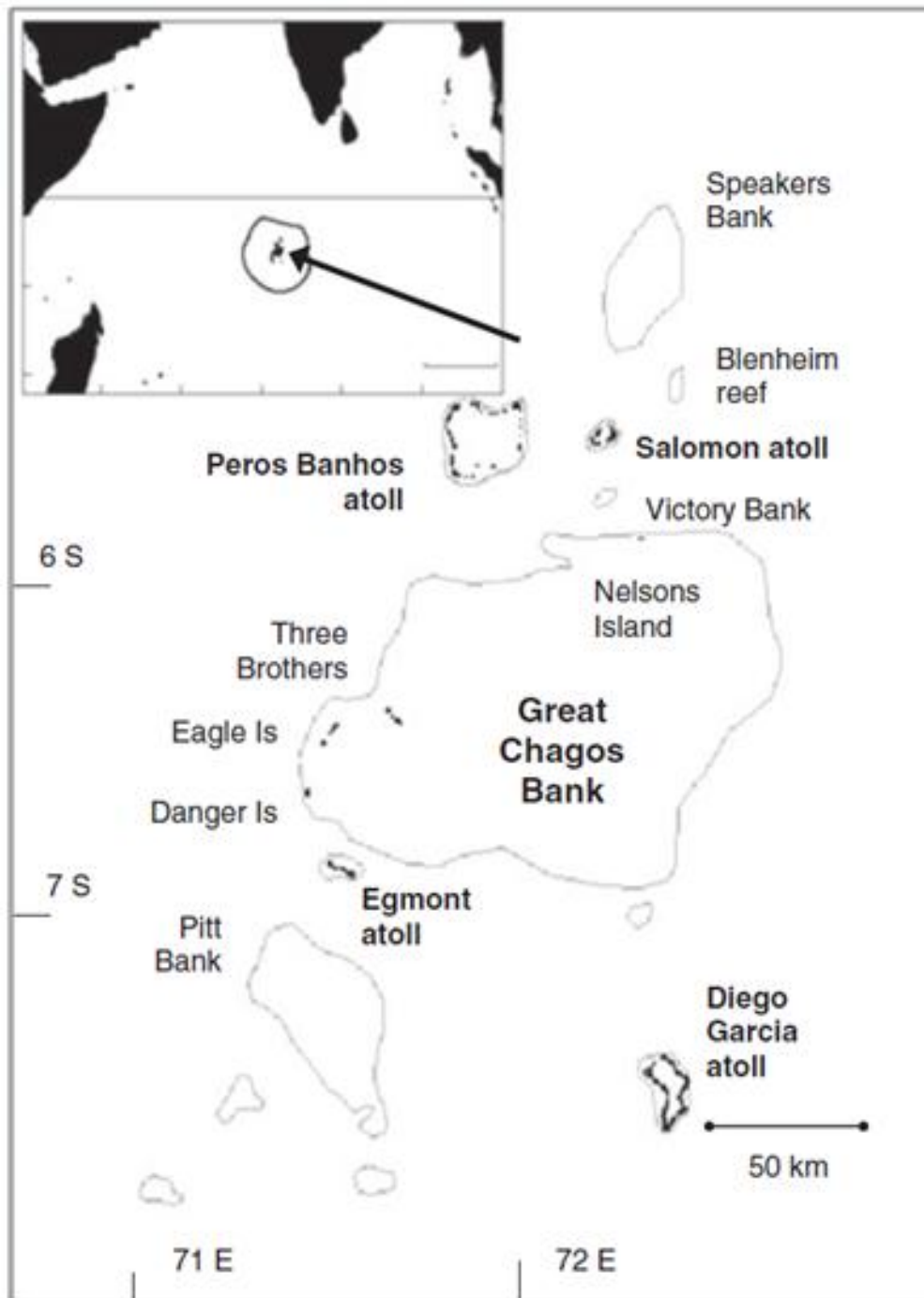
### *Relative importance of senses*

A comparison of the sizes of sensory brain regions may be useful as a proxy for the relative reliance on the different senses. By using this information to infer the relative importance of different sensory modalities, it may be possible to investigate the effect of sensory dominance on the behaviour of sharks in a natural setting in the proposed study. Lisney et al. (2007) investigated the changes in the relative size of the optic tecta, the olfactory bulbs and the anterior and posterior lateral line lobes (that receive input from the olfactory epithelium, eyes, ampullae of Lorenzini, and lateral line, respectively). They found that the relative size of the optic tecta and olfactory bulbs differed between juvenile and adult elasmobranchs. The patterns they observed suggest that the relative importance of these senses changes over the course of an animal's life, with olfactory ability possibly becoming more important than vision in adults.

### ***Study Site***

The footage to be used in this study was collected in April 2012 from several locations around the Chagos Archipelago, British Indian Ocean Territory. Located in the central Indian Ocean, approximately 500 km south of the Maldives and 1700 km south-west of Sri Lanka, the Chagos Archipelago is comprised of seven major atoll structures, 55 islands and 86 seamounts (Graham et al., 2010). Diego Garcia, the largest of the islands in the Archipelago, hosts a US Navy base and British administration for the territory, while the remaining islands are uninhabited and have been since the early 1970s (Graham et al., 2010).

In 2010, the Chagos Archipelago was established as the site of the world's largest no-take marine protected area (no-take MPA) representing 16% of the world's fully protected coral reef and 60% of the world's no-take MPA estate (Koldewey et al., 2010). The Archipelago encompasses not only the atolls but also 86 unfished seamounts, 243 deep knolls and important pelagic areas, providing an ideal site for ecological studies, and providing a reference site for comparisons between fished and unfished locations (Koldewey et al., 2010; Sheppard et al., 2012). The relatively natural state of this ecosystem has also been proposed as an ideal location for studies of animal behaviour in the natural environment free from human disturbance (Sheppard et al., 2012). Despite formal protection, there have been significant impacts on shark populations in this area (Anderson et al., 1998; Graham et al., 2010), and therefore the true state of these shark populations is unclear. The relatively low level of human impacts has been suggested as the reason for some rarely observed or unique behaviours of other species in the Chagos, reinforcing the area as an important reference site for ecological and behavioural studies (Sheppard et al., 2012). For example moray eels (*Gymnothorax pictus*), have been observed feeding on shorecrabs, leaping clear of the water in order to catch them (Graham et al., 2009). Shark poaching and bycatch in the tuna longline fishery prior to the introduction of the no-take MPA, indicate that there was a significant impact of humans on the Chagos shark population (Clarke et al., 2012). Given shark life history characteristics, there may therefore, be a delay before these populations recover and are considered to be in a truly natural state. Depending on the time scale associated with this lag, the proposed study will either describe natural behaviours of sharks or provide a baseline of shark behaviour which may change over time as shark populations revert to their natural state. Long term records of shark sightings by scientific divers in Chagos from 1975 to 2006 are available (Graham et al., 2010) and may provide an indication of when shark populations are again at a natural level.



*Figure 1:* Map of the Chagos Archipelago, the inset shows the position of the archipelago within the Indian Ocean and the reserve boundary. The main map shows; the major atolls with land (in bold), the islands of the Great Chagos Bank and and submerged reef atolls (not in bold) (taken from Sheppard et al. 2012).

### ***Review of Commonly Used Sampling Methods***

Studying sharks in their natural setting is challenging given that they are large predators and inhabit relatively remote environments that can be difficult to sample directly (Bres, 1993; Nelson, 1977). The methods employed in the study of shark behaviour, and behaviour in the

marine environment more generally, depend on the question being asked and the spatial and temporal scales at which the hypotheses are being tested. Current literature on shark behaviour includes lab studies of sharks in tanks involving direct observations of individuals and their interactions in this setting (Myrberg and Gruber, 1974), observational studies carried out by divers, many of which focus on agonistic displays (Johnson and Nelson, 1973; Smith et al., 2010), observational studies carried out from boats or cages (Sims et al., 2000; Sperone et al., 2010), studies of association patterns using photo ID techniques with sex and length estimations (Mourier et al., 2012), and broader scale tagging studies tracking movements for extended periods (Nelson, 1976; Vianna et al., 2013). However, there are a number of shortcomings in these techniques when considered for the purposes of this study.

Understanding of behaviour requires observation of interactions and behavioural responses in relatively natural settings. However, the size of most sharks and the costs associated with the maintenance of large animals in captivity reduce the feasibility of laboratory studies. In the field, sharks may avoid divers, especially those using open circuit scuba (Nelson, 1976), with diver proximity and number of divers in the water having a significant effect on shark behaviour (Smith et al., 2010). Additionally many species of sharks exhibit vertical migration patterns taking them below scuba depths for extended periods (Bres, 1993) and agonistic displays towards divers indicate that there is an element of risk involved in carrying out behavioural studies on sharks (Johnson and Nelson, 1973). Graham et al. (2010) also suggest that increased inquisitive behaviour of sharks in areas where divers are not common may skew results. Tagging studies are very useful in monitoring spatial movements of sharks over large temporal and spatial scales, however, they do not provide information on the finer scale behaviours and interactions of interest in this study. Watters et al. (2009) in a review of behavioural studies in zoos stated that observers who influence the behaviour of the animals they are studying create biased data sets and suggested video recordings as a method of avoiding observer effect.

Stereo baited remote underwater video systems (BRUVS) have been used in many studies of underwater assemblages (Cappo et al., 2004; Stobart et al., 2007). They are a cost effective, non-destructive method for sampling fish assemblages (Cappo et al., 2004) and can be operated in depths of up to 1200m (Zintzen et al.). They allow species identifications, length measurements, distance measurements, estimates of animal speed and habitat information to be collected and provide video footage of a range of species interacting with one another and the bait in a reproducible and controlled manner (Cappo et al., 2004; Harvey et al., 2007).

Despite this potential, stereo BRUVS have been used in relatively few behavioural studies of sharks (Ebner et al., 2009; Renchen et al., 2012). Remote video recordings proved useful in the study of behavioural interactions between thresher sharks and cleaner wrasses in the Philippines (Oliver et al., 2011) and handheld video cameras were used to record feeding behaviours in pelagic thresher sharks (Oliver et al., 2013). These studies may have been improved by the additional information that can be provided by stereo video observations (e.g. providing estimates of shark sizes and swimming speeds). Gruber and Myrberg (1977) stated that real progress in understanding shark behaviour will only be made when investigations have evolved to an analytical stage dependent on precise, accurate description, and quantitative data. This study aims to combine the usefulness of observations of shark behaviour gained from video footage with the metrics which can be derived from stereo-video systems, to allow detailed analysis and descriptions of behaviours to be combined with distance and length data.

Despite the apparent benefits of stereo BRUVS, there is no single sampling technique that does not introduce biases (Kingsford and Battershill, 1998). Potential biases associated with BRUVS include reliance on good visibility (Watson et al., 2005), the influence of the rig itself on behaviour, perhaps inducing inquisitive behaviour, the use of bait and the limited field of view meaning that only a subset of any individuals behaviours will be observed. However, any observational study conducted in the submarine environment is always going to be dependent on good visibility and any novel item introduced into the environment may induce inquisitive behaviour from animals, i.e. in the presence of boats or divers. To some degree, BRUVS may have a greater degree of control on this effect as the stimulus is identical in every replicate whereas diver behaviour etc may vary between replicates. The use of bait in this study is a possible source of error as it effectively removes the effect of the prey and may lead to unnatural responses in the sharks present which are not representative of typical foraging behaviour. However, the use of bait however, is required in order to ensure a large enough sample size. Care will therefore need to be taken in making any generalisations about foraging behaviour in these species, however, some species of sharks are known to scavenge (Dudley et al., 2000) and this study may provide an indication of this kind of foraging behaviour. The limited field of view is another bias of this method, however, a subset of behaviours will be captured within that field of view and with sufficient replication and sampling, the behaviours observed should be representative of all those exhibited within and outside the field of view.

Diurnal variation in behaviour also needs to be considered. The videos to be used in this study were collected during the daytime. Nelson (1977) suggests that many shark species are primarily night active and thus many of their interesting behaviours may occur under darkness. If this method can be conducted successfully during daylight hours there is scope to expand studies to investigate temporal changes in behaviour associated with time of day. This would remove the need for divers to be in the water and possible dangers associated with night diving.

### ***Knowledge Gap***

Bres (1993) suggested that more knowledge on almost every aspect of physiology, ecology and behaviour of sharks is required to determine how these species should be managed and protected. However, gathering these data is challenging. Foraging behaviour remains relatively poorly understood in large marine carnivores in general (Austin et al., 2006), studies of natural feeding behaviour in sharks are few and many of these are purely anecdotal (Motta and Wilga, 2001). Martin (2007) outlines a number of avenues of behavioural study which would provide benefits in consolidating theories and providing useful information to other sectors, including:

- Sound and video playback style experiments that test the functional significance of agonistic displays in sharks and the sensory modalities involved;
- Verification of studies of small sharks in controlled captive environments in the wild under more natural conditions;
- Further study of agonistic behaviour in sharks to increase safety in interactions between sharks and humans; and
- Study of sharks via direct observations to allow insights into more subtle aspects of social behaviour.

The proposed study provides a method of gathering behavioural data for a number of shark species in a relatively natural setting and allows video playback and analysis of these behaviours.

### **AIMS**

The overall aim of this study is to identify the factors affecting behavioural responses of sharks to bait and their interactions with one another as well as providing evidence for neuroecological theories of behaviour. I aim to provide evidence of behavioural differences

between species and ontogenetic shifts in behaviour within species. If differences are found, I then aim to use previously collected data on the brain morphology of the study species to provide support for inferences made about the behaviour of sharks based on their brain organisation and size. In order to do this, variation in relative brain size, the brain organisation and relative size of the different sensory brain area and any corresponding information on sensory ecology between species will be considered as explanatory variables in describing behavioural differences. I also aim simply to catalogue a range of behaviours exhibited by shark species in a natural setting.

Reflecting on these aims, it is hypothesised that:

1. Behavioural responses and interactions of sharks around BRUVS will vary between species and ontogenetically within species
2. Any differences found will be explained at least partially by relative brain size, brain organisation (with respect to the relative size of sensory brain regions and major brain regions) and phylogeny

## **SIGNIFICANCE AND OUTCOMES**

The use of remote sampling techniques in a relatively natural location to collect information on the foraging behaviour and interactions of sharks and the scales at which these behaviours vary is likely to lead to ecologically relevant observations. This will aid in making well informed and appropriate management and conservation decisions, as well as determining whether strategies are applicable to all shark species or whether they need to be tailored to individual species or clades and perhaps even to developmental stages within species.

Results on the way behaviours are exhibited and their frequencies vary with brain size, brain organisation and sensory ecology will provide evidence for the neurological basis of behaviour. Additionally, this study will test theories suggesting that differences in the neurology of elasmobranchs are linked not simply to their phylogeny but also to ecology and behaviour. This study is an ideal candidate for empirically testing these theories as it will compare several species of predator of varying sizes and levels of phylogenetic closeness under consistent conditions in relatively natural conditions. Results may also allow conclusions about foraging and predatory behaviour which could be important in foraging theories or may be applicable to other species.

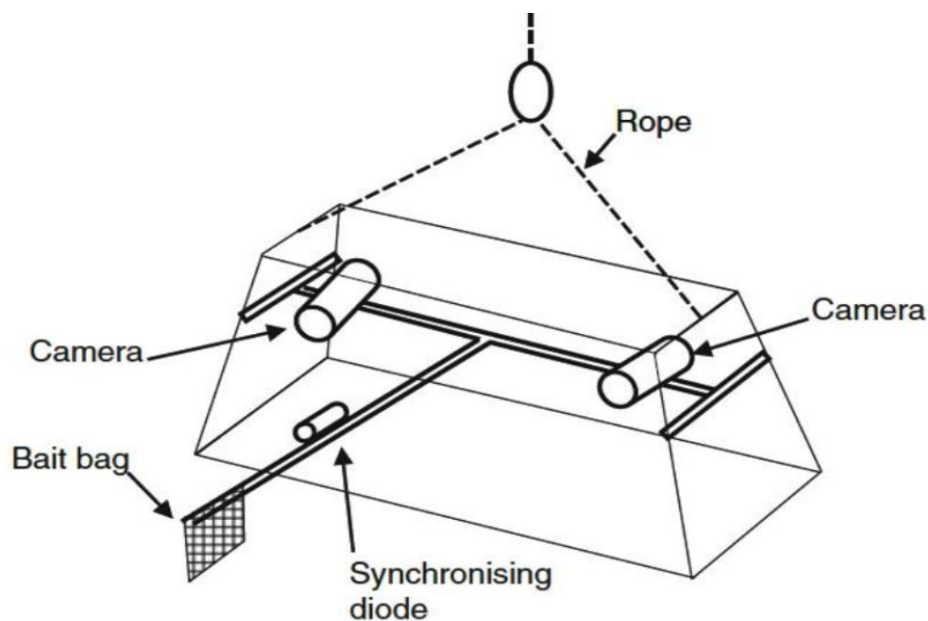


Additionally, the study of the behaviour of sharks may reveal evidence of previously undescribed behaviours or interactions that may provide useful information for improving conservation methods (e.g. by-catch reduction). Observations of agonistic behaviours may also add value in terms of mitigating negative interactions between sharks and humans or aid in the shark ecotourism industry (Martin, 2007).

## METHODOLOGY

### *Stereo-BRUVS*

The video footage utilised in this study was collected using stereo-BRUVS. Stereo-BRUVS consist of a metal frame onto which is mounted two video cameras in waterproof housings and a bait arm with bait bag attached (Figure 2). Prior to each deployment the bait bag was filled with 0.8-1kg of mashed pilchards for bait. The cameras were then readied and sealed in their housings, and then synchronised using a clapper board. Stereo-BRUVS were deployed at different sampling locations to film for one hour before being retrieved.



*Figure 2:* Schematic of a Stereo BRUVS, showing the metal frame, two video cameras in waterproof housings, bait arm, bait bag, synchronising diode and rope. The rigs used in this study did not use a synchronising diode.

### *Sampling Technique*

The footage used for this study was collected in April 2012 from several locations around the Chagos Archipelago in the central Indian Ocean (Figure 1) at a range of depths from 5.4 –

82.2 metres, and in a variety of habitats. Sightings of sharks were recorded in a total of 110 of the stereo BRUV drops.

### ***Video Analysis***

The videos collected will be analysed with the use of the computer program Event Measure. This program uses information on the angles of the stereo cameras to derive distance and length measurements, as well as allowing the logging of attributes such as species and behaviours. Each video will be analysed for one hour from the time of deployment. Three types of data will be recorded;

#### *1. Individual identification and body size estimates*

Individual identification is an important component to consider if the proposed methodology is to be effective. Individual sharks will be distinguished by size, sex and individual identifying marks and details (such as scars, fin tears and pigment spot patterns) (Myrberg and Gruber, 1974; Nelson, 1977). When a shark exits the field of view and there is reasonable doubt whether or not it is the same individual returning, these characteristics will be used to distinguish between individuals where possible. However, if an animal cannot be identified as “new” or an earlier identified animal, no measurement will be taken to avoid pseudo-replication (Hurlbert, 1984).

Body size estimates can be readily produced using Event Measure with reported precisions. Snout to tail fork measurements are used, these are obtained by clicking on the snout and tail fork in the view from the left hand camera of the rig, then repeating this procedure in the right hand frame (Figure 2). The program uses this information combined with previously determined calibration information to estimate the distance between the two points as well as the 3-dimensional position of the animal relative to the camera.

#### *2. Position at time $t$ at regular intervals to determine speeds and approaches to attractant/passes*

The 3-dimensional position of individuals at regular intervals will be determined by placing a point on the snout of the individual every 10 seconds throughout the time they are in frame. This period of 10 seconds is based on preliminary reviews of footage to ensure that sufficient positions are recorded. From these positions, I will be able to calculate distance from bait, swimming speeds, rate of approach to bait, number of passes, time spent at the bait, and other metrics.

### 3. Records of key behaviours

The Event Measure program allows behaviours identified in the ethogram to be recorded both temporally and spatially for each individual. Each time a key behaviour is observed a 3D point will be placed on the snout of the individual performing the behaviour and the behaviour and attributes of the individual will be logged in the attributes fields. The primary focus of this part of the project will be to identify and document interactions with the bait bag, interactions between individuals and display behaviours.

Ethograms are a primary tool for the semi-quantitative analysis of behavioural data (Schleidt et al., 1984). An ethogram is essentially a catalogue of behaviours or actions exhibited by an animal. An ethogram of foraging and display behaviours applicable to each study species will be developed. This will be developed based on previous literature of shark behaviours (Johnson and Nelson, 1973; Martin, 2007; Myrberg and Gruber, 1974; Smith et al., 2010; Sperone et al., 2010), preliminary viewing of the footage, relevance to the study and the ability to be objectively defined such that behaviours are consistently identified. Each behaviour will be assigned a clear and objective definition which will then be used during video analysis to log behaviours.

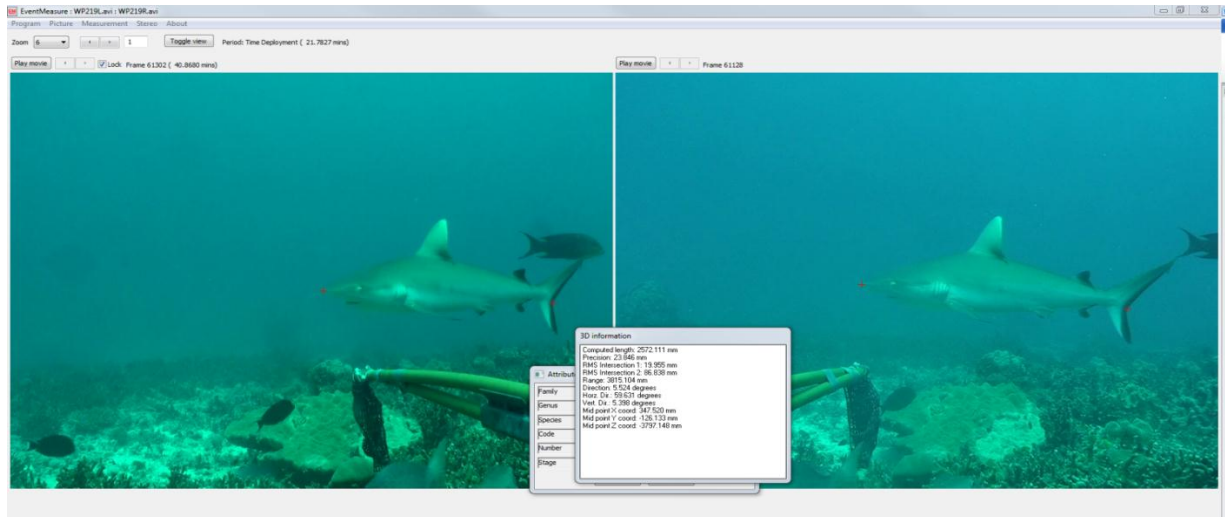


Figure 2: The Event Measure window showing views from the left and right cameras, measurement points (red) on the snout and tail fork in each frame and the measurements results window.

### Brain Morphology

Data required for comparisons of sensory ability and sensory brain morphometrics will be gathered primarily from previous studies. However, if any additional information is required,

existing preserved specimens may be accessed to gather data (Yopak and Collin pers. comm.). A range of brain characteristics will be considered in the analyses.

Firstly, to investigate the role of sensory dominance, the relative size of the different sensory brain areas will be compared as a proportion of total sensory brain mass (Wagner, 2002). This will involve assessing the relative volumes of the olfactory bulbs, optic tecta and anterior and posterior lateral line lobes that receive input from the olfactory epithelium, eyes, electroreceptors and lateral line, respectively. The relative size of major brain regions (telencephalon, diencephalon, mesencephalon, cerebellum and medulla) will also be considered based on their relative size as a proportion of total brain mass, of the. Relative brain size (total) will be calculated as a percentage of overall body mass (Yopak et al., 2007).

### ***Statistical Analysis***

Statistical analysis will rely heavily on the use of analyses of covariance (ANCOVA), to test how behaviours vary across species and variations in brain morphology whilst controlling for body size as our independent variables are a mix of categorical and continuous. Independent contrasts will be used to account for the effect of phylogeny (Felsenstein, 1985), when considering the effect of brain organisation and size. Chi square analyses may also be used where response variables are counts (i.e. number of passes) and logistic regression may be used for binary variables such as the presence or absence of a bite.

### ***Methodological Limitations***

There are a number of limitations and requirements of the methodology to be used. Stereo-BRUVS require good visibility to gather high quality video and allow accurate measurements to be made, however this is a limitation common to most observational studies in a marine environment and we will mitigate this by sampling only the video footage with sufficient visibility to make accurate measurements within 7 m of the bait bag. The limited frame of stereo-BRUVS also means that only a subset of the behaviours exhibited by individual sharks will be recorded. However, all interactions with the bait bag will be recorded and it is likely that with a large sample size, behaviours which occur within frame will be representative of those exhibited overall.

## **TIMETABLE**

**Table 2:** Proposed timetable indicating the timing of various aspects of the proposed project. Blue boxes indicate that the given activity will be conducted during the corresponding month.

Activity	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul
Project Outline												
Write Proposal												
Proposal seminar												
Finalise methods												
Video analysis												
Data Analysis												
Write thesis												
Revisions on thesis												
Coursework												

## BUDGET

*Table 1:* Anticipated expenditures associated with the proposed project.

Component	Estimated cost
Software Licensing	\$100
Costs associated with participating in a field trip in order to gain firsthand experience with camera rigs	\$300
Hard-drives	\$300
Printing costs	\$100
<b>Total</b>	<b>\$800</b>

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Original Article

# Honeybee cognitive ecology in a fluctuating agricultural setting of apple and pear trees

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Honeybees' plasticity to adjust responses according to environmental changes has been extensively studied in the laboratory but seldom along temporal variation in natural resources. Apple (*Malus domestica*) and pear (*Pyrus communis*) trees often coexist in mixed agricultural settings offering different resources, and their blooming periods are slightly shifted. This scenario provides an opportunity to study how changing environments influence the way the honeybees perceive, learn, discriminate, and use odor-resource information along successive flowering events. We found that honeybees preferred to gather pollen on pear flowers and nectar on apple. These individual preferences correlated with variations in the type of resources collected at the colony level according to changes in the floral market. Spontaneous proboscis extension response to pear and apple floral scents of bees captured at the hives located within the crops fluctuated according to changes in floral availability too. The capability of the proboscis extension response-trained honeybees to discriminate between both floral scents at the beginning or at the end of the flowering period was also found, although bees lose this ability when both flowers were fully available. These response patterns suggest that olfactory information experienced on flowers were memorized to predict reward yet susceptible of being reversed when the floral scents were no longer available in the orchard. Combining behavioral assays and field observations, we provide here an example of how honeybees process floral odor information to make decisions about resources in a fluctuating complex environment.

## INTRODUCTION

An approach to study animal behavior is the so-called cognitive ecology—concerned with how animals obtain and process information of their environment, relate it to themselves, and use it to make decisions (Dukas 1998; Healy and Braithwaite 2000; Chittka and Thomson 2001). An important point of this field is integrating ecology (i.e., interactions between organisms and their environment) and cognition (i.e., the neuronal processes through which animals acquire and make use of information) to understand how the surroundings influence the way an animal makes decisions according to their perceptual and learning capabilities.

Abundance and composition of flower species have profound ecological consequences on the insect pollinators because both can change over their short life span (Kearns and Inouye 1993; Willmer and Stone 2004). The ability of nectar and pollen foragers to perceive, learn, discriminate, and thereafter switch their foraging preferences among flower species is critical to adjusting their behavior in an ever-changing environment. Such a statement is supported by numerous studies that assess the relevance of insect behavioral plasticity in response to a wide range of factors that change unpredictably under laboratory conditions (Menzel 1999; Dyer and Chittka 2004; Giurfa 2007). However, few studies have analyzed such plasticity concerning natural floral resource fluctuations in space and time (Gerber et al. 1996; Raine and Chittka 2008).

It is well known that generalist bees, such as honeybees and bumblebees, assess the nectar quality of the flowers offered (von Frisch 1967; Waddington and Gottlieb 1990; Banschbach 1994; Molet et al. 2009), which forms the basis of the decision of whether to forage or not. When a forager bee perceives a food source as suitable (either a nectar or a pollen source), it can associate the surrounding floral cues with the reward and within a few visits

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## Adjacent coral reef habitats produce different underwater sound signatures

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**ABSTRACT:** The underwater sound generated by the organisms on a reef has been shown to provide an important orientation cue for a wide range of larval, juvenile and adult marine organisms. There is some preliminary evidence that some organisms can discriminate among different benthic habitats using sound cues over relatively short spatial ranges (i.e. within hundreds of metres); however, the divergence in the sound emitted from different habitats, often in close proximity to one another, is poorly described. Therefore, the sound emitted from single locations within 3 adjacent habitats, Fringing Reef, Back Reef and Lagoon, at Lizard Island on the Great Barrier Reef, Australia, were recorded during the new moon phase in early summer. Analyses of the sound recordings revealed differences among these 3 habitats in the temporal and frequency composition and in sound levels. Most of the spectral variability among the 3 habitats was observed below 800 Hz, where the duration of the dusk chorus differed between the 3 habitats. Some of these observed differences were due to the acoustic output of some key soniferous organisms dwelling in these habitats, especially snapping shrimp and fish species producing a pop sound. It is possible that these habitat-related differences in underwater sound are being used to remotely guide the movement of coastal organisms in relation to these habitats.

**KEY WORDS:** Reef sound · Fish sound · Habitat · Great Barrier Reef · Acoustic orientation

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### INTRODUCTION

Ambient underwater sound in the sea is composed of a combination of biotic and abiotic acoustic sources (Acosta et al. 1997, Cato & McCauley 2002). Abiotic sources of ambient underwater sound in the nearshore environment are mostly due to the effect of the wind and waves, and tend to produce sound of frequencies above 150 Hz. In contrast, sound from biotic sources covers a wide range of frequencies and is generated by a variety of marine life involved in activities such as feeding, reproductive displays, territorial defence and echolocation (Knudson et al. 1948, Tait 1962, Wenz 1962, Cato 1978, 1992, McCauley & Cato 2000, Radford et al. 2008a,b).

The fact that ambient underwater sound varies among locations in the marine environment has been known for many years (Urlick 1983). For example, the

spatial variability of underwater sound due to surf and ice movement (Wilson et al. 1985, Yang et al. 1987, Deane 2000) has been described, but there are limited data describing the acoustic environment in shallow-water coastal environments in tropical or temperate waters (Kennedy et al. 2010, Radford et al. 2010, Staaterman et al. 2013). Only 2 published studies have examined differences in the underwater acoustic environment in shallow coastal waters over small spatial scales (within kilometres) in typical temperate (Radford et al. 2010) and tropical reefs and associated habitats (Kennedy et al. 2010). Marked differences were found over relatively short spatial scales in the spectral and temporal composition of ambient sound associated with different coastal habitat types along a coastline in the temperate waters of New Zealand (Radford et al. 2010). Similarly, the frequency characteristics of the daytime sound pro-

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**APPENDIX III:** Brain organisation and body mass data. Major brain region values indicate their relative proportion of the total brain mass.

Species	Body mass (g)	Brain mass (g)	Brain to Body ratio (%)	Olfactory bulbs (%)	Telencephalon (%)	Diencephalon (%)	Mesencephalon (%)	Cerebellum (%)	Medulla oblongata (%)
<i>Carcharhinus albimarginatus</i> <sup>a,b</sup>	5000	25	0.50						
<i>Carcharhinus amblyrhynchos</i> <sup>c,d,e,f</sup>	25540	37	0.14	3.79	61.78	4.55	7.99	12.57	9.30
<i>Carcharhinus melanopterus</i> <sup>c,d,e,f</sup>	7650	19	0.25	5.99	54.87	5.82	7.40	14.90	11.07
<i>Galeocerdo cuvier</i> <sup>c,d,e,f</sup>	148600	27	0.02	27.05	36.51	5.11	8.60	12.32	10.44
<i>Triaenodon obesus</i> <sup>c,d,e,f</sup>	16300	16	0.10	3.09	55.10	5.18	8.71	15.40	12.56
<i>Nebrius ferrugineus</i> <sup>c,d,e,f</sup>	32200	16	0.05	4.95	55.41	4.98	4.17	20.83	9.66
<i>Sphyrna lewini</i> <sup>c,d,e,f</sup>	25000	50	0.20	5.83	50.62	4.19	6.15	22.33	10.89
<i>Sphyrna mokarran</i> <sup>c,d,e,f</sup>	148500	110	0.07	9.66	60.10	3.06	3.39	16.63	7.16

Source: a) Bauchot et al. 1995 b) Yopak 2012 c) Yopak et al. 2007 d) Yopak et al. 2010 e) Yopak & Lisney 2012 f) Yopak et al. (in review)